Websites:
http://www.sciencepub.net
http://www.sciencepub.net/report

Emails:
editor@sciencepub.net
reportopinion@gmail.com

Report and Opinion

MARSLAND PRESS
Multidisciplinary Academic Journal Publisher

Cell Growth and Division and Cancer Biology Research Literatures

Mark Herbert, PhD

World Development Institute
39-06 Main Street, Flushing, Queens, New York 11354, USA, ma8080@gmail.com

Abstract: Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries. This article introduces recent research reports as references in the cell growth and division and cancer biology research literatures and related studies.

[Mark H. **Cell Growth and Division and Cancer Biology Research Literatures.** *Rep Opinion* 2020;12(8):28-147]. ISSN 1553-9873 (print); ISSN 2375-7205 (online). http://www.sciencepub.net/report. 8. doi:10.7537/marsroj120820.08.

Key words: cancer; life; research; literature; cell

1. Introduction

Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries. This article introduces recent research reports as references in the cell growth and division and cancer biology research literatures and related studies.

The following introduces recent reports as references in the related studies.

Abasolo, I., et al. (2003). "Overexpression of adrenomedullin gene markedly inhibits proliferation of PC3 prostate cancer cells in vitro and in vivo." Mol Cell Endocrinol **199**(1-2): 179-187.

The expression of the gene encoding adrenomedullin (AM), a multifunctional peptide hormone, in the prostate is localized to the epithelial cells. Prostate cancer cells are derived from prostatic epithelial cells. To elucidate the potential role of the AM gene in prostate cancer progression, we have stably-transfected the PC3 human prostate cancer cell line with an AM gene expression vector. The AM-transfected PC3 sublines were studied along with parental and empty vector transfected PC3 cells as controls. The average level of AM in the conditioned media of AM-transfected cells was 0.959+/-0.113 nM, a physiologically relevant concentration. The ectopic expression of AM gene inhibited the proliferation of PC3 cells in culture dishes. In addition, anchorage-independent growth of the transfected sublines was virtually abolished in soft agar assays. Flow cytometry studies showed that overexpression of AM gene caused a very significant G (1)/G (0) cell cycle arrest. In vivo experiments demonstrated that AM gene expression markedly inhibited the growth of xenograft tumors in nude mice. Our in vivo and in vitro studies suggest that AM could strongly suppress the malignancy of prostate cancer cells, via autocrine and/or paracrine mechanisms.

Abdul-Wahab, K., et al. (1999). "Overexpression of insulin-like growth factor II (IGFII) in ZR-75-1 human breast cancer cells: higher threshold levels of receptor (IGFIR) are required for a proliferative response than for effects on specific gene expression." Cell Prolif **32**(5): 271-287.

Previous transfection experiments using a zinc-inducible expression vector have shown that overexpression of insulin-like growth factor II (IGFII) in MCF7 human breast cancer cells can reduce dependence on oestrogen for cell growth in vitro (DALY RJ, HARRIS WH, WANG DY, DARBRE PD. (1991) Cell Growth Differentiation 2, 457-464.). Parallel transfections now performed into another oestrogen-dependent human breast cancer cell line

(ZR-75-1) yielded three clones of transfected ZR-75-1 cells that produced levels of zinc-inducible IGFII mRNA and secreted mature IGFII protein similar to those found in the transfected MCF7 cells. However, unlike in MCF7 cells, no resulting effects were found on cell growth in the ZR-75-1 clones, even though the ZR-75-1 clones possessed receptors capable of binding 125I-IGFI and showed a growth response to exogenously added IGFII. Medium conditioned by the ZR-75-1 clones could stimulate growth of untransfected MCF7 cells, indicating that the secreted IGFII protein was bioactive. Furthermore, zinc-induced IGFII was capable of increasing both pS2 mRNA levels and CAT activity from a transiently transfected AP1-CAT gene in the ZR-75-1 clones. Constitutive co-overexpression of the protein processing enzyme PC2 resulted in reduced levels of large forms of zinc-inducible IGFII, but zinc treatment still produced no effect on cell growth rate. Finally, however, constitutive co-overexpression of the type I IGF receptor (IGFIR) did result in zinc-inducible increased basal cell growth and reduced dependence on oestrogen for cell growth. These results demonstrate that while overexpression of IGFII per se was sufficient to deregulate MCF7 cell growth, the ZR-75-1 cells are limited in their proliferative response by their intrinsic receptor levels. However, although the proliferative response was limited, molecular responses (expression of pS2 and AP1-CAT) were not limited, indicating that different cellular responses can have different threshold receptor level requirements.

Abeysinghe, H. R., et al. (2004). "The role of the THY1 gene in human ovarian cancer suppression based on transfection studies." Cancer Genet Cytogenet **149**(1): 1-10.

In our recent studies, the expression of the THY1 gene encoding a 25-28 kDa glycoprotein located at 11q23-q24, was found to be associated with complete tumor suppression of the ovarian cancer cell line SKOV-3 after the transfer of chromosome 11. These studies raised the possibility that THY1 maybe a candidate tumor suppressor gene for ovarian cancer. To investigate this, the complete cDNA sequence for THY1 was cloned and transfected into SKOV-3 ovarian cancer cells. The expression of THY1 in the transfectants was confirmed by Northern blot analysis, immunocytochemistry, and flow cytometry. Both SKOV-3-THY1 and SKOV-3-null cells were inoculated subcutaneously into severe combined immunodeficiency (SCID) mice to determine in vivo tumorigenicity. THY1 transfectants formed tumors, but overall tumor growth rate and tumor size was significantly reduced compared with their null counterparts. To further correlate THY1 expression with tumorigenicity, the THY1 antisense was transfected into the nontumorigenic clone, 11(C)9-8, which resulted in restoration of tumorigenicity. These data indicate that THY1 expression alone cannot suppress tumorigenicity; however, abrogation of THY1 expression from nontumorigenic cells can restore tumorigenesis. Taken together, the data suggest that THY1 is necessary but not sufficient to suppress ovarian tumorigenicity. Therefore, THY1 can be designated as a putative tumor suppressor gene for human ovarian cancer.

Afonja, O., et al. (2002). "RAR agonists stimulate SOX9 gene expression in breast cancer cell lines: evidence for a role in retinoid-mediated growth inhibition." Oncogene **21**(51): 7850-7860.

Retinoic acid receptors (RARs) are ligand-dependent transcription factors which are members of the steroid/thyroid hormone receptor gene family. RAR-agonists inhibit the proliferation of many human breast cancer cell lines, particularly those whose growth is stimulated by estradiol (E2) or growth factors. PCR-amplified subtractive hybridization was used to identify candidate retinoid-regulated genes that may be involved in growth inhibition. One candidate gene identified was SOX9, a member of the high mobility group (HMG) box gene family of transcription factors. SOX9 gene expression is rapidly stimulated by RAR-agonists in T-47D cells and other retinoid-inhibited breast cancer cell lines. In support of this finding, a database search indicates that SOX9 is expressed as an EST in breast tumor cells. SOX9 is known to be expressed in chondrocytes where it regulates the transcription of type II collagen and in testes where it plays a role in male sexual differentiation. RAR pan-agonists and the RARalpha-selective agonist Am580, but not RXR agonists, stimulate the expression of SOX9 in a wide variety of retinoid-inhibited breast cancer cell lines. RAR-agonists did not stimulate SOX9 in breast cancer cell lines which were not growth inhibited by retinoids. Expression of SOX9 in T-47D cells leads to cycle changes similar to those found with RAR-agonists while expression of a dominant negative form of SOX9 blocks RA-mediated cell cycle changes, suggesting a role for SOX9 in retinoid-mediated growth inhibition.

Ahn, W. S., et al. (2004). "Recombinant adenovirus-p53 gene transfer and cell-specific growth suppression of human cervical cancer cells in vitro and in vivo." Gynecol Oncol **92**(2): 611-621.

PURPOSE: We investigated the time-course expression patterns of p53 and E6 on cervical cancer cells to obtain a molecular level understanding of cell-dependent tumor growth suppression effects of

recombinant adenovirus expressing p53 in vitro and in vivo. METHODS: Four human papillomavirus (HPV)-infected human cervical cancer cell lines (HPV 16-positive cells, CaSki and SiHa cells; and HPV 18-positive cells, HeLa and HeLaS3 cells) were used. Also, HPV negative C33A and HT3 cell line that has a mutation on p53 gene were used. After infection with AdCMVp53, the cell growth inhibition was studied via cell count assay, MTT assay, and Neutral red assay. After transfecting AdCMVp53 and AdCMVLacZ into the cancer cells-xenografted nude mice, antitumor effects were investigated for 1 month, respectively. RESULTS: For each cervical cancer cell, IC50 was as follows; CaSki (68.5 multiplicity of infection, or MOI), SiHa (43.5 MOI), HeLa (31 MOI), HeLaS3 (42 MOI), C33A (21 MOI), and HT3 (62 MOI). In particular, complete inhibition of cell growth was observed at 125 MOI in both CaSki and SiHa cells. However, the complete inhibition was detected at 62.5 MOI in HeLa and HeLaS3. In contrast, at these MOI, no suppression of cell growth was observed when cells were infected with recombinant adenovirus expressing beta-gal as a negative control. The levels of p53 protein were notably expressed in CaSki and HeLa more than in SiHa and HeLaS3 on days 2 and 4. However, the p53 was only detected in HeLaS3 on day 6. In contrast, p53 expression was continually maintained in C33A and HT3 during the same periods. After transfection AdCMVp53 into CaSki- and SiHa-xenografted nude mice, the size of tumor was remarkably decreased in SiHa cells as compared to AdCMVLacZ transfection. CONCLUSION: The adenovirus-mediated p53 gene transfection was done effectively in vitro and in vivo. Also, the antitumor effects were accomplished via differential role of p53-specific apoptotic cell death, which is dependent upon the cervical cancer cell line.

Ahn, W. S., et al. (2003). "A major constituent of green tea, EGCG, inhibits the growth of a human cervical cancer cell line, CaSki cells, through apoptosis, G (1) arrest, and regulation of gene expression." DNA Cell Biol **22**(3): 217-224.

A constituent of green tea, (-)-epigallocatechin-3-gallate (EGCG) has been known to possess antiproliferative properties. In this study, we investigated the anticancer effects of EGCG in human papillomavirus (HPV)-16 associated cervical cancer cell line, CaSki cells. The growth inhibitory mechanism (s) and regulation of gene expression by EGCG were also evaluated. EGCG showed growth inhibitory effects in CaSki cells in a dose-dependent fashion, with an inhibitory dose (ID) (50) of approximately 35 microM. When CaSki cells were further tested for EGCG-induced apoptosis, apoptotic cells were significantly observed after 24 h at 100 microM EGCG. In contrast, an insignificant induction of apoptotic cells was observed at 35 microM EGCG. However, cell cycles at the G1 phase were arrested at 35 microM EGCG, suggesting that cell cycle arrests might precede apoptosis. When CaSki cells were tested for their gene expression using 384 cDNA microarray, an alteration in the gene expression was observed by EGCG treatment. EGCG downregulated the expression of 16 genes over time more than twofold. In contrast, EGCG upregulated the expression of four genes more than twofold, suggesting a possible gene regulatory role of EGCG. This data supports that EGCG can inhibit cervical cancer cell growth through induction of apoptosis and cell cycle arrest as well as regulation of gene expression in vitro. Furthermore, in vivo antitumor effects of EGCG were also observed. Thus, EGCG likely provides an additional option for a new and potential drug approach for cervical cancer patients.

Alkhalaf, M. and A. M. El-Mowafy (2003). "Overexpression of wild-type p53 gene renders MCF-7 breast cancer cells more sensitive to the antiproliferative effect of progesterone." J Endocrinol **179**(1): 55-62.

We have recently shown that growth inhibition of breast cancer cells by progesterone is due to the induction of cell differentiation, but not apoptosis. Because the tumor suppressor protein p53 plays a central role in normal cell growth and in tumor suppression, we have examined the effect of progesterone on the levels of this protein in MCF-7 cells. We show here that the antiproliferative effect of progesterone is accompanied with down-regulation of endogenous p53 protein. To study the effect of progesterone on cell growth in the presence of normal levels of p53 protein, we used transient transfection to overexpress p53 protein. MCF-7 cells were transfected with a p53 expressing vector that contains p53 human cDNA under the control of a cytomegalovirus promoter. Cell growth, cell viability, and apoptosis were analyzed in the transfected cells after six days of exposure to 100 nM progesterone. We show here that progesterone significantly enhances growth inhibition and apoptosis in MCF-7 cells overexpressing p53, but not in cells transfected with the control vector. These data suggest that re-establishing p53 function in MCF-7 breast cancer cells renders them more sensitive to the growth inhibitory effect of progesterone.

Allay, J. A., et al. (2000). "Adenovirus p16 gene therapy for prostate cancer." World J Urol **18**(2): 111-120.

Surgery, radiation, or hormone deprivation alone does not adequately affect local control of clinical or pathologic stage T3 prostate cancer. Lack of local cancer control ultimately leads to a higher incidence of

morbidity, distant metastasis, and decreased survival, with patients having disease-specific mortality exceeding 75%. Other novel therapies against this devastating and common disease are needed for the achievement of long-term local cancer control. For this purpose, therapeutic interventions should target prostate-cancer cells at the molecular and cellular level in ways not possible by current modalities of cancer treatment. Any strategy that can modify the biologic behavior of these cells may potentially have the most significant clinical impact. As prostate cancer represents an accumulation of genetic mutations that causes a prostate cell to lose the ability to control its growth, one new approach against prostate cancer may be gene therapy. Identification of key missing or mutated tumor-suppressor genes that, when replaced, may inhibit or destroy prostate-cancer cells may have the best chance of clinical success. One such gene appears to be tumor-suppressor gene p16 (also known as MTS1, INK4A, and CDKN2). Tumor-suppressor gene p16 is an important negative cell-cycle regulator whose functional loss may significantly contribute to malignant transformation and progression. Alterations in the p16 gene and its protein expression often occur in prostate cancer. An adenoviral vector containing wild-type p16 (Adp16) had a high transduction efficiency in prostate-cancer cells both in vitro and in vivo. Moreover, prostate tumors injected with Adp16 expressed p16 and the adenoviral vector expressed the transgene for up to 14 days. Wild-type p16 inhibited prostate-cancer proliferation in vitro and markedly suppressed tumors in vivo. Pathologic evaluation of the Adp16-treated tumors showed dose-dependent necrosis and fibrosis. Although the mechanism of p16 inhibition in cancer remains to be elucidated, senescence and apoptosis may both be important; however, the data suggest that p16-induced growth inhibition can function independently of the retinoblastoma gene product.

Anwer, K., et al. (2000). "Cationic lipid-based delivery system for systemic cancer gene therapy." Cancer Gene Ther **7**(8): 1156-1164.

A cationic lipid-based gene delivery system composed of N-[(1-(2,3-dioleyloxy)propyl)]-N-N-N-trimethylammonium chloride and cholesterol, at a 4:1 molar ratio, was developed for systemic administration. Plasmid biodistribution and expression were characterized in syngeneic mouse tumor model squamous cell carcinoma VII cells. A reporter gene expression plasmid was used for biodistribution of plasmid and expression. The results showed that lungs and primary tumors were transfected. Fluorescence microscopy showed that fluorescent-labeled transfection complexes were passively targeted to the tumor vasculature and that the endothelial cells internalized the plasmid. Transgene expression was characterized based on duration of expression and dosing schedule. In vivo gene transfer with an interleukin-12 expression plasmid yielded protein levels in blood, lungs, and primary tumor after intravenous administration. Efficacy studies showed that 15 microg of interleukin-12 plasmid was sufficient to produce a gene-specific inhibition of primary tumor growth. These results characterize the vascularity of the tumor model, characterize the in vivo gene transfer properties of the plasmid-based gene delivery system, and show that the transgene expression level was sufficient to elicit a biological response by inhibiting tumor growth.

Bai, J., et al. (1998). "Overexpression of CuZnSOD gene suppresses the growth of hepatocellular cancer cell line HepG2." Chin Med J (Engl) **111**(9): 789-792.

OBJECTIVE: To explore the inhibiting effect of superoxide dismutase (SOD) on the growth of hepatocellular cancer cell line HepG2. METHODS: By gene transfer technique, hepatocellular cancer cells (HepG2) were transfected with a retroviral vector containing human CuZnSOD cDNA. The elevated SOD gene expression of the transfected cells was compared with the parental and neo control cells. RESULTS: Compared to the control cells, cancer cells transfected with SOD gene showed an inhibited cell growth, a reduced number of cells in S phase and decreased clone forming ability in soft agar, as well as a smaller tumor size formed in nude mice. CONCLUSION: The overexpression of CuZnSOD gene could, to certain extent, suppress the cell growth of hepatocellular cancer cell line HepG2.

Bai, M., et al. (2001). "[Influence of suppressor gene p16 on retinoic acid inducing lung cancer cell A549 differentiation]." Zhonghua Jie He He Hu Xi Za Zhi **24**(9): 534-536.

OBJECTIVE: To investigate the role of suppressor gene p16 in the process of differential regulation of retinoic acid (RA) on the A549 lung cancer cells. METHODS: Tumor suppressor gene p16 was transfered into A549 cells and the cells were treated with all-trans retinoic acid (ATRA) at the dosage of 5 x 10(-6) mol/L for 4 d. After that, the proliferation and differentiation of A549 cells were examined by growth curve and cytometry analysis, the change of lung lineage-specific marker MUC1 was tested by immunohistochemical staining. Meanwhile, Western blot was used to observe the change of p16 protein expression in A549 cells treated with ATRA. RESULTS: ATRA could obviously inhibit the growth and induce the differentiation of A549 cells that were transfered with p16 gene. There were more cells

arrested in G1/G0 phase and the expression of MUC1 was markedly down-regulated than in control cells. The expression of p16 protein was up-regulated in A549 cells treated with ATRA. CONCLUSION: Suppressor gene p16 could enhance the effects of RA on proliferative suppression and differential induction of A549 cells.

Banerjee, A., et al. (1992). "Changes in growth and tumorigenicity following reconstitution of retinoblastoma gene function in various human cancer cell types by microcell transfer of chromosome 13." Cancer Res **52**(22): 6297-6304.

Functional loss of the retinoblastoma (RB) gene has been implicated in the initiation or progression of several human tumor types including cancer of the eye, bone, bladder, and prostate. To examine the consequence of adding one RB allele containing its normal regulatory elements back into representative examples of each of these cancer types, as well as to compare the results to those previously reported using various RB complementary DNA constructs, a neomycin resistant marked 13 chromosome was transferred by microcell fusion. Several attempts to obtain RB positive osteosarcoma cells failed. In addition, only one RB positive retinoblastoma clone was isolated. This clone contained many large cells, could not be maintained in long-term culture, and produced only RB negative tumors. Three RB positive bladder cancer cell clones were obtained, all of which grew slower in culture than their RB negative parental counterpart and did not form colonies in soft agar. Tumorigenicity was markedly suppressed in these clones. One clone yielded no tumors, and the other 2 clones produced only one small tumor each, both of which were RB negative. In contrast, the 2 RB positive prostate cancer cell clones isolated had no differences in their cell culture growth properties, including growth in soft agar compared to the parental cells. One of the clones was nontumorigenic, while the other clone produced 4 small tumors, all of which were RB positive. These results indicate that the transfer of one RB allele by microcell transfer produces different levels of growth inhibition as well as tumor suppression, depending on the cell type examined. In the case of prostate cancer, the function of the RB gene in tumor suppression appears to be independent from its growth regulatory function, since no growth inhibition in cell culture was noted in these cells, although tumor suppression was significant.

Bao, J. J., et al. (2002). "Reexpression of the tumor suppressor gene ARHI induces apoptosis in ovarian and breast cancer cells through a caspase-independent calpain-dependent pathway." Cancer Res **62**(24): 7264-7272.

ARHI, an imprinted putative tumor suppressor gene, encodes a M (r) 26,000 GTP-binding protein that is 60% homologous to ras and rap but has a dramatically different function. ARHI expression is down-regulated in a majority of breast and ovarian cancers. Using a dual adenovirus system, we have reexpressed ARHI in ovarian cancer and breast cancer cells that have lost ARHI expression. Reexpression of ARHI inhibited growth, decreased invasiveness, and induced apoptosis. At 5 days after infection with ARHI adenovirus, 30-45% of MDA-MB-231 breast cancer cells and 5-11% of SKOv3 ovarian cancer cells were apoptotic as judged by a terminal deoxynucleotidyl transferase-mediated nick end labeling assay and by Annexin V staining with flow cytometric analysis. Although poly (ADP-ribose) polymerase could be detected immunohistochemically in the nuclei of apoptotic cells, no activation of the effector caspases (caspase 3, 6, 7, or 12) or the initiator caspases (caspase 8 or 9) could be detected in cell lysates using Western blotting. When gene expression was analyzed on a custom cDNA array that contained 2304 known genes, infection with ARHI adenovirus up-regulated 15 genes relative to control cells infected with LacZ adenovirus. The greatest degree of mRNA up-regulation was observed in a Homo sapiens calpain-like protease. On Western blot analysis, calpain protein was increased 2-3-fold at 3-5 days after infection with ARHI adenovirus. No increase in calpain protein was observed after LacZ adenovirus infection. Calpain cleavage could be detected after ARHI reexpression, and inhibitors of calpain, but not inhibitors of caspase, partially prevented ARHI-induced apoptosis. Consequently, reexpression of ARHI in breast and ovarian cancer cells appears to induce apoptosis through a caspase-independent, calpain-dependent mechanism.

Bardon, S., et al. (1998). "Monoterpenes inhibit cell growth, cell cycle progression, and cyclin D1 gene expression in human breast cancer cell lines." Nutr Cancer **32**(1): 1-7.

Monoterpenes are found in the essential oils of many commonly consumed fruits and vegetables. These compounds have been shown to exert chemopreventive and chemotherapeutic activities in mammary tumor models and represent a new class of breast cancer therapeutic agents. In this study, we investigated the effects of limonene and limonene-related monoterpenes, perillyl alcohol and perillic acid, on cell growth, cell cycle progression, and expression of cyclin D1 cell cycle-regulatory gene in T-47D, MCF-7, and MDA-MB-231 breast cancer cell lines. Our results revealed that limonene-related monoterpenes caused a dose-dependent inhibition of cell proliferation. Of the three monoterpenes tested,

perillyl alcohol was the most potent and limonene was the least potent inhibitor of cell growth. The enantiomeric composition of limonene and perillyl alcohol did not interfere with their effect on cell growth. Sensitivity of breast cancer cell lines to monoterpenes was in the following order: T-47D > MCF-7 > MDA-MB-231. Growth inhibition induced by perillyl alcohol and perillic acid was associated with a fall in the proportion of cells in the S phase and an accumulation of cells in the G1 phase of the cell cycle. Finally, we showed that the effects of limonene-related monoterpenes on cell proliferation and cell cycle progression were preceded by a decrease in cyclin D1 mRNA levels.

Bardon, S. and L. Razanamahefa (1998). "Retinoic acid suppresses insulin-induced cell growth and cyclin D1 gene expression in human breast cancer cells." Int J Oncol **12**(2): 355-359.

We examined the effects of all-trans retinoic acid (RA) on the insulin-induced cell growth, cell cycle progression and cyclin D1 gene expression in breast cancer cells. RA exerted a dose-dependent growth inhibition on insulin-induced proliferation in T47D and MCF-7 hormone-dependent cell lines, whereas MDA-MB231 hormone-independent cells were not affected. The RA antagonism of insulin growth effect was associated with an inhibition of cell cycle progression and a suppression of insulin-induced cyclin D1 mRNA. The effect of RA on cyclin D1 mRNA was dose-dependent and was observed within 5 h of treatment when insulin response was maximal.

Bargou, R. C., et al. (1996). "Overexpression of the death-promoting gene bax-alpha which is downregulated in breast cancer restores sensitivity to different apoptotic stimuli and reduces tumor growth in SCID mice." J Clin Invest **97**(11): 2651-2659.

We have studied the expression of members of the bcl-2 family in human breast cancer. The expression pattern of these genes in breast cancer tissue samples was compared with the expression pattern in normal breast epithelium. No marked difference with regard to bcl-2 and bcl-xL expression was observed between normal breast epithelium and cancer tissue. In contrast, bax-alpha, a splice variant of bax, which promotes apoptosis, is expressed in high amounts in normal breast epithelium, whereas only weak or no expression could be detected in 39 out of 40 cancer tissue samples examined so far. Of interest, downregulation of bax-alpha was found in different histological subtypes. Furthermore, we transfected bax-alpha into breast cancer cell lines under the control of a tetracycline-dependent expression system. We were able to demonstrate for the first time that induction of bax expression in breast cancer cell lines restores sensitivity towards both serum starvation and APO-I/Fas-triggered apoptosis and significantly reduces tumor growth in SCID mice. Therefore, we propose that dysregulation of apoptosis might contribute to the pathogenesis of breast cancer at least in part due to an imbalance between members of the bcl-2 gene family.

Barron-Gonzalez, A. and I. Castro Romero (2004). "Re-expression of estrogen receptor alpha using a tetracycline-regulated gene expression system induced estrogen-mediated growth inhibition of the MDA-MB-231 breast cancer cell line." Biochem Cell Biol **82**(2): 335-342.

Estrogen receptor (ER)-negative breast carcinomas are often difficult to treat with antiestrogens. This work was performed to determine if the re-expression of the human ER alpha could restore the hormone response of these cells. We have transfected the human wild-type ER alpha to an ER-negative breast cancer cell line (MDA-MB-231) using a tetracycline-regulated gene expression system. We obtained a new cell line, MDA-A4-5/2. Cell count and flow cytometry "S" phase cell fraction showed that 17-beta-estradiol induced an inhibition on the proliferation of these cells; on the contrary, the antiestrogens ICI 182 780, and tamoxifen blocked this effect. Finally, we demonstrated an induction of the endogenous progesterone receptor gene when ER alpha was present. These results suggest that the re-expression of ER alpha in ER-negative breast cancer cells recreate, at least partially, a hormone-responsive phenotype and may be useful as a therapeutic approach to control this pathology.

Bateman, A. and H. P. Bennett (2009). "The granulin gene family: from cancer to dementia." Bioessays **31**(11): 1245-1254.

The growth factor progranulin (PGRN) regulates cell division, survival, and migration. PGRN is an extracellular glycoprotein bearing multiple copies of the cysteine-rich granulin motif. With PGRN family members in plants and slime mold, it represents one of the most ancient of the extracellular regulatory proteins still extant in modern animals. PRGN has multiple biological roles. It contributes to the regulation of early embryogenesis, to adult tissue repair and inflammation. Elevated PGRN levels often occur in cancers, and PGRN immunotherapy inhibits the growth of hepatic cancer xenografts in mice. Recent studies have demonstrated roles for PGRN in neurobiology. An autosomal dominant mutation in GRN, the gene for PGRN, leads to neuronal atrophy in the frontal and temporal lobes, resulting in the disease frontotemporal lobar dementia. In this review we will

discuss current knowledge of the multifaceted biology of PGRN.

Bergamaschi, A., et al. (2011). "Reversal of endocrine resistance in breast cancer: interrelationships among 14-3-3zeta, FOXM1, and a gene signature associated with mitosis." Breast Cancer Res **13**(3): R70.

INTRODUCTION: Despite the benefits of estrogen receptor (ER)-targeted endocrine therapies in breast cancer, many tumors develop resistance. 14-3-3 zeta/YWHAZ, a member of the 14-3-3 family of conserved proteins, is over-expressed in several types of cancer, and our previous work showed that high expression of 14-3-3zeta in ER-positive breast cancers was associated with a poor clinical outcome for women on tamoxifen. Therefore, we now probe the role of 14-3-3zeta in endocrine resistance, and we examine the functional dimensions and molecular basis that underlie 14-3-3zeta activities. METHODS: From analyses of four independent breast cancer microarray datasets from nearly 400 women, we characterized a gene signature that correlated strongly with high expression of 14-3-3zeta in breast tumors and examined its association with breast cancer molecular subtypes and clinical-pathological features. We investigated the effects of altering 14-3-3zeta levels in ER-positive, endocrine sensitive and resistant breast cancer cells on the regulation of 14-3-3zeta signature genes, and on cellular signaling pathways and cell phenotypic properties. RESULTS: The gene signature associated with high 14-3-3zeta levels in breast tumors encompassed many with functions in mitosis and cytokinesis, including aurora kinase-B, polo-like kinase-1, CDC25B, and BIRC5/survivin. The gene signature correlated with early recurrence and risk of metastasis, and was found predominantly in luminal B breast cancers, the more aggressive ER-positive molecular subtype. The expression of the signature genes was significantly decreased or increased upon reduction or overexpression of 14-3-3zeta in ER-positive breast cancer cells, indicating their coregulation. 14-3-3zeta also played a critical role in the regulation of FOXM1, with 14-3-3zeta acting upstream of FOXM1 to regulate cell division-signature genes. Depletion of 14-3-3zeta markedly increased apoptosis, reduced proliferation and receptor tyrosine kinase (HER2 and EGFR) signaling, and, importantly, reversed endocrine resistance. CONCLUSIONS: This study reveals that 14-3-3zeta is a key predictive marker for risk of failure on endocrine therapy and serves a pivotal role impacting growth factor signaling, and promoting cell survival and resistance to endocrine therapies. Targeting 14-3-3zeta and its coregulated proteins, such as FOXM1, should prove valuable in restoring endocrine sensitivity and reducing risk of breast cancer recurrence.

Boente, M. P., et al. (1998). "Suppression of diacylglycerol levels by antibodies reactive with the c-erbB-2 (HER-2/neu) gene product p185c-erbB-2 in breast and ovarian cancer cell lines." Gynecol Oncol **70**(1): 49-55.

Seven of 10 murine monoclonal antibodies reactive with the extracellular domain of p185c-erbB-2 inhibited the anchorage independent growth of the SKBr3 breast cancer cell line that overexpressed p185c-erbB-2. Significant inhibition (56-72%) of diacylglycerol (DAG) levels ($P < 0.0001$) was observed with the 10 antibodies that inhibited SKBr3 growth (RC1, NB3, RC6, PB3, 741F8, DB5, ID5), whereas the 3 antibodies (TA1, 520C9, 454C11) that failed to inhibit SKBr3 growth also failed to affect DAG levels. Thus, DAG levels correlated with antibody-mediated growth regulation for each of the 10 monoclonal reagents. Antibody-induced inhibition of anchorage-independent growth of SKBr3 could be reversed by incubation with phorbol myristate acetate. The ID5 antibody inhibited growth of the SKBr3, SKOv3, and OVCA 432 tumor cell lines, but not of OVCA 420, OVCA 429, and OVCA 433. DAG levels were significantly decreased after ID5 treatment of the SKBr3 and SKOv3 cell lines, but not the OVCA 420, OVCA 429, and OVCA 433 lines. In the 432 line, there was a decrease which did not reach significance. Consequently, changes in DAG levels correlated with growth regulation in 5 of 6 breast and ovarian carcinoma cell lines tested with a trend toward correlation in the sixth. Decreases in DAG may be one mediator of the growth regulatory signals produced by anti-p185c-erbB-2 antibodies.

Bottone, F. G., Jr., et al. (2004). "Gene modulation by Cox-1 and Cox-2 specific inhibitors in human colorectal carcinoma cancer cells." Carcinogenesis **25**(3): 349-357.

Cox-1 and Cox-2 specific inhibitors exert chemo-preventative activity. However, the exact mechanisms for this activity remain unclear. Increasing evidence suggests that non-steroidal anti-inflammatory drugs regulate gene expression, which may be responsible, in part, for this activity. In this study, human colorectal carcinoma HCT-116 cells were treated with the Cox-1 specific inhibitor SC-560 and the Cox-2 specific inhibitor SC-58125 to evaluate their ability to induce apoptosis, inhibit cell proliferation, inhibit growth on soft agar and modulate gene expression. The Cox-1 specific inhibitor, SC-560 significantly induced apoptosis and inhibited the growth of HCT-116 cells on soft agar, an in vitro assay for tumorigenicity. SC-58125 moderately induced apoptosis and inhibited

growth on soft agar at higher concentrations than were required for SC-560. Previously, we reported that the potent chemo-preventative drug sulindac sulfide altered the expression of eight genes including several transcription factors that may be linked to this drug's chemo-preventative activity. HCT-116 cells were treated with various concentrations of SC-560 or SC-58125 and changes in the expression of these eight genes were determined by real-time reverse transcription- polymerase chain reaction. SC-560 modulated mRNA expression of the eight genes studied. In contrast, SC-58125 required approximately 5-10-fold higher concentrations to achieve similar degrees of gene modulation in six of eight genes. Changes in protein expression by SC-560 also occurred for five of these genes with antibodies available (NAG-1, ATF3, C/EBPbeta, MAD2 and MSX1). In conclusion, this is the first report to suggest that like sulindac sulfide, the Cox-1 specific inhibitor SC-560 appears to elicit chemo-preventative activity by altering gene expression, while the chemo-preventative effects of SC-58125 are complex and probably work through these and other mechanisms, such as the inhibition of Cox-2.

Bougeret, C., et al. (2000). "Cancer gene therapy mediated by CTS1, a p53 derivative: advantage over wild-type p53 in growth inhibition of human tumors overexpressing MDM2." Cancer Gene Ther **7**(5): 789-798.

Recently, a new p53 derivative has been designed, namely chimeric tumor suppressor 1 (CTS1), in which the p53 domains that are known to mediate p53 inactivation have been replaced. In this study, the antitumoral activity of CTS1 mediated by adenovirus vector has been evaluated in comparison with a p53 adenovirus vector in various human tumor cell lines. In vitro, in terms of cell growth inhibition, the CTS1 vector was significantly (P <.01) more efficient (2- to 7-fold) than the p53 vector in tumor models overexpressing an inhibitor of p53, murine double minute-2. This result was confirmed in vivo in a pre-established tumor developed in nude mice. In an osteosarcoma model overexpressing murine double minute-2, we have shown a significantly (P <.05) higher tumor growth delay with the CTS1 vector compared with the p53 vector (25.6 days compared with 12.4 days). Furthermore, both in vitro and in vivo, we have shown that this higher inhibition of tumor growth with the CTS1 vector was correlated with a higher induction of apoptosis. Therefore, CTS1 is a potentially improved tumor suppressor gene for the treatment of human tumors resistant to wild-type p53 gene therapy.

Brand, K. (2002). "Cancer gene therapy with tissue inhibitors of metalloproteinases (TIMPs)." Curr Gene Ther **2**(2): 255-271.

Matrix metalloproteinases (MMPs) are of crucial importance for the invasive behavior of primary tumors and their metastases. MMP activity is regulated by the four naturally occurring tissue inhibitors of metalloproteinases (TIMPs). It has been shown that overexpression of TIMPs in tumors of various origins leads to reduced tumor growth and formation of metastases. More recently, antitumor efficacy by in vivo gene transfer of TIMPs has been reported in several clinically relevant animal models. This review analyses the therapeutic potential of the TIMPs from a cancer gene therapeutic point of view with particular emphasis on cell culture and in vivo data.

Brauweiler, A., et al. (2007). "RING-dependent tumor suppression and G2/M arrest induced by the TRC8 hereditary kidney cancer gene." Oncogene **26**(16): 2263-2271.

TRC8/RNF139 and von Hippel-Lindau (VHL) both encode E3 ubiquitin (Ub) ligases mutated in clear-cell renal carcinomas (ccRCC). VHL, inactivated in nearly 70% of ccRCCs, is a tumor suppressor encoding the targeting subunit for a Ub ligase complex that downregulates hypoxia-inducible factor-alpha. TRC8/RNF139 is a putative tumor suppressor containing a sterol-sensing domain and a RING-H2 motif essential for Ub ligase activity. Here we report that human kidney cells are growth inhibited by TRC8. Inhibition is manifested by G2/M arrest, decreased DNA synthesis and increased apoptosis and is dependent upon the Ub ligase activity of the RING domain. Tumor formation in a nude mouse model is inhibited by TRC8 in a RING-dependent manner. Expression of TRC8 represses genes involved in cholesterol and fatty acid biosynthesis that are transcriptionally regulated by the sterol response element binding proteins (SREBPs). Expression of activated SREBP-1a partially restores the growth of TRC8-inhibited cells. These data suggest that TRC8 modulation of SREBP activity comprises a novel regulatory link between growth control and the cholesterol/lipid homeostasis pathway.

Burnatowska-Hledin, M. A., et al. (2004). "T47D breast cancer cell growth is inhibited by expression of VACM-1, a cul-5 gene." Biochem Biophys Res Commun **319**(3): 817-825.

Vasopressin-activated calcium-mobilizing (VACM-1), a cul-5 gene, is localized on chromosome 11q22-23 close to the gene for Ataxia Telangiectasia in a region associated with a loss of heterozygosity in breast cancer tumor samples. To examine the biological role of VACM-1, we studied the effect of

VACM-1 expression on cellular growth and gene expression in T47D breast cancer cells. Immunocytochemistry studies demonstrated that VACM-1 was expressed in 0.6-6% of the T47D cells and localized to the nucleus of mitotic cells. Overexpressing VACM-1 significantly attenuated cellular proliferation and MAPK phosphorylation when compared to the control cells. In addition, VACM-1 decreased egr-1 and increased Fas-L mRNA levels. Further, egr-1 protein levels were significantly lower in the nuclear fraction from VACM-1 transfected cells when compared to controls. These data indicate that VACM-1 is involved in the regulation of cellular growth.

Burney, T. L., et al. (1994). "Partial growth suppression of human prostate cancer cells by the Krev-1 suppressor gene." Prostate **25**(4): 177-188.

A series of functional studies were performed to assess the potential role of the ras-related transformation suppressor gene, Krev-1, in suppressing prostate cancer cell growth. Three human prostate cancer cell lines, PC-3, TSU-Pr1, and DU-145 were transfected with a plasmid containing the Krev-1 cDNA and a neomycin resistance gene. Selected G418-resistant clones were isolated and expanded into cell lines. All cloned transfectants exhibited a significant reduction in their in vitro growth rates, i.e., longer doubling times, when compared to the parental cell lines. Molecular analysis of the Krev-1 cloned transfectants revealed that they all contained variable copy numbers of the Krev-1 gene and expressed high levels of Krev-1 mRNA transcript, as shown by Southern and Northern analysis, respectively. To determine whether the biological properties associated with tumorigenicity were changed in these Krev-1 transfectants, their growth characteristics were examined on the basis of their ability to a) form colonies in soft agar, and b) produce tumors in SCID mice. The majority of the Krev-1 transfectants from the PC-3 and TSU-Pr1 cell lines showed a substantially reduced ability to form colonies in soft agar and produced significantly smaller tumors when inoculated into SCID mice. In contrast, there was no significant reduction in the soft agar colony-forming ability or in vivo tumorigenicity of the DU-145 Krev-1 transfectants. These results suggest that the Krev-1 suppressor gene induces partial suppression of the malignant phenotype of human prostate cancer cells containing activated ras oncogenes.

Burns, F. J., et al. (2002). "The action of a dietary retinoid on gene expression and cancer induction in electron-irradiated rat skin." J Radiat Res **43 Suppl**: S229-232.

Current models of radiation carcinogenesis generally assume that the DNA is damaged in a variety of ways by the radiation and that subsequent cell divisions contribute to the conversion of the damage to heritable mutations. Cancer may seem complex and intractable, but its complexity provides multiple opportunities for preventive interventions. Mitotic inhibitors are among the strongest cancer preventive agents, not only slowing the growth rate of preneoplasias but also increasing the fidelity of DNA repair processes. Ionizing radiation, including electrons, is a strong inducer of cancer in rat skin, and dietary retinoids have shown potent cancer preventive activity in the same system. A non-toxic dietary dose of retinyl acetate altered gene expression levels 24 hours after electron irradiation of rat skin. Of the 8740 genes on an Affymetrix rat expression array, the radiation significantly (5 fold or higher) altered 188, while the retinoid altered 231, including 16 radiation-altered genes that were reversely altered. While radiation strongly affected the expression of stress response, immune/inflammation and nucleic acid metabolism genes, the retinoid most strongly affected proliferation-related genes, including some significant reversals, such as, keratin 14, retinol binding protein, and calcium binding proteins. These results point to reversal of proliferation-relevant genes as a likely basis for the anti-radiogenic effects of dietary retinyl acetate.

Butz, K., et al. (1999). "Induction of the p53-target gene GADD45 in HPV-positive cancer cells." Oncogene **18**(14): 2381-2386.

The E6 oncoprotein of human papillomaviruses (HPVs) has the potential to functionally antagonize p53. In several experimental model systems, ectopic expression of E6 can block the genotoxic induction of the growth inhibitory p53 target gene gadd45, suggesting that the inactivation of this pathway may play a major role for HPV-associated cell transformation. Here, we investigated whether this reflects the regulation of gadd45 expression in carcinoma-derived HPV-positive cells. We found that the gadd45 gene is efficiently induced by mitomycin C, cisplatin, and UV irradiation in a series of HPV-positive cervical cancer cell lines. Moreover, clear induction of gadd45 gene expression was also observed following treatment with gamma-irradiation, a pathway that is strictly dependent on functional p53. This contrasted with findings in human foreskin keratinocytes experimentally immortalized by expressing the HPV16 E6, E7, or E6/E7 oncogenes from the heterologous CMV promoter, where expression of the E6 gene was linked to a lack of gadd45 induction following gamma-irradiation. These results indicate (1) that the tumorigenic phenotype of

HPV-positive cancer cells is not linked to an inability to induce the gadd45 gene following DNA damage, (2) that experimental model systems in which the E6 gene is expressed ectopically and/or in a different cellular context do not necessarily reflect the regulation of p53-associated pathways in HPV-positive cancer cells and (3) that a pathway strictly depending on functional p53 is inducible in HPV-positive cancer cells, providing direct evidence that the endogenous p53 protein in these cells is competent to activate a cellular target gene, despite coexpression of the viral E6 oncogene.

Cahlin, C., et al. (2000). "Experimental cancer cachexia: the role of host-derived cytokines interleukin (IL)-6, IL-12, interferon-gamma, and tumor necrosis factor alpha evaluated in gene knockout, tumor-bearing mice on C57 Bl background and eicosanoid-dependent cachexia." Cancer Res **60**(19): 5488-5493.

MCG 101 tumors were implanted sc. on wild-type C57 Bl and gene knockout mice to evaluate the role of host-produced cytokines [interleukin (IL)-6, IL-12, IFNgamma, tumor necrosis factor (TNF) receptor 1, and TNF receptor 2] to explain local tumor growth, anorexia, and carcass weight loss in a well-defined model with experimental cachexia. Indomethacin was provided in the drinking water to explore interactions between host and tumor-derived prostaglandins and proinflammatory cytokines for tumor growth. Wild-type tumor-bearing mice developed cachexia because of rapid tumor growth, which were both attenuated in IL-6 gene knockouts. Similar findings were observed after provision of anti-IL-6 to wild-type tumor-bearing mice. Alterations in food intake were not directly related to systemic IL-6 but rather secondarily to IL-6-dependent tumor growth. The absence of host-derived IL-12, IFN-gamma, or the TNF receptor 1 or receptor 2 gene did not attenuate tumor growth or improve subsequent cachexia. Thus, carcass weight loss was not improved by the omission of host cytokine (TNF-alpha, IL-12, or IFN-gamma) except for IL-6. Systemic indomethacin provision decreased plasma prostaglandin E2 in five of six groups of gene knockout tumor-bearing mice, which was associated with improved carcass weight in these groups. Indomethacin seemed to improve food intake to a similar extent in both wild-type and gene knockouts, which agree with the speculation that eicosanoids are more important to explain anorexia than host cytokines. Our results demonstrate that host- and tumor-derived cytokines and prostaglandins interact with tumor growth and promote cachexia in a more complex fashion than usually presented based on previous information in studies on either anti-cytokine experiments in vivo or on gene knockouts with respect to a "single cytokine model." Overall, host cytokines were quantitatively less important than tumor-derived cytokines to explain net tumor growth, which indirectly explains subsequent cachexia and anorexia.

Calvo, A., et al. (2002). "Alterations in gene expression profiles during prostate cancer progression: functional correlations to tumorigenicity and down-regulation of selenoprotein-P in mouse and human tumors." Cancer Res **62**(18): 5325-5335.

To identify molecular changes that occur during prostate tumor progression, we have characterized a series of prostate cancer cell lines isolated at different stages of tumorigenesis from C3(1)/Tag transgenic mice. Cell lines derived from low- and high-grade prostatic intraepithelial neoplasia, invasive carcinoma, and a lung metastasis exhibited significant differences in cell growth, tumorigenicity, invasiveness, and angiogenesis. cDNA microarray analysis of 8700 features revealed correlations between the tumorigenicity of the C3(1)/Tag-Pr cells and changes in the expression levels of genes regulating cell growth, angiogenesis, and invasion. Many changes observed in transcriptional regulation in this in vitro system are similar to those reported for human prostate cancer, as well as other types of human tumors. This analysis of expression patterns has also identified novel genes that may be involved in mechanisms of prostate oncogenesis or serve as potential biomarkers or therapeutic targets for prostate cancer. Examples include the L1-cell adhesion molecule, metastasis-associated gene (MTA-2), Rab-25, tumor-associated signal transducer-2 (Trop-2), and Selenoprotein-P, a gene that binds selenium and prevents oxidative stress. Many genes identified in the Pr-cell line model have been shown to be altered in human prostate cancer. The comprehensive microarray data provides a rational basis for using this model system for studies where alterations of specific genes or pathways are of particular interest. Quantitative real-time reverse transcription-PCR for Selenoprotein-P demonstrated a similar down-regulation of the transcript of this gene in a subset of human prostate tumors, mouse tumors, and prostate carcinoma cell lines. This work demonstrates that expression profiling in animal models may lead to the identification of novel genes involved in human prostate cancer biology.

Campbell, I., et al. (2000). "Adenovirus-mediated p16INK4 gene transfer significantly suppresses human breast cancer growth." Cancer Gene Ther **7**(9): 1270-1278.

The p16INK4 tumor suppressor gene encodes a protein that inhibits cyclin-dependent kinase 4, and its homologous deletion is common in human breast cancer. p16INK4 gene transfer has been reported to be efficacious in inducing growth inhibition of various

human tumors such as brain, lung, prostate, and esophageal cancers. However, the efficiency of the p16INK4 gene with regard to growth inhibition of human breast cancer has not been studied extensively. To examine its tumor-suppressive function and its potential in breast cancer gene therapy, the wild-type p16INK4 gene was expressed in an adenovirus-mediated gene delivery system and introduced into breast cancer cell lines that do not express p16INK4 protein. Expression of the introduced p16INK4 blocked tumor cell entry into the S phase of the cell cycle, induced tumor cell apoptosis, and inhibited tumor cell proliferation both in vitro and in vivo. These results strongly suggest that p16INK4 is a tumor suppressor gene and suggest that it has potential utility in breast cancer gene therapy.

Cao, G., et al. (2001). "Adenovirus-mediated interferon-beta gene therapy suppresses growth and metastasis of human prostate cancer in nude mice." Cancer Gene Ther **8**(7): 497-505.

The purpose of this study was to determine the effects of interferon-beta (IFN-beta) gene transfer on the growth of PC3MM2 human prostate cancer cells in nude mice. Intralesional delivery of an adenoviral vector encoding murine IFN-beta (AdIFN-beta), but not a vector encoding bacterial beta-galactosidase (AdLacZ), suppressed PC3MM2 tumors in a dose-dependent manner. At the highest dose (2x10(9) plaque-forming units, PFU), a single injection of AdIFN-beta (but not AdLacZ) suppressed orthotopic PC3MM2 tumors and development of metastasis by 80%, and eradicated the tumors in 20% of mice. Immunohistochemical staining showed that AdIFN-beta-treated tumors contained fewer microvessels, fewer proliferating cells, and more apoptotic cells than did the control tumors. Compared with controls, tumors injected with AdIFN-beta expressed higher levels of IFN-beta and inducible nitric oxide synthase (iNOS) and lower levels of basic fibroblast growth factor (bFGF) and transforming growth factor beta1 (TGF-beta1). In vitro analysis indicated that expression of bFGF and TGF-beta1 in PC3MM2 cells could be suppressed by the nitric oxide donor sodium nitroprusside. These data suggest that intratumoral delivery of the IFN-beta gene with adenoviral vectors could be an effective therapy for prostate cancer and that tumor suppression by AdIFN-beta correlated with up-regulation of iNOS and down-regulation of angiogenesis.

Casey, G., et al. (1991). "Growth suppression of human breast cancer cells by the introduction of a wild-type p53 gene." Oncogene **6**(10): 1791-1797.

Mutations in the p53 gene are associated with a wide variety of human tumors, including those of the breast. To assess functionally the role of the p53 gene in the development of human breast cancer, we introduced either wild-type or mutant p53 cDNA into three human breast cancer cell lines by DNA transfection. The cell lines MDA-MB 468 and T47 D contain only single mutated copies of the p53 gene, whereas the status of p53 in the breast cancer cell line MCF 7 remains equivocal. Following transfection, MCF 7 cells continued to grow unaffected both in vitro and in vivo in the presence of high levels of expression of the exogenous wild-type p53 gene. In contrast, however, the continued expression of an exogenous wild-type p53 gene was incompatible with cellular growth in both the MDA-MB 468 and T47 D cell lines. Elevated levels of expression of the exogenous mutant p53 gene did not alter the growth of the cell lines in vitro. These data strongly suggest that the wild-type p53 gene can function as a suppressor of cellular growth in breast cancer cells. That the wild-type p53 gene does not suppress the growth of MCF 7 cells indicates that at least some human breast tumors can arise without functional inactivation of the p53 gene by mutation. These tumors may represent a separate prognostic group.

Casey, G., et al. (1993). "Functional evidence for a breast cancer growth suppressor gene on chromosome 17." Hum Mol Genet **2**(11): 1921-1927.

Rearrangements or deletions of chromosome 17 are the most frequently observed genetic changes identified in breast tumors. Molecular analyses suggest that in addition to the p53 gene on 17p13.1 there may be at least three other tumor suppressor genes on chromosome 17 involved in breast cancer. Regions of loss of heterozygosity (LOH) identified on 17p13.3 and 17q12-qter occur frequently in breast tumors, and the BRCA-1 gene has been mapped to 17q21 by genetic linkage analysis. Here we provide biological evidence for the presence of a growth suppressor gene (s) on chromosome 17 that results in the in vitro growth suppression of the p53 wild-type MCF 7 breast cancer cell line. We have introduced a normal chromosome 17 into MCF 7 cells by microcell-mediated chromosome transfer (MMCT), and demonstrate that cells growth arrest before 10 to 12 population doublings. In contrast, the introduction of a normal chromosome 13 had no effect upon growth of these cells either in vitro or in vivo. These data provide direct functional evidence for the presence of a growth suppressor gene (s) on chromosome 17, which is not p53, and which may represent one of several gene (s) that play a critical role in the development of breast cancer.

Celinski, S. A., et al. (2003). "Somatostatin receptor gene transfer inhibits established pancreatic cancer xenografts." J Surg Res **115**(1): 41-47.

BACKGROUND: Most human pancreatic adenocarcinoma cells do not express somatostatin receptors, and somatostatin does not inhibit the growth of these cancers. We have demonstrated previously that somatostatin inhibits the growth of pancreatic cancers expressing somatostatin receptor subtype-2 (SSTR2), but not receptor-negative cancers. SSTR2 expression may be an important tumor-suppressor pathway that is lost in human pancreatic cancer. We hypothesized that SSTR2 gene transfer would restore the growth-inhibitory response of human pancreatic cancer to somatostatin. MATERIALS AND METHODS: Palpable human pancreatic adenocarcinoma tumors were established on the backs of nude mice by subcutaneous injection of cultured cells (Panc-1). The animals were divided into 5 groups (n = 10/group). Group I served as an untreated control. Group II received an intramuscular injection of the long-acting somatostatin analogue Sandostatin LAR. Group III received Lac-Z expressing adenovirus via intraperitoneal injection. Group IV received SSTR2 expressing adenovirus via intraperitoneal injection. Group V received SSTR2 expressing adenovirus via intraperitoneal injection and an intramuscular injection of Sandostatin LAR. The rate of tumor growth was assessed with calipers. After 28 days, the animals were anesthetized and exsanguanated, and the tumors were excised and weighed. Plasma somatostatin and octreotide levels were measured by radioimmunoassay. Expression of cell-surface somatostatin-receptor protein and known tumor-suppressor proteins was determined by reverse transcriptase-polymerase chain reaction, Western blot, and immunohistochemistry. RESULTS: Systemic delivery of SSTR2-expressing adenovirus by intraperitoneal injection resulted in expression of SSTR2 protein in the subcutaneous human pancreatic cancers. Final tumor weight was significantly decreased in the groups expressing SSTR2 receptors compared to the other 3 groups. Treatment with Sandostatin LAR increased plasma octreotide levels as determined by radioimmunoassay, but had no significant effect on tumor growth. Western blot analysis revealed an up-regulation of the cyclin-dependent kinase inhibitors p27 and p16 in the SSTR2 transfected tumors. CONCLUSIONS: Expression of SSTR2 by human pancreatic cancer causes significant slowing of tumor growth by a mechanism independent of exogenous somatostatin. The mechanism may involve up-regulation of known tumor-suppressor proteins. Restoration of SSTR2 gene expression deserves further study as a potential gene-therapy strategy in human pancreatic cancer.

Chan, I., et al. (2008). "Progression elevated gene-3 promoter (PEG-Prom) confers cancer cell selectivity to human polynucleotide phosphorylase (hPNPase (old-35))-mediated growth suppression." J Cell Physiol **215**(2): 401-409.

The poor prognosis of pancreatic cancer patients using currently available therapies mandates novel therapeutics that combine anti-neoplastic potency with toxicity-minimizing cancer specificity. Employing an overlapping pathway screen to identify genes exhibiting coordinated expression as a consequence of terminal cell differentiation and replicative senescence, we identified human polynucleotide phosphorylase (hPNPase (old-35)), a 3',5'-exoribonuclease that exhibits robust growth-suppressing effects in a wide spectrum of human cancers. A limitation to the anti-neoplastic efficacy of hPNPase (old-35) relates to its lack of cancer specificity. The promoter of Progression Elevated Gene-3 (PEG-Prom), discovered in our laboratory via subtraction hybridization in a transformation progression rodent tumor model functions selectively in a diverse array of human cancer cells, with limited activity in normal cells. An adenovirus constructed with the PEG-Prom driving expression of hPNPase (old-35) containing a C-terminal Hemaglutinin (HA)-tag (Ad.PEG.hPNPase (old-35)) was shown to induce robust transgene expression, growth suppression, apoptosis, and cell-cycle arrest in a broad panel of pancreatic cancer cells, with minimal effects in normal immortalized pancreatic cells. hPNPase (old-35) expression correlated with arrest in the G (2)/M phase of the cell cycle and up-regulation of the cyclin-dependent kinase inhibitors (CDKI) p21(CIP1/WAF-1/MDA-6) and p27(KIP1). In a nude mouse xenograft model, Ad.PEG.hPNPase (old-35) injections effectively inhibited growth of human pancreatic cancer cells in vivo. These findings support the potential efficacy of combining a cancer-specific promoter, such as the PEG-Prom, with a novel anti-neoplastic agent, such as hPNPase (old-35), to create a potent, targeted cancer therapeutic, especially for a devastating disease like pancreatic cancer.

Chen, C., et al. (2002). "[The effects of C2 gene on growth of gastric cancer cells]." Zhonghua Nei Ke Za Zhi **41**(3): 149-152.

OBJECTIVE: To investigate the effect of a newly cloned full length gene, C2 gene, which encodes human eukaryotic translation initiation factor, on growth of gastric cancer cells. METHODS: A constructed eukaryotic vector carrying the full length of C2 gene was amplified, purified and transfected into gastric cancer cell line-SGC 7901. Expression of C2 protein was examined with fluorescence activated cell sorting (FACS) and immunohistochemical staining.

Growth curve of the C2 gene transfected gastric cancer cells was drawn with the results of MTT assay and cell count. RESULTS: FACS and immunohistochemical staining showed that C2 protein was expressed highly and stably in the C2 gene transfected cells. The growth curve of the C2 gene transfected cells by both MTT and cell count showed that the C2 gene transfected cells had less proliferation than the control cells. The growth cycle of transfected cells was 11 days, while the control was 7 days. CONCLUSION: C2 gene transfection may inhibit the growth of human gastric cancer cells. It might become a potential inhibitor of gastric cancer in the level of protein translation initiation.

Chen, J., et al. (2000). "[The effects of RA538 and antisense c-myc on cervical cancer cell lines with high expression of bcl-2 gene]." Zhonghua Zhong Liu Za Zhi **22**(4): 279-282.

OBJECTIVE: To study the effects of recombinant adenovirus with RA538 or antisense c-myc insert on cervical cancer cell lines with high expression of bcl-2 gene. METHODS: Cervical cancer cell lines HeLa and SiHa were transduced with full length bcl-2 cDNA by lipofectin and cell clones with stable expression of bcl-2 were selected. They were then transfected with recombinant adenovirus containing RA538 (Ad-RA538), antisense c-myc (Ad-ASc-myc) or LacZ (Ad-LacZ) gene. Morphologic and molecular changes of the transfected cancer cells were examined by light microscopy, MTT, RT-PCR and Northern blot. RESULTS: The bcl-2 cDNA was successfully transferred into HeLa and SiHa cells. Two cell lines, called HeLa-bcl2 and SiHa-bcl2 with high expression of bcl-2 gene were generated. Ad-RA538 and Ad-ASc-myc transfection both could inhibit cell growth and induce apoptosis of HeLa and SiHa cells, and inhibit their expression of c-myc and bcl-2 genes. However, although Ad-RA538 and Ad-ASc-myc had some inhibitory effect on bcl-2 and c-myc gene expression by HeLa-bcl2 and SiHa-bcl2 cells, they could only slightly inhibit cell growth and weakly induce apoptosis. CONCLUSION: Ad-RA538 and Ad-ASc-myc can to certain extent inhibit cell growth and induce apoptosis of two cervical cancer cell lines. They have little such effect when these cell lines have over-expressed bcl-2 by transduction of exogenous bcl-2 gene.

Chen, L., et al. (1996). "Sensitization of human breast cancer cells to cyclophosphamide and ifosfamide by transfer of a liver cytochrome P450 gene." Cancer Res **56**(6): 1331-1340.

The cancer chemotherapeutic agent cyclophosphamide (CPA) and its isomer ifosfamide (IFA) are alkylating agent prodrugs that require metabolism by liver cytochrome P450 (P450) enzymes for antitumor activity. The therapeutic effectiveness of these oxazaphosphorines is limited by the hematopoietic, renal, and cardiac toxicity that accompanies the systemic distribution of liver-derived activated drug metabolites. Transfer of a liver cytochrome P450 gene, CYP2B1, into human breast MCF-7 cancer cells is presently shown to greatly sensitize these cells to oxazaphosphorine toxicity as a consequence of the acquired capacity for intratumoral CPA and IFA activation. Thus, CPA and IFA were highly cytotoxic to MCF-7 cells following stable transfection of CYP2B1 but exhibited no toxicity to parental tumor cells or to a beta-galactosidase-expressing MCF-7 transfectant. This cytotoxicity could be appreciably blocked by the CYP2B1 inhibitor metyrapone. Cell cycle analysis revealed that CPA arrested the CYP2B1-expressing cells, but not CYP2B1-negative cells, at G (2)-M phase. A strong bystander cytotoxicity effect that does not require direct cell-cell contact was mediated by CYP2B1-expressing MCF-7 cells on non-CYP2B1 cells. Intratumoral CYP2B1 expression conferred a distinct therapeutic advantage when treating MCF-7 tumors grown in nude mice with CPA, as revealed by a 15-20-fold greater in vivo cytotoxicity, determined by tumor excision/colony formation assay, and by the substantially enhanced antitumor activity, monitored by tumor growth delay, for CYP2B1-e xpressing MCF-7 tumors as compared to CYP2B1-negative control tumors. These enhanced therapeutic effects were obtained without any apparent increase in host toxicity. To evaluate the extent to which a CPA/P450 gene therapy strategy may be generally applicable to other tumor cell types, a replication-defective recombinant adenovirus carrying the CYP2B1 gene driven by the cytomegalovirus (CMV) promotor ad.CMV-2B1 was constructed and used to infect a panel of human tumor cell lines. Ad.CMV-2B1 infection rendered each of the cell lines highly sensitive to CPA and IFA cytotoxicity, with substantial chemosensitization seen at multiplicities of infection as low as 10. The CPA/P450 prodrug activation system may thus serve as a useful paradigm for further development of novel cancer gene therapy strategies that utilize drug susceptibility genes to significantly potentiate the antitumor activity of conventional cancer chemotherapeutic agents.

Chen, M., et al. (2009). "CCDC62/ERAP75 functions as a coactivator to enhance estrogen receptor beta-mediated transactivation and target gene expression in prostate cancer cells." Carcinogenesis **30**(5): 841-850.

Human prostate cancer (PCa) and prostate epithelial cells predominantly express estrogen

receptor (ER) beta, but not ERalpha. ERbeta might utilize various ER coregulators to mediate the E2-signaling pathway in PCa. Here, we identified coiled-coil domain containing 62 (CCDC62)/ERAP75 as a novel ER coactivator. CCDC62/ERAP75 is widely expressed in PCa cell lines and has low expression in MCF7 cells. Both in vitro and in vivo interaction assays using mammalian two-hybrid, glutathione S-transferase pull-down and coimmunoprecipitation methods proved that ERbeta can interact with the C-terminus of CCDC62/ERAP75 via the ligand-binding domain. The first LXXLL motif within CCDC62/ERAP75 is required for the interaction between ERbeta and CCDC62/ERAP75. Electrophoretic mobility shift assay showed that CCDC62/ERAP75 can be recruited by the estrogen response element-ER complex in the presence of ligand. Furthermore, a chromatin immunoprecipitation assay demonstrated the hormone-dependent recruitment of CCDC62/ERAP75 within the promoter of the estrogen-responsive gene cyclin D1. In addition, using silencing RNA (siRNA) against endogeneous CCDC62/ERAP75, we demonstrated that inhibition of endogenous CCDC62/ERAP75 results in the suppression of ERbeta-mediated transactivation as well as target gene expression in LNCaP cells. More importantly, using the tet-on overexpression system, we showed that induced expression of CCDC62/ERAP75 can enhance the E2-regulated cyclin D1 expression and cell growth in LNCaP cells. Together, our results revealed the role of CCDC62/ERAP75 as a novel coactivator in PCa cells that can modulate ERbeta transactivation and receptor function.

Chen, S. M., et al. (2010). "RNA interference-mediated silencing of UBCH10 gene inhibits colorectal cancer cell growth in vitro and in vivo." Clin Exp Pharmacol Physiol **37**(5-6): 525-529.

1. UbcH10 is the cancer-related E2 ubiquitin-conjugating enzyme, and its overexpression has been demonstrated in a variety of malignancies. The aim of the present study is to silence UbcH10 gene by RNA interference (RNAi) and to observe its inhibitory effect on the colorectal cancer cell growth in vitro and in vivo. 2. We constructed the expression vector pGPU6/GFP/Neo/UbcH10-RNAi (pUbcH10-RNAi), which contained a UbcH10 short hairpin RNA expression cassette. Then the UbcH10 gene silencing cell lines LoVo/UbcH10-RNAi and HT-29/UbcH10-RNAi were established. Reverse transcription-polymerase chain reaction and western blot analysis were used to evaluate the expression of the UbcH10 gene. Cell Counting Kit-8 was used to assess properties of tumour cell growth in vitro. Flow cytometry was used to detect the effect of pUbcH10-RNAi on the cell cycle of colorectal cancer cells. Furthermore, the anti-tumour effects of pUbcH10-RNAi were evaluated in vivo in a nude mouse xenografts model. 3. Results demonstrated that UbcH10 gene expression was significantly decreased in pUbcH10-RNAi treated cells. Colorectal cancer cells growth was markedly suppressed in the pUbcH10-RNAi group compared with control conditions and colorectal cancer cells were arrested in the G2-M phase. In vivo, the downregulation of UbcH10 gene expression by pUbcH10-RNAi also inhibited tumour growth in a nude mice xenograft model. 4. Our study suggests that RNA interference-mediated silencing of UbcH10 gene has anti-tumour activity on colorectal cancer and might have therapeutic potential for the treatment of colorectal cancer.

Chen, W., et al. (2003). "Retroviral endostatin gene transfer inhibits human colon cancer cell growth in vivo." Chin Med J (Engl) **116**(10): 1582-1584.

OBJECTIVE: To investigate the therapeutic effect of retroviral endostatin gene transfer on the human colon cancer cell line, LoVo. METHODS: A retroviral vector pLESSN expressing secretable endostatin was constructed and packaged with a titer of 8.2 x 10(5) CFU/ml. A LoVo cell line was subjected to retrovirus-mediated endostatin gene transfer. The proviral integration of endostatin was analyzed with PCR. The function of endostatin was tested by MTT assay in vitro and a mouse xenograft model in vivo. RESULTS: After transfection and superinfection, amphotropic retrovirus was collected, and transduction with amphotropic retroviruses resulted in endostatin proviral integration. The endostatin secreted by transduced LoVo cells markedly inhibited cell growth up to 67% (P<0.001), compared with the control cells. The gene expression of endostatin in LoVo colon tumor cells significantly inhibited tumor growth in vivo. There was an 86% reduction in tumor size in the endostatin-transduced group, accompanied by a reduction in vessels, compared with the control group (P<0.01). CONCLUSION: Retroviruses can allow functional expression of the endostatin gene in human colon tumors, showing promise for an antitumor strategy using antiangiogenesis.

Chen, Y., et al. (2007). "Homeobox gene HOP has a potential tumor suppressive activity in human lung cancer." Int J Cancer **121**(5): 1021-1027.

The homeobox containing gene HOP (Homeodomain Only Protein) was identified in the developing heart and lung where it functions downstream of Nkx2.5 and Nkx2.1 to modulate cardiac and lung gene expression. Previously, we

found that HOP was downregulated in lung cancer. In this study, we constructed an expression vector containing the full-length cDNA of HOP and transfected it into a lung cancer cell line H2170. Stable transfection led to an increased expression of HOP confirmed by Northern blot analysis. HOP positive transfectants remarkably reduced the growth rate and the ability of anchorage-independent growth in soft agar, and moreover suppressed the tumor formation in nude mice compared to controls. Transient transfection of Nkx2.1 into H2170 resulted in the overexpression of HOP, and correspondingly, siRNA silencing of Nkx2.1 reduced the expression of HOP in lung cancer cells. Treatment with a differentiation modulating agent 5-bromodeoxyuridine (BrdU) led to restoration of HOP expression in a small cell lung cancer cell line H526. In 29 paired primary lung tumor samples, loss of heterozygosity (LOH) analysis was performed by using the 3 microsatellite markers D4S189, D4S231 and D4S392 around the region of chromosome 4q12 where HOP locates. LOH was only found in 4 out 23 cases (17.4%) indicating that allelic loss is a rare genetic event not responsible for the downregulation of HOP in lung cancer. Taken together, our data suggest that HOP is a potential tumor suppressor possibly involved in lung cancer differentiation, and functions downstream of Nkx2.1.

Cheng, Y., et al. (2011). "Evaluation of PPP2R2A as a prostate cancer susceptibility gene: a comprehensive germline and somatic study." Cancer Genet **204**(7): 375-381.

PPP2R2A, mapped to 8p21.2, codes for the alpha isoform of the regulatory B55 subfamily of protein phosphatase 2 (PP2A). PP2A is one of the four major serine/threonine phosphatases and is implicated in the negative control of cell growth and division. Because of its known functions and location within a chromosomal region where evidence for linkage and somatic loss of heterozygosity was found, we hypothesized that either somatic copy number changes or germline sequence variants in PPP2R2A may increase prostate cancer (PCa) risk. We examined PPP2R2A deletion status in 141 PCa samples using Affymetrix SNP arrays. It was found that PPP2R2A was commonly (67.1%) deleted in tumor samples, including a homozygous deletion in three tumors (2.1%). We performed a mutation screen for PPP2R2A in 96 probands of hereditary prostate cancer families. No high risk mutations were identified. In addition, we re-analyzed 10 SNPs of PPP2R2A in sporadic PCa cases and controls. No significant differences in the allele and genotype frequencies were observed among either PCa cases and controls or PCa aggressive and non-aggressive cases. Taken together, these results suggest that a somatic deletion rather than germline sequence variants of PPP2R2A may play a more important role in PCa susceptibility.

Chitambar, C. R., et al. (2006). "Expression of the hemochromatosis gene modulates the cytotoxicity of doxorubicin in breast cancer cells." Int J Cancer **119**(9): 2200-2204.

The antineoplastic agent doxorubicin inhibits cell growth through mechanisms that include an interaction with iron, resulting in the generation of cytotoxic reactive oxygen species (ROS). Prior studies have shown that the wild-type hemochromatosis gene (wt HFE) may downregulate iron uptake and alter iron homeostasis in cells. We therefore tested the hypothesis that expression of wt HFE would affect the cytotoxicity of doxorubicin. Human breast cancer MCF-7 cells were transfected with an expression plasmid for a FLAG-tagged wt HFE gene [fwtHFE (+) cells], to examine the impact of wt HFE expression on doxorubicin-induced apoptosis. Our results show that, in MCF-7 cells, fwtHFE expression resulted in a reduction in cellular iron uptake and a decrease in the growth inhibitory effects of doxorubicin. Two micromolar doxorubicin inhibited the growth of fwtHFE (+) and fwtHFE (-) MCF-7 cells by 34% and 61%, respectively. In parallel, doxorubicin induced caspase-3-like activity in fwtHFE (-) cells, but not in fwtHFE (+) cells. On analysis with a DCF fluorescence assay, ROS could be detected in fwtHFE (-) cells but not in fwtHFE (+) cells exposed to doxorubicin. Western blot analysis of breast biopsy samples from patients revealed immunoreactive HFE and transferrin receptor proteins in both normal and malignant breast tissues. Our studies suggest that HFE expression and its consequent effect on cellular iron homeostasis may modulate doxorubicin-induced oxidative stress and apoptosis in breast cancer cells. Further investigation is warranted to determine whether HFE expression in tumor cells impacts on the clinical efficacy of doxorubicin.

Cho-Chung, Y. S. (1996). "Protein kinase A-directed antisense restrains cancer growth: sequence-specific inhibition of gene expression." Antisense Nucleic Acid Drug Dev **6**(3): 237-244.

Increased expression of the RI alpha subunit of cAMP-dependent protein kinase type I has been shown in human cancer cell lines, in primary tumors, in cells after transformation, and in cells upon stimulation of growth. The sequence-specific inhibition of RI alpha gene expression by an antisense oligodeoxynucleotide results in the differentiation of leukemia cells and growth arrest of cancer cells of epithelial origin. A single-injection RI alpha antisense treatment in vivo also causes a reduction in RI alpha expression and inhibition of tumor growth. Tumor cells behave like

untransformed cells by making less protein kinase type I. The RI alpha antisense, which produces a biochemical imprint for growth control, requires infrequent dosing to restrain neoplastic growth in vivo.

Chu, I., et al. (2005). "The dual ErbB1/ErbB2 inhibitor, lapatinib (GW572016), cooperates with tamoxifen to inhibit both cell proliferation- and estrogen-dependent gene expression in antiestrogen-resistant breast cancer." Cancer Res **65**(1): 18-25.

Effective treatment of estrogen receptor (ER)-positive breast cancers with tamoxifen is often curtailed by the development of drug resistance. Antiestrogen-resistant breast cancers often show increased expression of the epidermal growth factor receptor family members, ErbB1 and ErbB2. Tamoxifen activates the cyclin-dependent kinase inhibitor, p27 to mediate G (1) arrest. ErbB2 or ErbB1 overexpression can abrogate tamoxifen sensitivity in breast cancer lines through both reduction in p27 levels and inhibition of its function. Here we show that the dual ErbB1/ErbB2 inhibitor, lapatinib (GW572016), can restore tamoxifen sensitivity in ER-positive, tamoxifen-resistant breast cancer models. Treatment of MCF-7(pr), T-47D, and ZR-75 cells with lapatinib or tamoxifen alone caused an incomplete cell cycle arrest. Treatment with both drugs led to a more rapid and profound cell cycle arrest in all three lines. Mitogen-activated protein kinase and protein kinase B were inhibited by lapatinib. The two drugs together caused a greater reduction of cyclin D1 and a greater p27 increase and cyclin E-cdk2 inhibition than observed with either drug alone. In addition to inhibiting mitogenic signaling and cell cycle progression, lapatinib inhibited estrogen-stimulated ER transcriptional activity and cooperated with tamoxifen to further reduce ER-dependent transcription. Lapatinib in combination with tamoxifen effectively inhibited the growth of tamoxifen-resistant ErbB2 overexpressing MCF-7 mammary tumor xenografts. These data provide strong preclinical data to support clinical trials of ErbB1/ErbB2 inhibitors in combination with tamoxifen in the treatment of human breast cancer.

Chung, B. H., et al. (2001). "Effects of docosahexaenoic acid and eicosapentaenoic acid on androgen-mediated cell growth and gene expression in LNCaP prostate cancer cells." Carcinogenesis **22**(8): 1201-1206.

There is some epidemiological support for a protective influence of omega-3 fatty acids against prostate cancer. We wanted to explore whether omega-3 polyunsaturated fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) can affect androgen receptor function in prostate cancer cells. Our study showed that both DHA and EPA inhibit androgen-stimulated cell growth. Androgenic induction of prostate-specific antigen (PSA) protein was repressed by DHA and EPA in a dose-dependent manner. The mRNA levels of five androgen up-regulated genes, PSA, ornithine decarboxylase, NKX 3.1, immunophilin fkbp 51 and Drg-1, were decreased with DHA treatment in the presence of androgens. Transfection experiments using a DNA vector containing androgen-responsive elements demonstrated that both DHA and EPA could interfere with transactivation activities of the androgen receptor (AR). However, western blot analysis of AR protein showed that DHA and EPA treatments did not change AR expression levels. Interestingly, the proto-oncoprotein c-jun was increased by DHA treatment. A transient transfection found that forced expression of c-jun inhibited AR transactivation activity. Thus, this study found that the inhibitory effects of omega-3 polyunsaturated fatty acids on AR-mediated actions are due, at least in part, to an increase in c-jun protein.

Church, J. G., et al. (1989). "Activation of the Na+/H+ antiport is not required for epidermal growth factor-dependent gene expression, growth inhibition or proliferation in human breast cancer cells." Biochem J **257**(1): 151-157.

Mitogen interaction with specific receptors in many cell types leads to activation of the Na+/H+ antiport and a resultant cytoplasmic alkalinization. Since amiloride inhibits both Na+/H+ exchange and cell proliferation, it has been hypothesized that activation of the antiport is an obligatory requirement for mitogenesis. However, concentrations of amiloride which inhibit the antiport also inhibit other cellular processes, including protein synthesis and phosphorylation. We have used an epidermal growth factor (EGF) receptor gene-amplified human breast cancer cell line, the growth of which is inhibited by high levels of EGF in culture (MDA-468) and a variant, the growth of which is stimulated by EGF (MDA-468-S4), along with two potent amiloride analogues to examine whether activation of the Na+/H+ antiport and cytoplasmic alkalinization is necessary for both EGF-dependent effects to occur. At concentrations of the amiloride analogues which block Na+/H+ exchange in both cell types by 76-98%, the EGF-dependent alterations in [3H]thymidine incorporation or induction in c-myc or c-fos gene transcription were unaltered. These results were confirmed by a lack of effect of the amiloride analogues on both the growth-stimulatory and growth-inhibitory effects on EGF in an anchorage-independent growth assay. Similarly, in pH-altered media that prevented normal cytoplasmic alkalinization, the response of both MDA-468 and MDA-468-S4 to EGF

activation was unaltered. In addition, activation of the Na+/H+ antiport alone was not sufficient to induce c-myc and c-fos transcription in either cell type. Taken together, these data suggest that neither the Na+/H+ antiport nor cytoplasmic alkalinization are necessary or sufficient for either EGF-dependent growth stimulation or growth inhibition in MDA-468 human breast cancer cells.

Cinar, B., et al. (2001). "Androgen receptor mediates the reduced tumor growth, enhanced androgen responsiveness, and selected target gene transactivation in a human prostate cancer cell line." Cancer Res **61**(19): 7310-7317.

The growth and development of the prostate gland are regulated by the androgen and the androgen receptor (AR). Despite our molecular understanding of the roles of the AR regulating; a downstream target gene transcription, the direct or indirect (stromally mediated) actions of the androgen in controlling prostate cell growth and differentiation are still unclear. In this report, an invasive; and metastatic human prostate tumor cell line, androgen-repressed human prostate cancer cell line (ARCaP), either transduced with wild-type human AR (hAR) or a control neomycin-resistant plasmid DNA, was used to evaluate the direct role of AR in regulating prostate tumor cell growth and gene transcription. Results showed that: (a) introduction of wild-type hAR to ARCaP cells restored positive androgen regulation of prostate tumor cell growth in vitro through an enhanced cell-cycle progression from G (0)/G (1) to S and G (2)-M phases; (b) hAR was shown to transactivate glucocorticoid-responsive element but not prostate-specific antigen promoter-directed reporter gene expression; and (c) hAR-transduced ARCaP cells exhibited reduced growth, invasion, and migratory behavior in vitro and tumor growth in vivo. These results suggest that the introduction of hAR into the invasive human prostate cancer ARCaP cell line restored its androgen-regulated cell growth, decreased the rate of tumor growth, and selectively activated AR target gene expression. These cellular functions in response to androgen are commonly associated with increased differentiation of prostate epithelial cells.

Cogoi, S., et al. (2013). "Guanidino anthrathiophenediones as G-quadruplex binders: uptake, intracellular localization, and anti-Harvey-Ras gene activity in bladder cancer cells." J Med Chem **56**(7): 2764-2778.

We prepared a series of anthrathiophenediones (ATPDs) with guanidino-alkyl side chains of different length (compounds 1, 10-13). The aim was to investigate their interaction with DNA and RNA G-quadruplexes, their uptake in malignant and nonmalignant cells, and their capacity to modulate gene expression and inhibit cell growth. Flow cytometry showed that the ATPDs enter more efficiently in malignant T24 bladder cells than in nonmalignant embryonic kidney 293 or fibroblast NIH 3T3 cells. In T24 malignant cells, compound 1, with two ethyl side chains, is taken up by endocytosis, while 12 and 13, with respectively propyl and butyl side chains, are transported by passive diffusion. The designed ATPDs localize in the cytoplasm and nucleus and tightly bind to DNA and RNA G-quadruplexes. They also decrease HRAS expression, increase the cell population in G0/G1, and strongly inhibit proliferation in malignant T24 bladder cells, but not in nonmalignant 293 or NIH 3T3 cells.

Collinet, P., et al. (2006). "In vivo expression and antitumor activity of p53 gene transfer with naked plasmid DNA in an ovarian cancer xenograft model in nude mice." J Obstet Gynaecol Res **32**(5): 449-453.

INTRODUCTION: Abnormalities in the p53 and p16 tumor suppressor genes are one of the most common occurrences associated with human neoplasia. Consequently, restoration of wild-type p53 or p16 functions is seen as a particularly promising approach for cancer gene therapy. In vitro and in vivo data have demonstrated that virus-mediated p53 gene transfer can induce active cell death and ovarian tumor regression. AIM: To evaluate the efficiency of intratumoral injection of naked DNA in tumor growth inhibition in an ovarian xenograft model. For that purpose, plasmid vectors encoding wild-type p53 (wt-p53) or p16 alone or in combination were used. METHODS: Nude mice were injected subcutaneously with the human ovarian adenocarcinoma cell line SKOV3. Three weeks after xenograft, tumor-bearing mice were injected twice a week with plasmid vectors carrying WT-p53 and/or WT-p16 cDNA. Empty plasmids and saline buffer were used as control. Tumor growth was monitored to evaluate the inhibition potential with p53 and/or p16 restoration. RESULTS: When compared to the control, intratumoral repeated injections of naked plasmid DNA encoding wt-p53 were inhibiting tumor growth. This inhibition was not observed with p16 and no synergy could be obtained between p53 and p16. p53 expression was restored in 84% of mice injected with plasmid encoding wt-p53. p16 expression was restored in 63% of mice injected with plasmid encoding p16. CONCLUSIONS: In this report we demonstrated that: (i) naked DNA represents an efficient gene transfer in the SKOV3 xenograft model; (ii) restoration of wt-p53 gene allows tumor growth inhibition; and (iii) this inhibition could be correlated with p53 expression as seen in 84% of treated mice after repeated naked DNA injections. These results allow us to envisage naked

DNA as a therapeutic adjuvant in ovarian cancer treatment, concomitantly with tumor resection and chemotherapy.

Connolly, J. M. and D. P. Rose (1998). "Enhanced angiogenesis and growth of 12-lipoxygenase gene-transfected MCF-7 human breast cancer cells in athymic nude mice." Cancer Lett **132**(1-2): 107-112.

Transfection of the estrogen-dependent and poorly invasive MCF-7 human breast cancer cell line so that it stably overexpressed 12-lipoxygenase and secreted high levels of 12-hydroxyeicosatetraenoic acid when cultured with arachidonate resulted in rapid growth in athymic nude mice when compared with the parental line. This enhanced acquisition of tumor mass was a result of both increased cell proliferation and reduced apoptotic cell death and was accompanied by high angiogenic activity.

Copper, M. P., et al. (1997). "All-trans retinoic acid induced gene expression and growth inhibition in head and neck cancer cell lines." Oral Oncol **33**(4): 270-274.

Retinoids are natural and synthetic analogues of vitamin A and have proven activity in various types of cancer. As for head and neck squamous cell cancer (HNSCC), retinoids are especially active in leukoplakia and in preventing second primary cancers. The aim of this study was to assess the growth inhibiting activity of all-trans retinoic acid (all-trans RA) in a panel of six head and neck squamous cell cancer cell lines and to correlate this response to the mRNA expression of factors related to differentiation and receptor mediated signal transduction. Three lines showed minimal, two moderate and one strong growth inhibition after 72 h exposure to all-trans RA. Three lines with a dissimilar response were selected for further studies, the measurement of mRNA expression by northern blotting. It was found that neither the expression nor the induction of retinoic acid receptor (RAR)-alpha and -gamma and retinoic X receptor-alpha mRNA war related to sensitivity. The mRNA expression of RAR-beta was too low to be measured in the three cell lines. The most sensitive cell line was, however, the only one that expressed mRNA of squamous differentiation markers. These data suggest a relationship between the retinoid sensitivity profile and the degree of cellular differentiation.

Creighton, C., et al. (2003). "Profiling of pathway-specific changes in gene expression following growth of human cancer cell lines transplanted into mice." Genome Biol **4**(7): R46.

BACKGROUND: Tumor cells cultured in vitro are widely used to investigate the molecular biology of cancers and to evaluate responses to drugs and other agents. The full extent to which gene expression in cancer cells is modulated by extrinsic factors and by the microenvironment in which the cancer cells reside remains to be determined. Two cancer cell lines (A549 lung adenocarcinoma and U118 glioblastoma) were transplanted subcutaneously into immunodeficient mice to form tumors. Global gene-expression profiles of the tumors were determined, based on analysis of expression of human genes, and compared with expression profiles of the cell lines grown in culture. RESULTS: A bioinformatics approach associated genes that showed changes in their expression levels with functional classes as defined by either the GO gene annotations or MeSH terms in the literature. The classes of genes expressed at higher levels in cells grown in vitro indicated increased cell division and metabolism, reflecting the more favorable environment for cell proliferation. In contrast, in vivo tumor growth resulted in upregulation of a significant number of genes involved in the extracellular matrix (ECM), cell adhesion, cytokine and metalloendopeptidase activity, and neovascularization. When placed in comparable tissue environments, the U118 cells and the A549 cells expressed different sets of ECM and cell adhesion-related genes, suggesting different mechanisms of extracellular interaction at work in the different cancers. CONCLUSIONS: Studies of this type allow us to examine the specific contribution of cancer cells to gene expression patterns within an in vivo tumor mixed with non-cancerous tissue.

Dai, C., et al. (2015). "Transcriptional activation of human CDCA8 gene regulated by transcription factor NF-Y in embryonic stem cells and cancer cells." J Biol Chem **290**(37): 22423-22434.

The cell division cycle associated 8 (CDCA8) gene plays an important role in mitosis. Overexpression of CDCA8 was reported in some human cancers and is required for cancer growth and progression. We found CDCA8 expression was also high in human ES cells (hESCs) but dropped significantly upon hESC differentiation. However, the regulation of CDCA8 expression has not yet been studied. Here, we characterized the CDCA8 promoter and identified its cis-elements and transcription factors. Three transcription start sites were identified. Reporter gene assays revealed that the CDCA8 promoter was activated in hESCs and cancer cell lines. The promoter drove the reporter expression specifically to pluripotent cells during early mouse embryo development and to tumor tissues in tumor-bearing mice. These results indicate that CDCA8 is transcriptionally activated in hESCs and cancer cells. Mechanistically, two key activation elements, bound

by transcription factor NF-Y and CREB1, respectively, were identified in the CDCA8 basic promoter by mutation analyses and electrophoretic motility shift assays. NF-Y binding is positively correlated with promoter activities in different cell types. Interestingly, the NF-YA subunit, binding to the promoter, is primarily a short isoform in hESCs and a long isoform in cancer cells, indicating a different activation mechanism of the CDCA8 transcription between hESCs and cancer cells. Finally, enhanced CDCA8 promoter activities by NF-Y overexpression and reduced CDCA8 transcription by NF-Y knockdown further verified that NF-Y is a positive regulator of CDCA8 transcription. Our study unearths the molecular mechanisms underlying the activation of CDCA8 expression in hESCs and cancer cells, which provides a better understanding of its biological functions.

Damon, S. E., et al. (2001). "Transcriptional regulation of insulin-like growth factor-I receptor gene expression in prostate cancer cells." Endocrinology **142**(1): 21-27.

A marked decrease in the type 1 insulin-like growth factor (IGF) receptor (IGF-IR) occurs in prostate epithelial cells during transformation from the benign to the metastatic state. One of the principal regulators of IGF-IR gene expression, the WT1 tumor suppressor, is expressed in prostate cancer and in prostate cancer cell lines. The purpose of this study was to determine whether the decrease in IGF-IR expression was transcriptionally regulated, and whether WT1 action may be involved in the repression of the IGF-IR gene in prostate cancer cells. The P69 cell line was derived by immortalization of human primary prostate epithelial cells with simian virus-40 T antigen and is rarely tumorigenic. The M12 line was derived from the P69 line by selection for tumor formation in nude mice and is tumorigeneic and metastatic. P69 cells express 20,000 IGF-IR/cell, whereas M12 cells express 3,500 IGF-IR/cell. These differences in receptor number are reflected in proportional differences in IGF-IR mRNA levels. To assess IGF-IR promoter activity in these cell lines, each was transiently transfected with luciferase reporter vectors containing the IGF-IR gene transcription start site and 476 bp of 5'-flanking sequence, 640 bp of 5'-untranslated region sequence, or both regions. The promoter activity of the full-length construct was 50% lower ($P < 0.01$) in M12 cells compared with P69 cells, the activity of the 5'-flanking region construct was 53% lower ($P < 0.0001$), and that of the 5'-untranslated region construct was 36% lower ($P = 0.01$). P69 clones stably transfected with a WT1 expression vector exhibited decreased expression of the endogenous IGF-IR gene and decreased promoter activity in transient transfection assays with IGF-IR promoter constructs containing multiple WT1 binding sites. The observed reduction in endogenous IGF-IR expression was sufficient to inhibit IGF-I-stimulated cell proliferation. These data suggest that most of the decreased expression of the IGF-IR seen in malignant prostate epithelium is the result of transcriptional repression of the IGF-IR gene, and that this repression may be due in part to the increased expression of the WT1 tumor suppressor in metastatic prostate cancer.

Davidson, A., et al. (1996). "Regulation of VIP gene expression in general. Human lung cancer cells in particular." J Mol Neurosci **7**(2): 99-110.

Vasoactive intestinal peptide (VIP) is a neuropeptide of multiple functions affecting development and aging. In cancer, for example, VIP was found to function as an autocrine growth factor in nonsmall cell lung cancer (NSCLC) promotion. Furthermore, a VIP hybrid antagonist (neurotensin (6-11)-VIP (7-28)) was found to inhibit NSCLC growth. In the present study, the expression of VIP mRNA was studied using human lung cancer cells. RNA prepared from 19 cell lines was fractionated by 1% agarose gel electrophoresis followed by blotting onto nitrocellulose membranes and hybridization to a VIP-specific RNA probe. VIP mRNA was detected in about 50% of the cell lines tested with a greater abundance in NSCLC. Cultures of the NSCLC NCI-H727 cell line were treated with forskolin, an activator of cyclic AMP (cAMP), and separately with the tumor promoter phorbol 12-myristate 13-acetate (PMA). Northern blot hybridization analysis showed an increase in VIP mRNA levels after 4 h treatment with 50 microM forskolin. Incubation with PMA also showed a significant increase in the levels of VIP transcripts. Cultures were then incubated with PMA in the presence of actinomycin D, a transcription blocker. Results indicated that PMA treatment may induce both VIP mRNA synthesis as well as VIP mRNA stabilization, and suggested a 4-5 h half-life for the VIP mRNA in the absence of PMA. Thus, lung cancer tumor proliferation may be regulated, in part, at the level of VIP gene expression.

Davies, H., et al. (2002). "Mutations of the BRAF gene in human cancer." Nature **417**(6892): 949-954.

Cancers arise owing to the accumulation of mutations in critical genes that alter normal programmes of cell proliferation, differentiation and death. As the first stage of a systematic genome-wide screen for these genes, we have prioritized for analysis signalling pathways in which at least one gene is mutated in human cancer. The RAS RAF MEK ERK

MAP kinase pathway mediates cellular responses to growth signals. RAS is mutated to an oncogenic form in about 15% of human cancer. The three RAF genes code for cytoplasmic serine/threonine kinases that are regulated by binding RAS. Here we report BRAF somatic missense mutations in 66% of malignant melanomas and at lower frequency in a wide range of human cancers. All mutations are within the kinase domain, with a single substitution (V599E) accounting for 80%. Mutated BRAF proteins have elevated kinase activity and are transforming in NIH3T3 cells. Furthermore, RAS function is not required for the growth of cancer cell lines with the V599E mutation. As BRAF is a serine/threonine kinase that is commonly activated by somatic point mutation in human cancer, it may provide new therapeutic opportunities in malignant melanoma.

De Schrijver, E., et al. (2003). "RNA interference-mediated silencing of the fatty acid synthase gene attenuates growth and induces morphological changes and apoptosis of LNCaP prostate cancer cells." Cancer Res **63**(13): 3799-3804.

Fatty acid synthase (FASE), a key enzyme in the biosynthesis of fatty acids, is markedly overexpressed in many human epithelial cancers, rendering it an interesting target for antineoplastic therapy. Here, using the potent and highly sequence-specific mechanism of RNA interference (RNAi), we have silenced the expression of FASE in lymph node carcinoma of the prostate (LNCaP) cells. RNAi-mediated down-regulation of FASE expression resulted in a major decrease in the synthesis of triglycerides and phospholipids and induced marked morphological changes, including a reduction in cell volume, a loss of cell-cell contacts, and the formation of spider-like extrusions. Furthermore, silencing of the FASE gene by RNAi significantly inhibited LNCaP cell growth and ultimately resulted in induction of apoptosis. Importantly and in striking contrast with LNCaP cells, RNAi-mediated inhibition of FASE did not influence growth rate or viability of nonmalignant cultured skin fibroblasts. These data indicate that RNAi opens new avenues toward the study of the role of FASE overexpression in tumor cells and provides an interesting and selective alternative to chemical FASE inhibitors in the development of antineoplastic therapy.

Deftos, L. J. (1998). "Granin-A, parathyroid hormone-related protein, and calcitonin gene products in neuroendocrine prostate cancer." Prostate Suppl **8**: 23-31.

BACKGROUND: The importance of the expression of granin A (GRN-A, chromogranin-A), calcitonin (CT) gene products (CGPs), and parathyroid hormone-related protein (PTHrP) has become appreciated in the neuroendocrine (NE) differentiation of prostate cancer. We have studied the prostate expression of these three NE cell products with in vivo and in vitro methods. METHODS: GRN-A secretion was measured by immunoassay in serum samples from patients with prostate cancer. Immunohistology procedures were used to assess GRN-A, CGPs, and PTHrP expression in paraffin-embedded prostate tissue samples. Serum and tumor findings were evaluated according to the patient's clinical status. All three substances were also studied in prostate cancer cell cultures. RESULTS: GRN-A, PTHrP, and CGPs were all secreted products of prostate cancer. Our studies demonstrated that GRN-A can serve as a prostate cancer serum and tumor marker with clinical value for both diagnosis and prognosis. Elevated serum GRN-A levels identified patients with prostate cancer, including some who did not have elevated serum prostate-specific antigen (PSA) levels. Serum GRN-A concentrations also had prognostic value for prostate cancer. PTHrP and CGPs were expressed in prostate cancer in addition to GRN-A, and all three were secreted by prostate cells in culture. Each had effects on prostate cell growth. CONCLUSIONS: GRN-A, PTHrP, and CGPs are produced and secreted by prostate cells. These three NE cell products can serve as tumor and markers for prostate cancer that have diagnostic and prognostic value. In addition, their derived peptides regulate prostate cell growth. However, studies more conclusive than the preliminary observations of our group and of other investigators are needed to define the roles of PTHrP, GRN-A, and CGPs in prostate cancer.

DeYoung, M. P., et al. (2003). "Down's syndrome-associated Single Minded 2 gene as a pancreatic cancer drug therapy target." Cancer Lett **200**(1): 25-31.

We report here a pancreatic cancer drug therapy utility of a gene involved in Down's syndrome. Single Minded 2 gene (SIM2) from Down's Syndrome Critical Region was expressed in pancreatic cancer-derived cell lines as well as in tumor tissues, but not in the normal pancreas. A related member of the SIM family, SIM1, did not show similar specificity. Inhibition by antisense technology of one of the isoforms of SIM2, the short-form (SIM2-s) expression in the CAPAN-1 pancreatic cancer cell line, caused a pronounced growth inhibition and induced cell death through apoptosis. The specificity of antisense was inferred from inhibition of SIM2-s mRNA but not the related members of SIM family. In view of the high mortality rate of pancreatic cancer patients, these findings have important implications for the future of pancreatic cancer treatment.

Dirican, E., et al. (2016). "Mutation distributions and clinical correlations of PIK3CA gene mutations in breast cancer." Tumour Biol **37**(6): 7033-7045.

Breast cancer (BCa) is the most common cancer and the second cause of death among women. Phosphoinositide 3-kinase (PI3K) signaling pathway has a crucial role in the cellular processes such as cell survival, growth, division, and motility. Moreover, oncogenic mutations in the PI3K pathway generally involve the activation phosphatidylinositol-4,5-bisphosphate 3-kinase-catalytic subunit alpha (PIK3CA) mutation which has been identified in numerous BCa subtypes. In this review, correlations between PIK3CA mutations and their clinicopathological parameters on BCa will be described. It is reported that PIK3CA mutations which have been localized mostly on exon 9 and 20 hot spots are detected 25-40 % in BCa. This relatively high frequency can offer an advantage for choosing the best treatment options for BCa. PIK3CA mutations may be used as biomarkers and have been major focus of drug development in cancer with the first clinical trials of PI3K pathway inhibitors currently in progress. Screening of PIK3CA gene mutations might be useful genetic tests for targeted therapeutics or diagnosis. Increasing data about PIK3CA mutations and its clinical correlations with BCa will help to introduce new clinical applications in the near future.

D'Orazi, G., et al. (2000). "Exogenous wt-p53 protein is active in transformed cells but not in their non-transformed counterparts: implications for cancer gene therapy without tumor targeting." J Gene Med **2**(1): 11-21.

BACKGROUND: Expression of exogenous wild-type p53 (wt-p53) protein in tumor cells can suppress the transformed phenotype whereas it does not apparently induce detrimental effects in non-transformed cells. This observation may provide a molecular basis for p53-mediated gene therapy of p53-sensitive cancers without the need for tumor targeting. METHODS: To understand the molecular mechanisms responsible for this different behavior in tumor versus normal cells, biochemical and functional analyses of exogenous wt-p53 protein were performed on non-transformed C2C12 myoblasts and their transformed counterparts, the C2-ras cells. RESULTS: The exogenous wt-p53 protein, which induced persistent growth arrest only in transformed C2-ras cells, was shown to be significantly more stable in transformed than in non-transformed cells. This different stability was due to different p53 proteolytic degradation. Moreover, constitutively, exogenous wt-p53 protein was found to be transcriptionally active only in C2-ras cells but it could also be activated in C2C12 cells by genotoxic damage. CONCLUSIONS: Non-transformed C2C12 cells present regulatory system (s) which control the expression and the activity of exogenously expressed wt-p53 protein probably through degradation and maintenance in a latent form. This regulatory system is lost/inactivated upon transformation.

Draus, J. M., et al. (2001). "p53 gene transfer does not enhance E2F-1-mediated apoptosis in human colon cancer cells." Exp Mol Med **33**(4): 209-219.

E2F-1 and p53 are sequence specific transcription factors that are intimately involved in the regulation of the cell cycle. In addition to their role in cell cycle control, both E2F-1 and p53 have been identified as tumor suppressors and mediators of apoptosis. We have shown previously that adenoviral-mediated E2F-1 overexpression induces efficient apoptosis in colon adenocarcinoma cells. Previous reports have suggested that E2F-1 and p53 cooperate to mediate apoptosis and therefore, in this study, we examined the efficacy of combination gene therapy using adenovirus vectors expressing E2F-1 and p53 in human colon adenocarcinoma cell lines, HT-29 and SW620 (both mutant p53). Cells were treated by mock infection or infection with adenoviral vectors expressing b-galactosidase (LacZ), E2F-1, p53 or a combination of E2F-1 and p53. IC25 concentrations of each virus were estimated and used for each treatment in order to detect any synergistic or cooperative effects on tumor cell death in the combination therapy. By 5 days post infection, E2F-1-overexpressing cells exhibited growth inhibition and approximately 40-50% cell death in both cell lines. Co-expression of p53 with E2F-1 abrogated E2F-1-mediated growth inhibition and cell death. Cell cycle analysis revealed that overexpression of E2F-1 resulted in an accumulation of cells in G2/M phase, while overexpression of p53 resulted in a G1 phase accumulation. However, co-expression of E2F-1 and p53 counteracted each other as fewer cells accumulated in G1 and G2/M when compared to either p53 or E2F-1 alone. Furthermore, co-expression of p53 with E2F-1 resulted in decreased levels of E2F-1 protein expression. Mechanistically, upregulation of the CDK inhibitory protein, p21(WAF1/CIP1), was demonstrated in HT-29 cells following overexpression of either E2F-1, p53 or the combination E2F-1/p53 therapy. However, in SW620 cells, only the cells infected with Ad-p53 alone or in combination resulted in upregulation of p21(WAF1/CIP1). These results suggest that p53 and p21(WAF1/CIP1) may cooperate to inhibit the expression and activity of E2F-1. In conclusion, combination adenoviral vector-mediated E2F-1 and p53 gene transfer was not therapeutically

advantageous in this in vitro model of human colon adenocarcinoma.

Duan, R., et al. (2002). "Estrogen regulation of c-fos gene expression through phosphatidylinositol-3-kinase-dependent activation of serum response factor in MCF-7 breast cancer cells." Biochem Biophys Res Commun **294**(2): 384-394.

17Beta-estradiol (E2) induces proliferation and c-fos gene expression in MCF-7 cells and both responses are partially blocked by wortmannin and LY294002 which are inhibitors of phosphatidylinositol-3-kinase (PI3-K). Analysis of the c-fos gene promoter shows that the effects of wortmannin and LY294002 are associated with inhibition of E2-induced activation through the serum response factor (SRF) motif within the proximal serum response element at -325 and -296. E2 activates constructs containing multiple copies of the SRF (pSRF) and a GAL4-SRF fusion protein; these responses are accompanied by PI3-K-dependent phosphorylation of Akt and inhibited by wortmannin/LY294002, the antiestrogen ICI 182780, but not by the mitogen-activated protein kinase kinase (MAPKK) inhibitor PD98059. Using a series of kinase inhibitors and dominant negative kinase expression plasmids, it was shown that the non-genomic activation of SRF by E2 was associated with src-ras-PI3-K pathway, thus, demonstrating hormonal activation of the SRE through src-ras activation of both PI3-K- and MAPK-dependent signaling pathways.

Duxbury, M. S., et al. (2004). "Systemic siRNA-mediated gene silencing: a new approach to targeted therapy of cancer." Ann Surg **240**(4): 667-674; discussion 675-666.

OBJECTIVE: RNA interference (RNAi), mediated by small interfering RNA (siRNA), silences genes with a high degree of specificity and potentially represents a general approach for molecularly targeted anticancer therapy. The aim of this study was to evaluate the ability of systemically administered siRNA to silence gene expression in vivo and to assess the effect of this approach on tumor growth using a murine pancreatic adenocarcinoma xenograft model. SUMMARY BACKGROUND DATA: Carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6) is widely overexpressed in human gastrointestinal cancer. Overexpression of CEACAM6 promotes cell survival under anchorage independent conditions, a characteristic associated with tumorigenesis and metastasis. METHODS: CEACAM6 expression was quantified by real-time polymerase chain reaction (PCR) and Western blot. Mice (n = 10/group) were subcutaneously xenografted with 2 x 10 BxPC3 cells (which inherently overexpress CEACAM6). Tumor growth, CEACAM6 expression, cellular proliferation (Ki-67 immunohistochemistry), apoptosis, angiogenesis (CD34 immunohistochemistry), and survival were compared for mice administered either systemic CEACAM6-specific or control single-base mismatch siRNA over 6 weeks, following orthotopic tumor implantation. RESULTS: Treatment with CEACAM6-specific siRNA suppressed primary tumor growth by 68% versus control siRNA ($P < 0.05$) and was associated with a decreased proliferating cell index, impaired angiogenesis and increased apoptosis in the xenografted tumors. CEACAM6-specific siRNA completely inhibited metastasis (0% of mice versus 60%, $P < 0.05$) and significantly improved survival, without apparent toxicity. CONCLUSIONS: Our data demonstrate the efficacy of systemically administered siRNA as a therapeutic modality in experimental pancreatic cancer. This novel therapeutic strategy may be applicable to a broad range of cancers and warrants investigation in patients with refractory disease.

Eastham, J. A., et al. (1995). "In vivo gene therapy with p53 or p21 adenovirus for prostate cancer." Cancer Res **55**(22): 5151-5155.

We introduced the gene for wild-type human p53 or p21, a critical downstream mediator of p53-induced growth suppression, into a p53-deficient mouse prostate cancer cell line using a recombinant adenoviral vector (Ad5CMV-p53 or Ad5CMV-p21). Elevated levels of endogenous mouse p21 mRNA provided evidence for the functional activity of virally transduced p53. Functional activity of viral-transduced p21 was demonstrated through immunoprecipitation of cellular protein extracts, which showed that the viral-transduced p21 associates with cyclin-dependent kinase 2 and was sufficient to down-regulate the activity of the cyclin-dependent kinase by approximately 65%. In vitro growth assays revealed significantly higher growth suppression after Ad5CMV-p21 infection compared to Ad5CMV-p53. In vivo studies in syngeneic male mice with established s.c. prostate tumors demonstrated that the rate of growth and final tumor volume were reduced to a much greater extent in mice that received intratumor injection of Ad5CMV-p21 compared to Ad5CMV-p53. In addition, the survival of host animals bearing tumors that were infected with Ad5CMV-p21, but not Ad5CMV-p53, was significantly extended. These data suggest that Ad5CMV-p21 may be effective as a therapeutic agent for prostate cancer.

Edamura, K., et al. (2007). "Adenovirus-mediated REIC/Dkk-3 gene transfer inhibits tumor growth and metastasis in an orthotopic prostate cancer model." Cancer Gene Ther **14**(9): 765-772.

We had previously reported that REIC/Dkk-3, a member of the Dickkopf (Dkk) gene family, works as a tumor suppressor. In this study, we evaluated the therapeutic effects of an intratumoral injection with adenoviral vector encoding REIC/Dkk-3 gene (Ad-REIC) using an orthotopic mouse prostate cancer model of RM-9 cells. We also investigated the in vivo anti-metastatic effect and in vitro anti-invasion effect of Ad-REIC gene delivery. We demonstrated that the Ad-REIC treatment inhibited prostate cancer growth and lymph node metastasis, and prolonged mice survival in the model. These therapeutic responses were consistent with the intratumoral apoptosis induction and in vitro suppression of cell invasion/migration with reduced matrix metalloprotease-2 activity. We thus concluded that in situ Ad-REIC/Dkk-3 gene transfer may be a promising therapeutic intervention modality for the treatment of prostate cancer.

Eggen, T., et al. (2012). "Increased gene expression of the ABCC5 transporter without distinct changes in the expression of PDE5 in human cervical cancer cells during growth." Anticancer Res **32**(8): 3055-3061.

Carcinoma of the uterine cervix represents the second most frequent female malignancy worldwide, but few biochemical tumour markers have been implemented into clinical practice. Elevated extracellular guanosine 3', 5'-cyclic monophosphate (cGMP) levels have been reported to be a sensitive, early and reliable marker for screening relapse in carcinoma of the uterine cervix. The mechanism behind this observation remains unknown. The possibility exists that the cancer cells develop resistance to the antiproliferative effect of high intracellular cGMP levels. The enhanced cGMP expression may originate from either an increase in cellular export capacity by increased expression of member 5 in subfamily C of ATP-Binding-Cassette transporters (ABCC5), or increased substrate (cGMP) levels for this pump. The latter situation occurs with increased expression of inducible nitric oxide synthase (iNOS) and/or soluble guanylyl cyclase (sGC) and/or reduced expression of member 5 of the cyclic nucleotide phosphodiesterases (PDE5). Four transformed human cell lines derived from carcinomas of the uterine cervix (C-4 I, C-33 A, SiHa and ME-180 cells) and one non-transformed human cell line (WI-38) were included in the study in order to unveil which biokinetic components are involved. The expressions of iNOS, sGC, PDE5 and ABCC5 in the initial and final phase of the exponential growth curve were compared. Assuming that the WI-38 control cells mimic the situation in a normal tissue, iNOS remains un-expressed during proliferation, and the expression of sGC is low but shows a clear increase during exponential growth. PDE5 is highly expressed and increases (approximately 130%) during growth whereas ABCC5 exhibited low to moderate expression, with a moderate increase (approximately 40%) during growth. The malignant cells exhibited moderate ABCC5 expression with a distinct increase during exponential growth, whereas PDE5 expression remained virtually unchanged. Dysregulation of the cGMP biokinetics in growing malignant cells may account for the elevation of extracellular cGMP observed in patients with carcinoma of the uterine cervix.

Elledge, R. M. and D. C. Allred (1994). "The p53 tumor suppressor gene in breast cancer." Breast Cancer Res Treat **32**(1): 39-47.

Alterations of the p53 tumor suppressor gene are the most common genetic changes found so far in breast cancer, suggesting that the gene plays a central role in the development of the disease. p53 functions as a negative regulator of cell growth, and alterations in the gene lead to loss of this negative growth regulation and more rapid cell proliferation. A number of independent groups using different methods of detection have shown that p53 alterations are associated with more aggressive tumor biologic factors and a poorer prognosis in breast cancer patients. Because of its possible role in the regulation of apoptosis and response to DNA damage, p53 status could also be a predictive marker for response to hormonal or chemotherapy.

Ellen, T. P., et al. (2008). "NDRG1, a growth and cancer related gene: regulation of gene expression and function in normal and disease states." Carcinogenesis **29**(1): 2-8.

N-myc downstream-regulated gene 1 (NDRG1) is an intracellular protein that is induced under a wide variety of stress and cell growth-regulatory conditions. NDRG1 is up-regulated by cell differentiation signals in various cancer cell lines and suppresses tumor metastasis. Despite its specific role in the molecular cause of Charcot-Marie-Tooth type 4D disease, there has been more interest in the gene as a marker of tumor progression and enhancer of cellular differentiation. Because it is strongly up-regulated under hypoxic conditions, and this condition is prevalent in solid tumors, its regulation is somewhat complex, governed by hypoxia-inducible factor 1 alpha (HIF-1alpha)- and p53-dependent pathways, as well as its namesake, neuroblastoma-derived myelocytomatosis, and probably many other factors, at the transcriptional and translational levels, and through mRNA stability. We survey the data for clues to the NDRG1 gene's mechanism and for indications that the

NDRG1 gene may be an efficient diagnostic tool and therapy in many types of cancers.

Elshami, A. A., et al. (1995). "The role of immunosuppression in the efficacy of cancer gene therapy using adenovirus transfer of the herpes simplex thymidine kinase gene." Ann Surg **222**(3): 298-307; 307-210.

OBJECTIVE: To determine whether the immune system limits or improves the therapeutic efficacy of an adenovirus vector expressing the herpes simplex thymidine kinase (HSVtk) gene in a subcutaneous tumor model. BACKGROUND DATA: Enhanced immune reactions against tumors may be therapeutically useful. However, recent studies with adenoviral vectors show that immune responses limit the efficacy and persistence of gene expression. The effect of the immune response on cancer gene therapy with HSVtk gene delivery by an adenovirus vector followed by treatment with ganciclovir is unclear. METHODS: After adenoviral transduction of a Fischer rat syngeneic mesothelioma cell line with the HSVtk gene in vitro, subcutaneous flank tumors were established. The ability of the HSVtk/ganciclovir system to inhibit tumor growth was compared among normal Fischer rats, immunodeficient nude rats, and Fischer rats immunosuppressed with cyclosporin. RESULTS: HSVtk/ganciclovir therapy was more effective in nude rats and immunosuppressed Fischer rats than in immunocompetent Fischer rats. CONCLUSION: These results indicate that the immune response against adenovirally transduced cells limits the efficacy of the HSVtk/ganciclovir system and that immunosuppression appears to be a useful adjunct. These findings have important implications for clinical trials using currently available adenovirus vectors as well as for future vector design.

Evoy, D., et al. (1997). "In vivo adenoviral-mediated gene transfer in the treatment of pancreatic cancer." J Surg Res **69**(1): 226-231.

Gene therapy may allow targeted delivery of tumoricidal drugs to treat pancreatic cancer. Cytosine deaminase (CD) is a bacterial enzyme that converts the nontoxic agent 5-fluorocytosine (5FC) to the active chemotherapeutic agent 5-fluorouracil (5FU). Neoplastic cells induced to express the CD gene treated with 5FC may generate locally high concentrations of 5FU while minimising systemic toxicity. Replication deficient adenovirus vector carrying the CD gene (AdCMV.CD) was tested for therapeutic efficacy against the murine pancreatic carcinoma cell line Pan02. Pan02 cells were infected in vitro with AdCMV.CD or null vector (Ad.-Null) and were examined for expression of CD messenger RNA (mRNA) (Northern blot) and CD enzymatic function (spectrophotometry). mRNA transcripts of the CD gene increased in a dose-dependent manner after infection with AdCMV.CD. Conversion of 5FC to 5FU at a multiplicity of infection (MOI) of 20 was measured to be 51% after a 48-hr incubation. Growth inhibition was measured by MTT assay and thymidine uptake. Pan02 growth in vitro treated with AdCMV.CD and 5FC was inhibited by 80% as compared to cells treated with Ad.Null and 5FC. An in vivo model of pancreatic cancer was established by injecting 2.5 x 10(5) PAN02 cells subcutaneously into the flanks of C57BL/ 6 mice. Seven days later AdCMV.CD was injected into each tumor and 5FC was administered for 10 days. Treatment of mice with AdCMV.CD and 5FC inhibited tumor growth compared to mice who received AdCMV.CD only or 5FC only. These data demonstrate the therapeutic efficacy of an enzyme prodrug strategy in experimental pancreatic cancer.

Ezawa, I., et al. (2016). "Novel p53 target gene FUCA1 encodes a fucosidase and regulates growth and survival of cancer cells." Cancer Sci **107**(6): 734-745.

The tumor suppressor p53 functions by inducing the transcription of a collection of target genes. We previously attempted to identify p53 target genes by microarray expression and ChIP-sequencing analyses. In this study, we describe a novel p53 target gene, FUCA1, which encodes a fucosidase. Although fucosidase, alpha-l-1 (FUCA1) has been reported to be a lysosomal protein, we detected it outside of lysosomes and observed that its activity is highest at physiological pH. As there is a reported association between fucosylation and tumorigenesis, we investigated the potential role of FUCA1 in cancer. We found that overexpression of FUCA1, but not a mutant defective in enzyme activity, suppressed the growth of cancer cells and induced cell death. Furthermore, we showed that FUCA1 reduced fucosylation and activation of epidermal growth factor receptor, and concomitantly suppressed epidermal growth factor signaling pathways. FUCA1 loss-of-function mutations are found in several cancers, its expression is reduced in cancers of the large intestine, and low FUCA1 expression is associated with poorer prognosis in several cancers. These results show that protein defucosylation mediated by FUCA1 is involved in tumor suppression.

Falzon, M. and J. Zong (1998). "The noncalcemic vitamin D analogs EB1089 and 22-oxacalcitriol suppress serum-induced parathyroid hormone-related peptide gene expression in a lung cancer cell line." Endocrinology **139**(3): 1046-1053.

PTH-related peptide (PTHrP) mediates the syndrome of humoral hypercalcemia of malignancy, a frequent complication of squamous cell carcinomas of the lung. This study was undertaken to determine whether 1,25-dihydroxyvitamin D3 [1,25-(OH)2D3] and two nonhypercalcemic analogs, EB1089 and 22-oxa-1,25-(OH)2D3 (22-oxacalcitriol), suppress serum- and epidermal growth factor (EGF)-induced PTHrP gene expression in a human lung squamous cancer cell line, NCI H520. PTHrP expression was up-regulated by serum and EGF in a concentration- and time-dependent manner. Nuclear run-on analysis showed that this induction was mediated via a transcriptional mechanism, and that sequences within promoter 1 were responsible. All three vitamin D3 compounds decreased both basal and serum- and EGF-induced steady state PTHrP messenger RNA and secreted peptide levels. These effects were again mediated via a transcriptional mechanism through sequences within promoter 1. All three vitamin D3 compounds also decreased the proliferation of NCI H520 cells in a concentration- and time-dependent manner. 1,25-(OH)2D3 is hypercalcemic in vivo. However, the noncalcemic analogs EB1089 and 22-oxa-1,25-(OH)2D3 have therapeutic potential, as they suppress not only the basal but also the growth factor-stimulated levels of PTHrP in a cancer cell line associated with hypercalcemia.

Fan, M., et al. (2006). "Diverse gene expression and DNA methylation profiles correlate with differential adaptation of breast cancer cells to the antiestrogens tamoxifen and fulvestrant." Cancer Res **66**(24): 11954-11966.

The development of targeted therapies for antiestrogen-resistant breast cancer requires a detailed understanding of its molecular characteristics. To further elucidate the molecular events underlying acquired resistance to the antiestrogens tamoxifen and fulvestrant, we established drug-resistant sublines from a single colony of hormone-dependent breast cancer MCF7 cells. These model systems allowed us to examine the cellular and molecular changes induced by antiestrogens in the context of a uniform clonal background. Global changes in both basal and estrogen-induced gene expression profiles were determined in hormone-sensitive and hormonal-resistant sublines using Affymetrix Human Genome U133 Plus 2.0 Arrays. Changes in DNA methylation were assessed by differential methylation hybridization, a high-throughput promoter CpG island microarray analysis. By comparative studies, we found distinct gene expression and promoter DNA methylation profiles associated with acquired resistance to fulvestrant versus tamoxifen. Fulvestrant resistance was characterized by pronounced up-regulation of multiple growth-stimulatory pathways, resulting in estrogen receptor alpha (ERalpha)-independent, autocrine-regulated proliferation. Conversely, acquired resistance to tamoxifen correlated with maintenance of the ERalpha-positive phenotype, although receptor-mediated gene regulation was altered. Activation of growth-promoting genes, due to promoter hypomethylation, was more frequently observed in antiestrogen-resistant cells compared with gene inactivation by promoter hypermethylation, revealing an unexpected insight into the molecular changes associated with endocrine resistance. In summary, this study provides an in-depth understanding of the molecular changes specific to acquired resistance to clinically important antiestrogens. Such knowledge of resistance-associated mechanisms could allow for identification of therapy targets and strategies for resensitization to these well-established antihormonal agents.

Fazeli, A., et al. (1997). "Phenotype of mice lacking functional Deleted in colorectal cancer (Dcc) gene." Nature **386**(6627): 796-804.

The DCC (Deleted in colorectal cancer) gene was first identified as a candidate for a tumour-suppressor gene on human chromosome 18q. More recently, in vitro studies in rodents have provided evidence that DCC might function as a receptor for the axonal chemoattractant netrin-1. Inactivation of the murine Dcc gene caused defects in axonal projections that are similar to those observed in netrin-1-deficient mice but did not affect growth, differentiation, morphogenesis or tumorigenesis in mouse intestine. These observations fail to support a tumour-suppressor function for Dcc, but are consistent with the hypothesis that DCC is a component of a receptor for netrin-1.

Fazzone, W., et al. (2009). "Histone deacetylase inhibitors suppress thymidylate synthase gene expression and synergize with the fluoropyrimidines in colon cancer cells." Int J Cancer **125**(2): 463-473.

Despite recent therapeutic advances, the response rates to chemotherapy for patients with metastatic colon cancer remain at approximately 50% with the fluoropyrimidine, 5-fluorouracil (5-FU), continuing to serve as the foundation chemotherapeutic agent for the treatment of this disease. Previous studies have demonstrated that overexpression of thymidylate synthase (TS) is a key determinant of resistance to 5-FU-based chemotherapy. Therefore, there is a significant need to develop alternative therapeutic strategies to overcome TS-mediated resistance. In this study, we demonstrate that the histone deacetylase inhibitors (HDACi) vorinostat and LBH589 significantly downregulate TS gene expression in a

panel of colon cancer cell lines. Downregulation of TS was independent of p53, p21 and HDAC2 expression and was achievable in vivo as demonstrated by mouse xenograft models. We provide evidence that HDACi treatment leads to a potent transcriptional repression of the TS gene. Combination of the fluoropyrimidines 5-FU or FUdR with both vorinostat and LBH589 enhanced cell cycle arrest and growth inhibition. Importantly, the downstream effects of TS inhibition were significantly enhanced by this combination including the inhibition of acute TS induction and the enhanced accumulation of the cytotoxic nucleotide intermediate dUTP. These data demonstrate that HDACi repress TS expression at the level of transcription and provides the first evidence suggesting a direct mechanistic link between TS downregulation and the synergistic interaction observed between HDACi and 5-FU. This study provides rationale for the continued clinical evaluation of HDACi in combination with 5-FU-based therapies as a strategy to overcome TS-mediated resistance.

Feldman, A. L., et al. (2000). "Antiangiogenic gene therapy of cancer utilizing a recombinant adenovirus to elevate systemic endostatin levels in mice." Cancer Res **60**(6): 1503-1506.

Gene therapy represents a possible alternative to the chronic delivery of recombinant antiangiogenic proteins to cancer patients. Inducing normal host tissues to produce high circulating levels of these proteins may be more effective than targeting antiangiogenic genes to tumor tissue specifically. Previously reported gene therapy approaches in mice have achieved peak circulating endostatin levels of 8-33 ng/ml. Here we report plasma endostatin levels of 1770 ng/ml after administration of a recombinant adenovirus. Growth of MC38 adenocarcinoma, which is relatively resistant to adenoviral infection, was inhibited by 40%. These findings encourage gene delivery approaches that use the host as a "factory" to produce high circulating levels of antiangiogenic agents.

Feldman, R. J., et al. (2003). "Pdef expression in human breast cancer is correlated with invasive potential and altered gene expression." Cancer Res **63**(15): 4626-4631.

Ets transcription factors control multiple biological processes, including cell proliferation, differentiation, apoptosis, angiogenesis, transformation, and invasion. Pdef is an Ets transcription factor originally identified in prostate tissue. We demonstrate that human Pdef is expressed at high levels primarily in tissues with high epithelial cell content, including prostate, colon, and breast. We also determined that Pdef protein is reduced in human invasive breast cancer and is absent in invasive breast cancer cell lines. We next assessed the functional consequences of these observations. Significantly, expression of Pdef in breast cancer cells leads to inhibition of invasion, migration, and growth. Expression of Pdef also results in the down-regulation of urokinase-type plasminogen activator and activation of the promoter of the tumor suppressor gene, MASPIN: Growth-suppressive effects of Pdef expression are mediated in part by a G (0)-G (1) cell cycle arrest associated with elevated p21 levels. Collectively, these results indicate that Pdef loss may alter the expression of genes controlling progression to invasive breast cancer.

Filmus, J., et al. (1985). "MDA-468, a human breast cancer cell line with a high number of epidermal growth factor (EGF) receptors, has an amplified EGF receptor gene and is growth inhibited by EGF." Biochem Biophys Res Commun **128**(2): 898-905.

Epidermal growth factor (EGF) has been noted to stimulate proliferation of a variety of normal and malignant cells including those of human breast epithelium. We report here that MDA-468, a human breast cancer cell line with a very high number of EGF receptors, is growth-inhibited at EGF concentrations that stimulate most other cells. The basis for the elevated receptor level is EGF receptor gene amplification and over-expression. An MDA-468 clone selected for resistance to EGF-induced growth inhibition shows a number of receptors within the normal range. The results are discussed in relation to a threshold model for EGF-induced growth inhibition.

Filmus, J., et al. (1987). "Epidermal growth factor receptor gene-amplified MDA-468 breast cancer cell line and its nonamplified variants." Mol Cell Biol **7**(1): 251-257.

We have recently reported (J. Filmus, M. N. Pollak, R. Cailleau, and R. N. Buick, Biochem. Biophys. Res. Commun. 128:898-905, 1985) that MDA-468, a human breast cancer cell line with a high number of epidermal growth factor (EGF) receptors, has an amplified EGF receptor gene and is growth inhibited in vitro pharmacological doses of EGF. We have derived several MDA-468 clonal variants which are resistant to EGF-induced growth inhibition. These clones had a number of EGF receptors, similar to normal human fibroblasts, and had lost the EGF receptor gene amplification. Karyotype analysis showed that MDA-468 cells had an abnormally banded region (ABR) in chromosome 7p which was not present in the variants. It was shown by in situ hybridization that the amplified EGF receptor sequences were located in that chromosome, 7pABR. Five of the six variants studied were able to generate tumors in nude mice, but their growth rate was

significantly lower than that of tumors derived from the parental cell line. The variant that was unable to produce tumors was found to be uniquely dependent on EGF for growth in soft agar.

Fisher, W. E., et al. (1996). "Expression of the somatostatin receptor subtype-2 gene predicts response of human pancreatic cancer to octreotide." Surgery **120**(2): 234-240; discussion 240-231.

BACKGROUND: Somatostatin inhibits proliferation of many solid tumors. The current study examines whether inhibition of the growth of pancreatic cancer by the somatostatin analog, octreotide, requires tumor expression of somatostatin receptors. METHODS: We studied five human pancreatic cancer cell lines, Capan-1, Capan-2, CAV, MIA PaCa-2, and Panc-1. Solid tumors were established in nude mice (n = 20/cell line) by flank injection of tumor cells. Subcutaneous octreotide (500 micrograms/kg/day) was administered by osmotic pumps to 10 of the animals in each group, and the other 10 received control infusions of saline solution. On day 36, the tumors were excised and weighed. Plasma levels of the putative trophic peptides cholecystokinin, epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1), and insulin were assessed by radioimmunoassay. Each of the five cell lines was assayed for the presence of cell surface somatostatin receptors by using whole cell competitive binding assays with 125I-somatostatin. Expression of the somatostatin receptor subtype-2 (SSR2) gene was determined with reverse transcriptase-polymerase chain reactions. Southern blot hybridization was used to assess the presence of the SSR2 gene. RESULTS: Octreotide inhibited tumor growth in the MIA PaCa-2 group (512 +/- 75 mg control versus 285 +/- 71 mg treated; $p < 0.05$) but had no significant effect on tumor weight in the other four cell lines. Plasma levels of cholecystokinin, epidermal growth factor, insulin-like growth factor-1, and insulin were not altered by chronic octreotide infusion. Cell surface somatostatin receptors and SSR2 gene expression were detected only in the MIA PaCa-2 tumors. The gene for the SSR2 receptor was found in all five tumor lines. CONCLUSIONS: Octreotide-mediated inhibition of pancreatic cancer growth is dependent on expression of somatostatin receptors. The expression of somatostatin receptors should be considered in the design and interpretation of clinical trials with somatostatin analogs for treatment of pancreatic cancer.

Fisher, W. E., et al. (2002). "Somatostatin receptor subtype 2 gene therapy inhibits pancreatic cancer in vitro." J Surg Res **105**(1): 58-64.

BACKGROUND: Most human pancreatic adenocarcinoma cells do not express somatostatin receptors and somatostatin does not inhibit the growth of these cancers. We have demonstrated previously that somatostatin inhibits the growth of pancreatic cancers expressing somatostatin receptor subtype 2 (SSR2) but not receptor-negative cancers. SSR2 expression may be an important tumor suppressor pathway that is lost in human pancreatic cancer. We hypothesized that SSR2 gene transfer would restore the growth inhibitory response of human pancreatic cancer to somatostatin. METHODS: We created adenoviral constructs containing the SSR2 or Lac-Z gene and transfected somatostatin receptor-negative human pancreatic cancer cells (Panc-1). Presence of functional cell surface SSR2 protein was assessed by whole-cell competitive binding assays. Parental cells, Lac-Z-transfected, and SSR2-transfected cells were cultured in the presence and absence of somatostatin. The rate of cell growth was determined by direct cell counting using a hemacytometer (n = 8 wells/group). Cells were analyzed for expression of tumor suppressor proteins by Western blot. RESULTS: Panc-1 cells transfected with the SSR2 transgene demonstrated high-affinity specific binding of (125)I-somatostatin at physiologic concentrations. Expression of somatostatin receptors caused 60% inhibition of cell growth compared with the Lac-Z virus-treated controls ($P < 0.05$ by Kruskal-Wallis/Bonferroni). There was no additional inhibition of cell proliferation with exogenous somatostatin. Furthermore, addition of somatostatin ligand antibody did not diminish the effect of SSR2 expression on cell proliferation. Western blot analysis revealed an upregulation of the cyclin-dependent kinase inhibitor p27 in the SSR2-transfected cells. CONCLUSIONS: Expression of SSR2 by human pancreatic cancer causes significant slowing of cell division by a mechanism independent of somatostatin. The mechanism may involve upregulation of known tumor suppressor proteins. Restoration of SSR2 gene expression deserves further study as a potential gene therapy strategy in human pancreatic cancer.

Forsti, A., et al. (2003). "Polymorphisms in the estrogen receptor beta gene and risk of breast cancer: no association." Breast Cancer Res Treat **79**(3): 409-413.

Polymorphisms in the estrogen receptor beta (ERbeta) gene may influence the cellular growth regulating effects of estradiol. In this first association study about breast cancer risk and polymorphisms in the ERbeta gene we have screened 219 Finnish sporadic breast cancer cases and 248 ethnically matched male controls. No difference in the allele

distribution of the six studied polymorphisms was found between the breast cancer and control groups.

Franco, O. E., et al. (2003). "Phenylacetate inhibits growth and modulates cell cycle gene expression in renal cancer cell lines." Anticancer Res **23**(2B): 1637-1642.

BACKGROUND: Phenylacetate (PA), an aromatic fatty acid, is now undergoing evaluation as a potential anticancer reagent. Our previous study showed that PA induces cell growth inhibition in prostate cancer cells. Here, we investigated whether PA is effective against three renal cancer cell lines in vitro. MATERIALS AND METHODS: The cell viability of PA-treated renal carcinoma cell lines (Caki-1, Os-RC-2 and RCC10) was assessed by trypan-blue exclusion and cell cycle distribution by flow cytometry. The cell cycle-regulatory protein expression was evaluated by Western blot, immunoprecipitation and kinase assay. RESULTS: Growth inhibition occurred with PA treatment at a dose of 2-5 mM and an increased percentage of cells in G1 after 24 hours of exposure. Reduced phosphorylation of the retinoblastoma protein (Rb) and CDK2 activity, increased expression of p21Cip1 and enhanced binding of p21Cip1 to CDK2 were observed following treatment with PA. CONCLUSION: Overall, these results suggest that p21Cip1 is a critical target in PA-mediated cell growth inhibition in RCC cells playing a key role in CDK2 inactivation, hypophosphorylation of pRb and subsequent G1 cell cycle arrest.

Frandsen, T. L., et al. (2001). "Direct evidence of the importance of stromal urokinase plasminogen activator (uPA) in the growth of an experimental human breast cancer using a combined uPA gene-disrupted and immunodeficient xenograft model." Cancer Res **61**(2): 532-537.

Several studies have indicated an interaction between tumor cells and infiltrating stromal cells regarding the urokinase plasminogen activation (uPA) system. By developing combined uPA gene-disrupted and immunodeficient mice, we have studied the role of stromal uPA for the growth of the MDA-MB-435 BAG human tumor xenograft. Subcutaneous tumor growth and lung metastasis were compared between wild-type immunodeficient mice and mice with the combined deficiencies. Tumor growth was evaluated by volume measurements and plasma beta-galactosidase activity and metastasis was evaluated by counting lung surface metastases. Although no differences appeared in primary tumor take between the two groups of mice, a significant difference was observed in primary tumor growth, with tumors in uPA-/- mice growing significantly more slowly. In addition, a nonsignificant trend toward fewer lung metastases in uPA-/- mice was observed. The present data points to a critical role of stromal-derived uPA in the primary tumor growth of MDA-MB-435 BAG xenografts, whereas only a trend toward fewer lung metastases in uPA gene-disrupted mice was found.

Frasor, J., et al. (2003). "Profiling of estrogen up- and down-regulated gene expression in human breast cancer cells: insights into gene networks and pathways underlying estrogenic control of proliferation and cell phenotype." Endocrinology **144**(10): 4562-4574.

Estrogens are known to regulate the proliferation of breast cancer cells and to alter their cytoarchitectural and phenotypic properties, but the gene networks and pathways by which estrogenic hormones regulate these events are only partially understood. We used global gene expression profiling by Affymetrix GeneChip microarray analysis, with quantitative PCR verification in many cases, to identify patterns and time courses of genes that are either stimulated or inhibited by estradiol (E2) in estrogen receptor (ER)-positive MCF-7 human breast cancer cells. Of the >12,000 genes queried, over 400 showed a robust pattern of regulation, and, notably, the majority (70%) were down-regulated. We observed a general up-regulation of positive proliferation regulators, including survivin, multiple growth factors, genes involved in cell cycle progression, and regulatory factor-receptor loops, and the down-regulation of transcriptional repressors, such as Mad4 and JunB, and of antiproliferative and proapoptotic genes, including B cell translocation gene-1 and -2, cyclin G2, BCL-2 antagonist/killer 1, BCL 2-interacting killer, caspase 9, and TGFbeta family growth inhibitory factors. These together likely contribute to the stimulation of proliferation and the suppression of apoptosis by E2 in these cells. Of interest, E2 appeared to modulate its own activity through the enhanced expression of genes involved in prostaglandin E production and signaling, which could lead to an increase in aromatase expression and E2 production, as well as the decreased expression of several nuclear receptor coactivators that could impact ER activity. Our studies highlight the diverse gene networks and metabolic and cell regulatory pathways through which this hormone operates to achieve its widespread effects on breast cancer cells.

Frebourg, T., et al. (1992). "Germ-line mutations of the p53 tumor suppressor gene in patients with high risk for cancer inactivate the p53 protein." Proc Natl Acad Sci U S A **89**(14): 6413-6417.

Germ-line mutations in the p53 tumor suppressor gene have been observed in patients with Li-Fraumeni syndrome, brain tumors, second malignancies, and

breast cancers. It is unclear whether all of these mutations have inactivated p53 and thereby provide an increased risk for cancer. Therefore, it is necessary to establish the biological significance of these germ-line mutations by the functional and structural analysis of the resulting mutant p53 proteins. We analyzed the ability of seven germ-line mutant proteins observed in patients with Li-Fraumeni syndrome, second primary neoplasms, or familial breast cancer to block the growth of malignant cells and compared the structural properties of the mutant proteins to that of the wild-type protein. Six of seven missense mutations disrupted the growth inhibitory properties and structure of the wild-type protein. One germ-line mutation retained the features of the wild-type p53. Genetic analysis of the breast cancer family in which this mutation was observed indicated that this germ-line mutation was not associated with the development of cancer. These results demonstrate that germ-line p53 mutations observed in patients with Li-Fraumeni syndrome and with second malignancies have inactivated the p53 tumor suppressor gene. The inability of the germ-line p53 mutants to block the growth of malignant cells can explain why patients with these germ-line mutations have an increased risk for cancer. The observation of a functionally silent germ-line mutation indicates that, before associating a germ-line tumor suppressor gene mutation with cancer risk, it is prudent to consider its functional significance.

Fredlund, E., et al. (2012). "The gene expression landscape of breast cancer is shaped by tumor protein p53 status and epithelial-mesenchymal transition." Breast Cancer Res **14**(4): R113.

INTRODUCTION: Gene expression data derived from clinical cancer specimens provide an opportunity to characterize cancer-specific transcriptional programs. Here, we present an analysis delineating a correlation-based gene expression landscape of breast cancer that identifies modules with strong associations to breast cancer-specific and general tumor biology. METHODS: Modules of highly connected genes were extracted from a gene co-expression network that was constructed based on Pearson correlation, and module activities were then calculated using a pathway activity score. Functional annotations of modules were experimentally validated with an siRNA cell spot microarray system using the KPL-4 breast cancer cell line, and by using gene expression data from functional studies. Modules were derived using gene expression data representing 1,608 breast cancer samples and validated in data sets representing 971 independent breast cancer samples as well as 1,231 samples from other cancer forms. RESULTS: The initial co-expression network analysis resulted in the characterization of eight tightly regulated gene modules. Cell cycle genes were divided into two transcriptional programs, and experimental validation using an siRNA screen showed different functional roles for these programs during proliferation. The division of the two programs was found to act as a marker for tumor protein p53 (TP53) gene status in luminal breast cancer, with the two programs being separated only in luminal tumors with functional p53 (encoded by TP53). Moreover, a module containing fibroblast and stroma-related genes was highly expressed in fibroblasts, but was also up-regulated by overexpression of epithelial-mesenchymal transition factors such as transforming growth factor beta 1 (TGF-beta1) and Snail in immortalized human mammary epithelial cells. Strikingly, the stroma transcriptional program related to less malignant tumors for luminal disease and aggressive lymph node positive disease among basal-like tumors. CONCLUSIONS: We have derived a robust gene expression landscape of breast cancer that reflects known subtypes as well as heterogeneity within these subtypes. By applying the modules to TP53-mutated samples we shed light on the biological consequences of non-functional p53 in otherwise low-proliferating luminal breast cancer. Furthermore, as in the case of the stroma module, we show that the biological and clinical interpretation of a set of co-regulated genes is subtype-dependent.

Fromigue, O., et al. (2003). "Gene expression profiling of normal human pulmonary fibroblasts following coculture with non-small-cell lung cancer cells reveals alterations related to matrix degradation, angiogenesis, cell growth and survival." Oncogene **22**(52): 8487-8497.

Increasing evidence supports a major role for the microenvironment in carcinoma formation and progression. The influence of the stroma is partly mediated by signalling between epithelial tumor cells and neighboring fibroblasts. However, the molecular mechanisms underlying these interactions are largely unknown. To mimic the initial steps of invasive carcinoma in which tumor cells come in contact with normal stromal cells, we used a coculture model of non-small-cell lung cancer tumor cells and normal pulmonary fibroblasts. Using DNA filter arrays, we first analysed the overall modification of gene expression profile after a 24 h period of coculture. Next, we focused our interest on the transcriptome of the purified fibroblastic fraction of coculture using both DNA filter arrays and a laboratory-made DNA microarray. These experiments allowed the identification of a set of modulated genes coding for growth and survival factors, angiogenic factors, proteases and protease inhibitors, transmembrane receptors, kinases and transcription regulators that can

potentially affect the regulation of matrix degradation, angiogenesis, invasion, cell growth and survival. This study represents to our knowledge the first attempt to dissect early global gene transcription occurring in a tumor-stroma coculture model and should help to understand better some of the molecular mechanisms involved in heterotypic signalling between epithelial tumor cells and fibroblasts.

Fukushima, M., et al. (2007). "Combination of non-viral connexin 43 gene therapy and docetaxel inhibits the growth of human prostate cancer in mice." Int J Oncol **30**(1): 225-231.

Docetaxel (DTX) is used for the treatment of advanced hormone refractory prostate cancer. Connexin 43 (Cx43) is a tumor suppressor gene, and transfection of the Cx43 gene increases sensitivity to several chemotherapeutic agents. The objective of this study was to evaluate the effectiveness of combination therapy of Cx43-expressing plasmid DNA (pCMV-Cx43) and DTX both in vitro and in vivo using a non-viral vector in human prostate cancer PC-3 cells. Transfection of pCMV-Cx43 into the cells neither inhibited tumor growth nor increased gap junctional intercellular communication; however, combination therapy of pCMV-Cx43 and DTX significantly inhibited cell growth. Forced expression of Cx43 in the cells induced apoptotic cells by down-regulation of Bcl-2 expression and significantly more up-regulation of caspase-3 activity than either treatment alone. The combination of repeated intratumoral injection of pCMV-Cx43 (10 microg/tumor) with non-viral vector and a single intravenous injection of DTX (15 mg/kg) was compared with a repeated injection of Cx43 alone and a single injection of DTX alone on PC-3 tumor xenografts. Significant antitumoral effects were observed in mice receiving combined treatment, compared with DTX alone. The data presented here provide a rational strategy for treating patients with advanced hormone refractory prostate cancer.

Gao, A. C., et al. (2000). "Enhanced GBX2 expression stimulates growth of human prostate cancer cells via transcriptional up-regulation of the interleukin 6 gene." Clin Cancer Res **6**(2): 493-497.

Previous studies demonstrated that the GBX2 homeobox gene is consistently overexpressed in cultured human prostate cancer cell lines. In this study, the human GBX2 cDNA was cloned and a quantitative reverse transcription-PCR method used to demonstrate that GBX2 mRNA expression is enhanced in approximately 70% of human prostate cancer tissues compared with normal human prostate tissues. Purified recombinant GBX2 protein binds specifically to an ATTA motif within the promoter of the interleukin 6 (IL-6) gene. Using an antisense approach, down-regulation of the expression of GBX2 correlated with decreased expression of IL-6 and an inhibition of tumorigenicity of PC3 human prostate cancer cells. In addition, in vitro growth of the antisense clones was partially restored by exogenous addition of recombinant IL-6 protein to the culture media. These data demonstrated that enhanced GBX2 expression results in a stimulation of malignant growth of prostate cancer cells and that part of this stimulation involves up-regulation in the transcription of the IL-6 gene.

Gao, N., et al. (2001). "[Transfection of wild-type p14ARF gene leads to growth inhibition of human lung cancer cell lines]." Zhongguo Fei Ai Za Zhi **4**(1): 15-19.

BACKGROUND: To examine whether wild-type p14ARF gene is a candidate suppressor gene for lung cancer. METHODS: Human lung cancer cell lines having various endogenous backgrounds in INK4a, p53 and Rb genes were used as the recipients of the wild-type p14ARF gene. The expression of p14ARF mRNA and protein was detected with RT-PCR, immunohistochemistry and Western blot after G418 selection. Clones which expressed both p14ARF mRNA and protein were identified and selected for further experiements. By comparing with the parental and negative control cells treated with empty vectors, the effects of exogenously transfected p14ARF on cell division rate, cell cycle distribution and morphologic alteration were analyzed. In vivo evaluation of the growth rate was also made with the experiment of nude mice tumor formation. RESULTS: Upon transfection with p14ARF gene, cells were arrested at G1 or G1/G2 phase of cell cycle in three wtp53 lung cancer cell lines and their proliferation rates were also inhibited. CONCLUSIONS: Human wild-type p14ARF gene has suppressive effect on abnormal proliferation of lung cancer cells, especially in some wtp53 lung cancer cells, and it might be an ideal candidate for gene therapy of human lung cancer.

Gao, N., et al. (2001). "The exogenous wild-type p14ARF gene induces growth arrest and promotes radiosensitivity in human lung cancer cell lines." J Cancer Res Clin Oncol **127**(6): 359-367.

The cyclin-dependent kinase inhibitor p16INK4a encoded by the INK4A/CDKN2A/MTS1 gene is a frequent target of 9p21 inactivation in human lung cancers. The p14ARF transcript, which is an alternative spliced form of this locus, is also altered or deleted in a proportion of human lung cancers and has been shown to inhibit cell cycle progression as an endogenous cellular regulator of the p53 protein, raising the possibility that it might constitute an additional lung tumor suppressor gene at the 9p21 locus. To test the candidacy of p14ARF as a lung

cancer suppressor and assess the role it plays in radiosensitivity, we transfected the wild-type p14ARF gene into four cell lines which had various endogenous gene backgrounds of INK4A-/p53+/RB+ (A549 and H460), INK4A+/p53+/RB- (H446) as well as p14ARF+/p53-/RB+ (Calu-1). We found that transfection of p14ARF is related to an obvious growth inhibition in all wtp53 cell lines, regardless of INK4A/ARF and RB status. Although it has been shown that p53-induced G1 checkpoint in response to DNA damage by ionizing radiation is p14ARF-independent, we found the radiosensitivity of two p14ARF-deficient cell lines was increased after p14ARF gene transfer. The results indicated that cell cycle redistribution after acquiring the exogenous gene might be the main explanation for the enhanced sensitization. An increased radiation-induced apoptotic proportion in one cell line also suggested a fortified p53 function that might be triggered by the restored p14ARF protein.

Garcia-Olmo, D. C., et al. (2008). "Loss of a reporter gene for green fluorescent protein during tumor progression suggests the recruitment of host cells in rats with experimentally induced colon cancer." Histol Histopathol **23**(10): 1205-1211.

The interactions between a host's normal cells and tumor cells appear to be of significant importance during the development of tumors. In the present study, we examined this issue using a cancer model in vivo in which tumor cells were tagged with a reporter gene for green fluorescent protein (GFP). We used a model of colon cancer in immunocompetent rats, which were given a subcutaneous injection of tumor cells that had been transfected with a gene for GFP. We found that the number of fluorescent cells decreased with the progression of the primary tumors and that lymph node and lung metastases were never macroscopically fluorescent. No GFP-encoding sequences were detected by PCR in many of the long-term primary tumors, in most lymph node metastases (86%) and in all lung metastases, whereas the detection of mutated k-ras, which identified such cells as tumor cells, was always positive. To explain these findings, we present a brief review of the literature and postulate that tumor growth did not occur exclusively as a result of the division of the injected cells, but also involved recruitment of host cells.

Gattoni-Celli, S., et al. (1988). "Partial suppression of anchorage-independent growth and tumorigenicity in immunodeficient mice by transfection of the H-2 class I gene H-2Ld into a human colon cancer cell line (HCT)." Proc Natl Acad Sci U S A **85**(22): 8543-8547.

Many human tumors, particularly those of epithelial origin, appear to express greatly reduced levels of major histocompatibility complex class I antigens on their surface. It has been previously reported that the class I gene H-2Ld, introduced into adenovirus type 12-transformed mouse cells, induces reversal of oncogenesis in immunocompetent BALB/c mice. We have tested the hypothesis that the H-2Ld gene, when transfected into HCT colon cancer cells, may alter their transformed phenotype. Two H-2Ld transfectants, HCT-Ii and HCT-If, were found to exhibit a markedly reduced-to-virtually suppressed ability to form colonies in soft agar in comparison to a transfectant (HCTh) carrying only the neomycin-resistance gene. We also compared the tumorigenicity of HCTh vs. HCT-If cells in two different strains of immunodeficient mice: nude (T-) and triple-deficient mutants (T-, NK-, B-). At 28 days postinjection of 10(7) and 10(6) cells, the size and growth rate of HCT-If tumors were greatly reduced compared to HCTh cells. Therefore, as assayed in immunodeficient animals, expression of the class I H-2Ld gene in HCT cells appears to correlate with partial suppression of the tumorigenic phenotype, suggesting that the expression of a transfected class I gene may by itself alter the phenotype of the recipient cell and that such phenotypic changes may be independent of the immune system.

Ge, Y., et al. (2002). "Effects of adenoviral gene transfer of C. elegans n-3 fatty acid desaturase on the lipid profile and growth of human breast cancer cells." Anticancer Res **22**(2A): 537-543.

BACKGROUND: Current evidence from both experimental and human studies indicates that omega-6 polyunsaturated fatty acids (n-6 PUFAs) promote breast tumor development, whereas long-chain n-3 polyunsaturated fatty acids (n-3 PUFAs) exert suppressive effects. The ratio of n-6 to n-3 fatty acids appears to be an important factor in controlling tumor development. Human cells usually have a very high n-6/n-3 fatty acid ratio because they cannot convert n-6 PUFAs to n-3 PUFAs due to lack of an n-3 desaturase found in C. elegans. MATERIALS AND METHODS: Adenoviral strategies were used to introduce the C. elegans fat-1 gene encoding an n-3 fatty acid desaturase into human breast cancer cells followed by examination of the n-6/n-3 fatty acid ratio and growth of the cells. RESULTS: Infection of MCF-7 cells with an adenovirus carrying the fat-1 gene resulted in a high expression of the n-3 fatty acid desaturase. Lipid analysis indicated a remarkable increase in the levels of n-3 PUFAs accompanied with a large decrease in the contents of n-6 PUFAs, leading to a change of the n-6/n-3 ratio from 12.0 to 0.8. Accordingly, production of the eicosanoids derived from n-6 PUFA was

reduced significantly in cells expressing the fat-1 gene. Importantly, the gene transfer induced mass cell death and inhibited cell proliferation. CONCLUSION: The gene transfer of the n-3 fatty acid desaturase, as a novel approach, can effectively modify the n-6/n-3 fatty acid ratio of human tumor cells and provide an anticancer effect, without the need of exogenous n-3 PUFA supplementation. These data also increase the understanding of the effects of n-3 fatty acids and the n-6/n-3 ratio on cancer prevention and treatment.

Geisen, C., et al. (2000). "Growth inhibition of cervical cancer cells by the human retinoic acid receptor beta gene." Int J Cancer **85**(2): 289-295.

Transcription of the retinoic receptor beta (RARbeta) gene is activated in a ligand-dependent manner by the retinoic acid receptor alpha. Reduced RARbeta gene expression and loss of ligand inducibility are frequently observed in human carcinoma cells indicating that such alterations might contribute to carcinogenesis. In this study we have analyzed the influence of RARbeta on cervical cancer cell growth. Transfection of HeLa cells with RARbeta expression plasmids resulted in reduced clonal cell growth in the presence of retinoic acid (RA). RA-induced growth inhibition in HeLa x fibroblast hybrid cells was partially relieved by a dominant-negative RARbeta mutant. HeLa clones stably expressing a RARbeta transgene under control of the human beta-actin promoter [HeLa (RARbeta)] were established and analyzed for transgene-mediated growth alterations in vitro and in vivo. Anchorage-independent growth of the HeLa (RARbeta) lines was indistinguishable from that of control cells in the absence of RA, but strongly impaired after RA treatment. Reduced tumor growth of HeLa (RARbeta) clones was associated with high RARbeta protein levels. Somatic cell fusion experiments revealed that the loss of ligand inducibility of RARbeta gene expression in HeLa cells cannot be complemented by fusion with other cervical cancer cell lines. Our data indicate, firstly, that RARbeta is a negative regulator of tumor cell growth and, secondly, that cancer-associated defects in RARbeta gene expression are caused by stable, non-complementable silencing mechanisms.

Gery, S., et al. (2006). "The circadian gene per1 plays an important role in cell growth and DNA damage control in human cancer cells." Mol Cell **22**(3): 375-382.

The Per1 gene is a core clock factor that plays an essential role in generating circadian rhythms. Recent data reveal that major biological pathways, including those critical to cell division, are under circadian control. We report here that Per1 provides an important link between the circadian system and the cell cycle system. Overexpression of Per1 sensitized human cancer cells to DNA damage-induced apoptosis; in contrast, inhibition of Per1 in similarly treated cells blunted apoptosis. The apoptotic phenotype was associated with altered expression of key cell cycle regulators. In addition, Per1 interacted with the checkpoint proteins ATM and Chk2. Ectopic expression of Per1 in human cancer cell lines led to significant growth reduction. Finally, Per1 levels were reduced in human cancer patient samples. Our results highlight the importance of circadian regulation to fundamental cellular functions and support the hypothesis that disruption of core clock genes may lead to cancer development.

Gery, S., et al. (2002). "TMEFF2 is an androgen-regulated gene exhibiting antiproliferative effects in prostate cancer cells." Oncogene **21**(31): 4739-4746.

We have identified a gene that is highly expressed in the androgen-dependent prostate cancer cell line, LNCaP. Sequence analysis revealed that it was identical to a recently cloned gene designated TMEFF2, which encodes a transmembrane protein containing an epidermal growth factor (EGF)-like motif and two follistatin domains. This gene was highly expressed only in primary samples of normal prostate and prostate cancer as well as normal brain. Expression of the gene was controlled by androgen as shown by dihydrotestosterone markedly increasing TMEFF2 expression in LNCaP cells. Also, androgen-dependent human prostate cancer xenografts (CWR22) expressed high levels of TMEFF2 and these levels markedly decreased by day 10 after castration of the mice. Furthermore, a large number of androgen-dependent xenografts (CWR22, LuCaP-35, LAPC-4AD, LAPC-9AD) exhibited higher levels of TMEFF2 mRNA than androgen-independent xenografts (CWR22R, LAPC-3AI, LAPC-4AI, LAPC-9AI). Ectopic expression of TMEFF2 in DU145 and PC3 cells resulted in their prominent inhibition of growth. Taken together, the results demonstrate that TMEFF2 is a androgen-regulated gene, which can suppress growth of prostate cancer cells and our xenograft data show that escape of prostate cancer cells from androgen modulation causes them to decrease their expression of this gene, which may result in their more malignant behavior.

Glondu, M., et al. (2002). "Down-regulation of cathepsin-D expression by antisense gene transfer inhibits tumor growth and experimental lung metastasis of human breast cancer cells." Oncogene **21**(33): 5127-5134.

Overexpression of cathepsin-D in primary breast cancer has been associated with rapid development of

clinical metastasis. To investigate the role of this protease in breast cancer growth and progression to metastasis, we stably transfected a highly metastatic human breast cancer cell line, MDA-MB-231, with a plasmid containing either the full-length cDNA for cathepsin-D or a 535 bp antisense cathepsin-D cDNA fragment. Clones expressing antisense cathepsin-D cDNA that exhibited a 70-80% reduction in cathepsin-D protein, both intra- and extracellularly compared to controls, were selected for further experiments. These antisense-transfected cells displayed a reduced outgrowth rate when embedded in a Matrigel matrix, formed smaller colonies in soft agar and presented a significantly decreased tumor growth and experimental lung metastasis in nude mice compared with controls. However, manipulating the cathepsin-D level in the antisense cells has no effect on their in vitro invasiveness. These studies demonstrate that cathepsin-D enhances anchorage-independent cell proliferation and subsequently facilitates tumorigenesis and metastasis of breast cancer cells. Our overall results provide the first evidence on the essential role of cathepsin-D in breast cancer, and support the development of a new cathepsin-D-targeted therapy.

Goodison, S., et al. (2007). "Exogenous mycoplasmal p37 protein alters gene expression, growth and morphology of prostate cancer cells." Cytogenet Genome Res **118**(2-4): 204-213.

We previously showed that the Mycoplasma hyorhinis-encoded protein p37 can promote invasion of cancer cells in a dose-dependent manner, an effect that was blocked by monoclonal antibodies specific for p37. In this study, we further elucidated changes in growth, morphology and gene expression in prostate cancer cell lines when treated with exogenous p37 protein. Incubation with recombinant p37 caused significant nuclear enlargement, denoting active, anaplastic cells and increased the migratory potential of both PC-3 and DU145 cells. Microarray analysis of p37-treated and untreated cells identified eight gene expression clusters that could be broadly classified into three basic patterns. These were an increase in both cell lines, a decrease in either cell line or a cell line-specific differential trend. The most represented functional gene categories included cell cycle, signal transduction and metabolic factors. Taken together, these observations suggest that p37 potentiates the aggressiveness of prostate cancer and thus molecular events triggered by p37 maybe target for therapy.

Gope, R. and M. L. Gope (1992). "Abundance and state of phosphorylation of the retinoblastoma susceptibility gene product in human colon cancer." Mol Cell Biochem **110**(2): 123-133.

In an effort to understand the possible role of Rb in cellular growth control, we have investigated the abundance and the state of phosphorylation of Rb protein (pRb) in normal and colon tumor cell lines as well as in matched colon tumors, adenomas and adjoining normal colonic mucosa. Resting normal human fibroblast cell lines were found to have only unphosphorylated pRb and phosphorylation of pRb occurred when the cells entered G1-S phase. In general, the colon tumor tissues had at least 1.5-2.0 fold increase in the abundance of pRb and 1.5-2.5 fold increase in the percentage of its phosphorylation as compared to the corresponding normal colonic mucosa. Whereas, the adenomas had similar pRb level and its phosphorylation status as observed in the normal colonic mucosa. The actively growing tumor cell lines had approximately two fold higher total pRb than normal cell lines. Although, the percentage of phosphorylated form in growing tumor cell lines as well as normal cell lines were almost equal, it was still considerably higher than normal colonic mucosa. Moreover, DNA binding assay revealed reduced binding affinity of pRb from colon tumor cell line SW480 as compared to the normal cell line WI38. These results suggest that the abundance of pRb and its phosphorylation level may have a role in the cellular growth control in human colonic epithelium.

Gordon, E. M., et al. (2018). "Cell cycle checkpoint control: The cyclin G1/Mdm2/p53 axis emerges as a strategic target for broad-spectrum cancer gene therapy - A review of molecular mechanisms for oncologists." Mol Clin Oncol **9**(2): 115-134.

Basic research in genetics, biochemistry and cell biology has identified the executive enzymes and protein kinase activities that regulate the cell division cycle of all eukaryotic organisms, thereby elucidating the importance of site-specific protein phosphorylation events that govern cell cycle progression. Research in cancer genomics and virology has provided meaningful links to mammalian checkpoint control elements with the characterization of growth-promoting proto-oncogenes encoding c-Myc, Mdm2, cyclins A, D1 and G1, and opposing tumor suppressor proteins, such as p53, pRb, p16(INK4A) and p21(WAF1), which are commonly dysregulated in cancer. While progress has been made in identifying numerous enzymes and molecular interactions associated with cell cycle checkpoint control, the marked complexity, particularly the functional redundancy, of these cell cycle control enzymes in mammalian systems, presents a major challenge in discerning an optimal locus for therapeutic intervention in the clinical management of cancer. Recent advances in genetic engineering, functional genomics and clinical oncology converged in

identifying cyclin G1 (CCNG1 gene) as a pivotal component of a commanding cyclin G1/Mdm2/p53 axis and a strategic locus for re-establishing cell cycle control by means of therapeutic gene transfer. The purpose of the present study is to provide a focused review of cycle checkpoint control as a practicum for clinical oncologists with an interest in applied molecular medicine. The aim is to present a unifying model that: i) clarifies the function of cyclin G1 in establishing proliferative competence, overriding p53 checkpoints and advancing cell cycle progression; ii) is supported by studies of inhibitory microRNAs linking CCNG1 expression to the mechanisms of carcinogenesis and viral subversion; and iii) provides a mechanistic basis for understanding the broad-spectrum anticancer activity and single-agent efficacy observed with dominant-negative cyclin G1, whose cytocidal mechanism of action triggers programmed cell death. Clinically, the utility of companion diagnostics for cyclin G1 pathways is anticipated in the staging, prognosis and treatment of cancers, including the potential for rational combinatorial therapies.

Goto, H., et al. (2001). "Gene therapy utilizing the Cre/loxP system selectively suppresses tumor growth of disseminated carcinoembryonic antigen-producing cancer cells." Int J Cancer **94**(3): 414-419.

Recent clinical trials of cancer gene therapy have shown encouraging results for controlling localized tumors. However, to control metastatic or disseminated tumor cells, further modification of vectors is required to enhance specificity and infectivity against targets. We investigated whether utilization of the Cre recombinase (Cre)/loxP system contributes to enhanced antitumor effects together with minimal adverse reactions in specific gene therapy against disseminated carcinoembryonic antigen (CEA)-producing cancer cells in the peritoneal cavity of mice. CEA-producing cancer would be a good therapeutic target because it is found in lung, stomach and colon sites, which account for most cancers. We constructed a pair of recombinant adenoviral vectors (Ads), one of which expresses the Cre gene under the control of the CEA promoter (Ad.CEA-Cre); the other expresses the herpes simplex virus thymidine kinase (HSV-TK) gene (Ad.lox-TK), or the beta-galactosidase gene (beta-gal) by Cre (Ad.lox-beta-gal). Intraperitoneal coinjection of Ad.CEA-Cre and Ad.lox-beta-gal into mice with peritonitis carcinomatosa by CEA-producing tumor cells showed selective expression of the beta-gal gene in tumor foci. Coinfection of Ad.CEA-Cre and Ad.lox-TK followed by ganciclovir (GCV) administration significantly suppressed the total tumor weight in the peritoneal cavity of the mice to 13% of that of the untreated mice and 22% of that of the mice treated with Ad.CEA-TK/GCV, an Ad that expressed the HSV-TK gene driven by the CEA promoter alone. Moreover, treatment with Ad.CEA-Cre and Ad.lox-TK/GCV completely suppressed tumors in 4 of 10 (40%) mice without significant weight loss, although 2 of 10 mice treated with Ad.CAG-TK/GCV, an adenovirus vector that strongly but nonspecifically expressed the TK gene, died due to severe side effects including diarrhea, weight loss and liver dysfunction. These findings suggest that cell type-specific gene therapy using the Cre/loxP system is effective against disseminated cancer cells without significant side effects.

Greco, E., et al. (2002). "Retrovirus-mediated herpes simplex virus thymidine kinase gene transfer in pancreatic cancer cell lines: an incomplete antitumor effect." Pancreas **25**(2): e21-29.

INTRODUCTION: The transfer of drug-susceptible (suicide) genes to tumor cells by retroviral or adenoviral vectors is a novel approach to the treatment of human tumors. AIMS: To ascertain the antitumor effect of retroviral transduction of the pancreatic cancer cell lines MIA PaCa 2, CAPAN-1, PANC1, and PSN1 with the herpes simplex virus thymidine kinase (HSV-TK) gene. METHODOLOGY: The vector carried a neoselectable marker gene, the human interleukin-2 gene, an internal ribosome entry coding site, and the region coding HSV-TK. RESULTS: Twenty micromoles or less of ganciclovir did not modify nontransduced TK- cell growth, whereas > or =100 micromol completely inhibited TK- cell growth, indicating that this dosage is cytotoxic per se. The 4 TK- and the 4 transduced cell lines were treated daily with 0.001, 0.01, 0.1, 1, 10, and 20 micromol of ganciclovir for 13 days. CAPAN-1 cell growth was completely inhibited by 0.1 micromol of ganciclovir; higher doses were required to kill PANC1 (10 micromol) and PSN1 (20 micromol). MIA PaCa 2 cell growth decreased following a 20-micromol ganciclovir dosing. The bystander effect was great in the CAPAN-1 cell line and moderate in PANC1; no bystander effect was recorded in MIA PaCa 2 and PSN1 cell lines. CONCLUSION: Gene therapy with HSV-TK for pancreatic cancer seems effective in only a limited number of tumor-derived cell lines, and this limits its application in vivo.

Grzmil, M., et al. (2004). "Up-regulated expression of the MAT-8 gene in prostate cancer and its siRNA-mediated inhibition of expression induces a decrease in proliferation of human prostate carcinoma cells." Int J Oncol **24**(1): 97-105.

In order to analyze differential gene expression of putative prostate tumor markers we compared the

expression levels of >400 cancer-related genes using the cDNA array technique in a set of prostate tumors and matched normal prostate tissues. Up-regulated expression of mammary tumor 8 kDa protein (MAT-8), complement component C1S (C1S), ferritin heavy chain (FTH1), peptidyl-prolyl cis-trans isomerase A (PPIA), RNA-binding protein regulatory subunit DJ-1 protein (DJ-1) and vacuolar ATP synthase subunit F (ATP6V1F) was determined in prostate carcinoma and confirmed by using quantitative real-time RT-PCR analyses. Furthermore, quantitative real time RT-PCR on intact RNAs from 11 paired laser microdissected epithelial tissue samples confirmed up-regulated MAT-8 expression in 6 out of 11 prostate tumors. To determine the function of MAT-8 in vitro, human PC-3 and LNCaP prostate carcinoma cells were transfected with small interfering double-stranded RNA (siRNA) oligonucleotides against the MAT-8 gene leading to a specific down-regulation of MAT-8 expression. In addition, suppression of MAT-8 expression caused a significant decrease in cellular proliferation of both prostate cancer cell lines, whereas invasive capacity and cellular apoptosis remained unaffected. Taken together, our results indicate that the human MAT-8 gene contains the potential to serve as a prostate cancer expression marker and that MAT-8 plays an important role in cellular growth of prostate carcinomas.

Guan, X., et al. (2012). "[Effect of CDK2-AP1 gene over-expression on proliferation and cell cycle regulation of breast cancer cell line MCF-7]." Zhong Nan Da Xue Xue Bao Yi Xue Ban **37**(10): 990-996.

OBJECTIVE: To over-express cyclin-dependent kinase 2-associated protein 1 (CDK2-AP1) gene, and investigate its effect on the proliferation and cell cycle regulation in breast cancer cell line MCF-7. METHODS: CDK2-AP1 gene coding region was cloned into lentivirus vector. Lentivirus particles were infected into MCF-7 cells to upregulate the expression of CDK2-AP1 gene. The expression level of CDK2-AP1 was detected at both mRNA and protein levels by real-time PCR and Western blot. MTT assay, colony formatting assay, and flow cytometry were performed to detect the change of proliferation and cell cycle in MCF-7 cells. We examined the expression of cell cycle associated genes (CDK2, CDK4, P16Ink4A, and P21Cip1/Waf1) followed by CDK2-AP1 over-expression by Western blot. RESULTS: CDK2-AP1 gene was up-regulated significantly at both mRNA (6.94 folds) and protein level. MTT based growth curve, colony formatting assay and flow cytometry showed that CDK2-AP1 over-expression lentivirus inhibited the proliferation of MCF-7 cells with statistical difference ($P<0.05$). In addition, with CDK2-AP1 over-expression, MCF-7 cells were arrested in G1 phase accompanied by apoptosis. Western blot showed that the expression level of P21Cip1/Waf1 and P16 Ink4A was upregulated, while the expression level of CDK2 and CDK4, members of the CDK family, was downregulated. CONCLUSION: CDK2-AP1 gene plays a cancer suppressor role in breast cancer. Its function includes inhibiting the proliferation of MCF-7 cells and arresting the cell cycle in G1 phase.

Guimaraes, D. A. B., et al. (2017). "Pitaya Extracts Induce Growth Inhibition and Proapoptotic Effects on Human Cell Lines of Breast Cancer via Downregulation of Estrogen Receptor Gene Expression." Oxid Med Cell Longev **2017**: 7865073.

Breast cancer is one of the most prevalent cancers in the world and is also the leading cause of cancer death in women. The use of bioactive compounds of functional foods contributes to reduce the risk of chronic diseases, such as cancer and vascular disorders. In this study, we evaluated the antioxidant potential and the influence of pitaya extract (PE) on cell viability, colony formation, cell cycle, apoptosis, and expression of BRCA1, BRCA2, PRAB, and Eralpha in breast cancer cell lines (MCF-7 and MDA-MB-435). PE showed high antioxidant activity and high values of anthocyanins (74.65 +/- 2.18). We observed a selective decrease in cell proliferation caused by PE in MCF-7 (ER (+)) cell line. Cell cycle analysis revealed that PE induced an increase in G0/G1 phase followed by a decrease in G2/M phase. Also, PE induced apoptosis in MCF-7 (ER (+)) cell line and suppressed BRCA1, BRCA2, PRAB, and Eralpha gene expression. Finally, we also demonstrate that no effect was observed with MDA-MB-435 cells (ER (-)) after PE treatment. Taken together, the present study suggests that pitaya may have a protective effect against breast cancer.

Guo-Chang, F. and W. Chu-Tse (2000). "Transfer of p14ARF gene in drug-resistant human breast cancer MCF-7/Adr cells inhibits proliferation and reduces doxorubicin resistance." Cancer Lett **158**(2): 203-210.

The INK4a/ARF locus on human chromosome 9p21 encodes two tumor suppressors, p16INK4a and p14ARF, that restrain cell growth by affecting the functions of the retinoblastoma protein and p53, respectively. Overexpression of ARF results in cell cycle arrest in both G1 and G2. To elucidate the effect of p14ARF gene on multidrug-resistant tumor cells, we transferred a p14ARF cDNA into p53-mutated MCF-7/Adr human breast cancer cells. In this report we demonstrated for the first time that p14ARF expression was able to greatly inhibit the MCF-7/Adr cell proliferation. Furthermore, p14ARF expression

resulted in decrease of MDR-1 mRNA and P-glycoprotein production, which linked to the reducing resistance of MCF-7/Adr cells to doxorubicin. These results imply that drug resistance might be effectively reversed by the wild-type p14ARF expression in human breast cancer cells.

Guzey, M., et al. (2004). "Vitamin D3 modulated gene expression patterns in human primary normal and cancer prostate cells." J Cell Biochem **93**(2): 271-285.

The vitamin D receptor (VDR) is a member of the steroid/retinoid receptor superfamily of nuclear receptors and has potential tumor-suppressive functions in prostate and other cancer types. Vitamin D3 (VD3) exerts its biological actions by binding within cells to VDR. The VDR then interacts with specific regions of the DNA in cells, and triggers changes in the activity of genes involved in cell division, cell survival, and cellular function. Using human primary cultures and the prostate cancer (PCa) cell line, ALVA-31, we examined the effects of VD3 under different culture conditions. Complete G0/G1 arrest of ALVA-31 cells and approximately 50% inhibition of tumor stromal cell growth was observed. To determine changes in gene expression patterns related to VD3 activity, microarray analysis was performed. More than approximately 20,000 genes were evaluated for twofold relative increases and decreases in expression levels. A number of the gene targets that were up- and down-regulated are related to potential mechanisms of prostatic growth regulation. These include estrogen receptor (ER), heat shock proteins: 70 and 90, Apaf1, Her-2/neu, and paxillin. Utilizing antibodies generated against these targets, we were able to confirm the changes at the protein level. These newly reported gene expression patterns provide novel information not only potential markers, but also on the genes involved in VD3 induced apoptosis in PCa.

Hamada, K., et al. (1996). "Adenovirus-mediated transfer of a wild-type p53 gene and induction of apoptosis in cervical cancer." Cancer Res **56**(13): 3047-3054.

In most cervical cancers, the function of p53 is down regulated. To explore the potential use of p53 in gene therapy for cervical cancer, we introduced wild-type p53 into cervical cancer cell lines via a recombinant adenoviral vector, Ad5CMV-p53, and analyzed its effects on cell and tumor growth. The transduction efficiencies of all cell lines were 100% at a multiplicity of infection of 100 or greater. The p53 protein was detected in Ad5CMV-p53-infected cells. Protein expression peaked at day 3 after infection and lasted 15 days. The Ad5CMV-p53-infected cells underwent apoptosis, and cell growth was greatly suppressed. The Ad5CMV-p53 treatment significantly reduced the volumes of established s.c. tumors in vivo. These results indicate that transfection of cervical cancer cells with the wild-type p53 gene via Ad5CMV-p53 is a potential novel approach to the therapy of cervical cancer.

Hamaguchi, M., et al. (2002). "DBC2, a candidate for a tumor suppressor gene involved in breast cancer." Proc Natl Acad Sci U S A **99**(21): 13647-13652.

A previously uncharacterized gene, DBC2 (deleted in breast cancer), was cloned from a homozygously deleted region at human chromosome 8p21. DBC2 contains a highly conserved RAS domain and two putative protein interacting domains. Our analyses indicate that DBC2 is the best candidate tumor suppressor gene from this region. It lies within the epicenter of the deletions and is homozygously deleted in 3.5% (7/200) of breast tumors. Mutation analysis of DBC2 led to discovery of two instances of somatic missense mutations in breast tumor specimens, whereas no missense mutations were found in other candidates from the region. Unlike other genes in the region, expression of DBC2 is often extinguished in breast cancer cells or tissues. Moreover, our functional analysis revealed that DBC2 expression in breast cancer cells lacking DBC2 transcripts causes growth inhibition. By contrast, expression of a somatic mutant discovered in a breast cancer specimen does not suppress the growth of breast cancer cells.

Hammamieh, R., et al. (2007). "Differential effects of omega-3 and omega-6 Fatty acids on gene expression in breast cancer cells." Breast Cancer Res Treat **101**(1): 7-16.

Essential fatty acids have long been identified as possible oncogenic factors. Existing reports suggest omega-6 (omega-6) essential fatty acids (EFA) as pro-oncogenic and omega-3 (omega-3) EFA as anti-oncogenic factors. The omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), inhibit the growth of human breast cancer cells while the omega-6 fatty acids induces growth of these cells in animal models and cell lines. In order to explore likely mechanisms for the modulation of breast cancer cell growth by omega-3 and omega-6 fatty acids, we examined the effects of arachidonic acid (AA), linoleic acid (LA), EPA and DHA on human breast cancer cell lines using cDNA microarrays and quantitative polymerase chain reaction. MDA-MB-231, MDA-MB-435s, MCF-7 and HCC2218 cell lines were treated with the selected fatty acids for 6 and 24 h. Microarray analysis of gene expression profiles in the breast cancer cells treated with both classes of fatty acids discerned essential

differences among the two classes at the earlier time point. The differential effects of omega-3 and omega-6 fatty acids on the breast cancer cells were lessened at the late time point. Data mining and statistical analyses identified genes that were differentially expressed between breast cancer cells treated with omega-3 and omega-6 fatty acids. Ontological investigations have associated those genes to a broad spectrum of biological functions, including cellular nutrition, cell division, cell proliferation, metastasis and transcription factors etc., and thus presented an important pool of biomarkers for the differential effect of omega-3 and omega-6EFAs.

Han, Y., et al. (2007). "The zinc finger domain of Wilms' tumor 1 suppressor gene (WT1) behaves as a dominant negative, leading to abrogation of WT1 oncogenic potential in breast cancer cells." Breast Cancer Res **9**(4): R43.

INTRODUCTION: There is growing evidence that the Wilms' tumor 1 suppressor gene (WT1) behaves as an oncogene in some forms of breast cancer. Previous studies have demonstrated that the N-terminal domain of WT1 can act as a dominant negative through self-association. In the studies presented here we have explored the potential for the zinc finger domain (ZF) of WT1 to also have dominant-negative effects, and thus further our understanding of this protein. METHODS: Using full-length and ZF-only forms of WT1 we assessed their effect on the WT1 and c-myc promoter using luciferase and chromatin immunoprecipitation assays. The gene expression levels were determined by quantitative real-time RT-PCR, northern blot and western blot. We also assessed the effect of the ZF-only form on the growth of breast cancer cell lines in culture. RESULTS: Transfection with WT1-ZF plasmids resulted in a stronger inhibition of WT1 promoter than full-length WT1 in breast cancer cells. The WT1-ZF form lacking the lysine-threonine-serine (KTS) insert (ZF - KTS) can bind to the majority of WT1 consensus sites throughout the WT1 promoter region, while the ZF containing the insert (ZF + KTS) form only binds to sites in the proximal promoter. The abundances of endogenous WT1 mRNA and protein were markedly decreased following the stable expression of ZF - KTS in breast cancer cells. The expressions of WT1 target genes, including c-myc, Bcl-2, amphiregulin and TERT, were similarly suppressed by ZF - KTS. Moreover, WT1-ZF - KTS abrogated the transcriptional activation of c-myc mediated by all four predominant isoforms of WT1 (including or lacking alternatively spliced exons 5 and 9). Finally, WT1-ZF - KTS inhibited colony formation and cell division, but induced apoptosis in MCF-7 cells. CONCLUSION: Our observations strongly argue that the WT1-ZF plasmid behaves as a dominant-negative regulator of the endogenous WT1 in breast cancer cells. The inhibition on proliferation of breast cancer cells by WT1-ZF - KTS provides a potential candidate of gene therapy for breast cancer.

Hang, Y., et al. (2005). "Suppression of gastric cancer growth by adenovirus-mediated transfer of the PTEN gene." World J Gastroenterol **11**(15): 2224-2229.

AIM: To investigate the tumor-suppressive effect of the phosphatase and tensin homologue deleted from chromosome (PTEN) in human gastric cancer cells that were wild type for PTEN. METHODS: Adenoviruses expressing PTEN or luciferase as a control were introduced into gastric cancer cells. The effect of exogenous PTEN gene on the growth and apoptosis of gastric cancer cells that are wtPTEN were examined in vitro and in vivo. RESULTS: Adenovirus-mediated transfer of PTEN (Ad-PTEN) suppressed cell growth and induced apoptosis significantly in gastric cancer cells (MGC-803, SGC-7901) carrying wtPTEN in comparison with that in normal gastric epithelial cells (GES-1) carrying wtPTEN. This suppression was induced through downregulation of the Akt/PKB pathway, dephosphorylation of focal adhesion kinase and mitogen-activated protein kinase and cell-cycle arrest at the G2/M phase but not at the G1 phase. Furthermore, treatment of human gastric tumor xenografts (MGC-803, SGC-7901) with Ad-PTEN resulted in a significant ($P<0.01$) suppression of tumor growth. CONCLUSION: These results indicate a significant tumor-suppressive effect of Ad-PTEN against human gastric cancer cells. Thus, Ad-PTEN may be used as a potential therapeutic strategy for treatment of gastric cancers.

Hausner, P., et al. (1999). "The "comparative growth assay": examining the interplay of anti-cancer agents with cells carrying single gene alterations." Neoplasia **1**(4): 356-367.

We have developed a "comparative growth assay" that complements current assays of drug effects based on cytotoxicity. A co-culture of two cell lines, one of which is fluorescently labeled, is exposed to a cytotoxic agent and the proportion of fluorescent cells is compared with that of a baseline unexposed co-culture. For demonstration purposes, two HCT116 cell lines (an hMLH1 homozygous and an hMLH1 heterozygous mutant), altered by insertion of vector alone or the same vector carrying an insert for the expression of enhanced green fluorescent protein (EGFP), were exposed to numerous "anti-cancer" agents. The assay was further validated in a system of two cell lines differing only in the expression of the

breast cancer resistance protein (BRCP). The assay allowed the estimation of the duration of action of a particular agent. Assessment of the agent's differential activity over a given time in culture could be expressed as a selection rate, which we chose to describe on an "average selection per day" basis. We conclude that this assay: 1) provides insight into the differential dynamic effects of chemotherapeutic agents or radiation; and 2) allows, through the use of matched cell lines, the investigation of critical physiologic features that govern cell sensitivity.

Haussler, O., et al. (1999). "Cell proliferation, apoptosis, oncogene, and tumor suppressor gene status in adenosis with comparison to benign prostatic hyperplasia, prostatic intraepithelial neoplasia, and cancer." Hum Pathol **30**(9): 1077-1086.

There is scant information on the cell proliferation, apoptosis, oncogenes, and tumor suppressor genes status in adenosis. Forty-eight foci of adenosis were studied with immunohistochemistry for MIB-1; c-erbB-2, c-erbB-3, bcl-2 oncogenes; and p53. To evaluate apoptosis, the TdT dUTP nick end labeling (TUNEL) method was applied. Results were compared with the same studies on benign prostatic hyperplasia (BPH) (n = 20), low-grade prostatic intraepithelial neoplasia (PIN) (n = 10); high-grade PIN (n = 20), Gleason sum 2 to 6 cancer (n = 16); and Gleason sum 7 to 10 cancer (n = 22). MIB-1 proliferation index was lowest in BPH, followed by adenosis, low-grade prostatic intraepithelial neoplasia (PIN), low-grade cancer, high-grade PIN, and high-grade cancer. The apoptotic rate was generally low in all groups, although it was higher in PIN and cancer. In BPH and adenosis, bcl-2 was absent in luminal cells. In low- and high-grade PIN, both basal and luminal cells expressed bcl-2, whereas in cancer, expression was found in only 1 case (3%). C-erbB-2 showed absent or low values for cancer and adenosis, whereas it was commonly expressed in BPH and low- and high-grade PIN. Low expression in adenosis was also found with c-erbB-3 (6%) compared with all other groups. Expression of p53 was confined to cancer. Despite a significantly higher proliferation index rate compared with BPH, adenosis showed a markedly lower proliferating index when compared with low-grade PIN, high-grade PIN, and cancer. Expression of the oncogenes c-erbB-2 and cerbB-3 was very low in adenosis, and the staining pattern for bcl-2 was similar to that of BPH. These results provide additional evidence to that of prior studies that adenosis is a histological small acinar proliferation more akin to BPH than high-grade PIN or adenocarcinoma.

Hayashi, S., et al. (1997). "Inhibition of establishment of hepatic metastasis in mice by combination gene therapy using both herpes simplex virus-thymidine kinase and granulocyte macrophage-colony stimulating factor genes in murine colon cancer." Cancer Gene Ther **4**(6): 339-344.

Herpes simplex virus-thymidine kinase (HS-tk) gene therapy with ganciclovir (GCV) treatment has been reported to inhibit the tumor growth, which is applied to the gene therapy targeted to the malignant brain tumor. To suppress the tumor growth completely, the authors designed the HS-tk gene therapy in combination with granulocyte macrophage-colony stimulating factor (GMCSF) gene using the hepatic metastatic model of murine colon cancer. The transduction of the HS-tk gene in combination with the GMCSF gene, followed by GCV, showed a complete inhibition of hepatic metastases of murine colon cancer, which was significantly superior to that of HS-tk gene alone. The growth of cancer cells transduced with both HS-tk and GMCSF genes was inhibited in vitro, and long-lasting antitumor immunity after hepatic metastasis of cancer cells transduced with both HS-tk and GMCSF genes was acquired. It is suggested that HS-tk gene therapy in combination with the GMCSF gene is effective for the complete inhibition of hepatic metastasis of murine colon cancer.

Hayashida, Y., et al. (2005). "PPP1R3 gene (protein phosphatase 1) alterations in colorectal cancer and its relationship to metastasis." Oncol Rep **13**(6): 1223-1227.

The PPP1R3 gene is located on chromosome 7q31, and encodes protein phosphatase 1 (regulatory 3). It has been reported that the inactivation of various phosphatase proteins causes abnormalities in cell division and cell growth systems. We analyzed alterations in the PPP1R3 gene and its relationship to tumor progression and metastasis. Deletion mutants of exons containing mutations were prepared and assayed for intranuclear transcription activity. SSCP analysis of PPP1R3 showed abnormal patterns in 6 (12%) of the 50 colorectal cancers. DNA sequencing of the 6 samples showing abnormal SSCP patterns confirmed point mutations in exon 4 in 4 samples, and in exon 1 in 2 samples. PPP1R3 gene alterations correlated with lymph node and liver metastases. Enhancement of luciferase activity by the full PPP1R3 gene was confirmed. However, when point mutation-containing exon 1 or 4 deletion mutants were examined for luciferase activity, enhancement of activity was decreased in the exon 1 deletion mutants, while no enhancement of the activity was noted in the exon 4 deletion mutants. These findings suggest that protein phosphatase 1 (regulatory 3) protein is involved in intracellular processes in some colorectal cancers and may play a role in metastasis.

He, C., et al. (2002). "[Action of apoptosis-induced ligand gene in relation to tumor necrosis factor on human colon cancer cell line HT29]." Zhonghua Zhong Liu Za Zhi **24**(2): 133-136.

OBJECTIVE: To evaluate the gene therapeutic efficiency of apoptosis-inducing ligand (TRAIL) related to tumor necrosis factor on human colon cancer cell line HT29. METHODS: Human colon cancer cell line HT29 was transfected with adenovirus-mediated TRAIL gene Ad/GT-TRAIL. The morphological changes, cell growth and apoptosis were measured by phase contrast microscope, MTT method and flow cytometry. RESULTS: Obvious morphological changes in HT29 cells was induced by Ad/GT-TRAIL and Ad/PGK-GV16. The cell suppression percentage and the percentage of apoptotic cells were 54.3% and 11.1%, respectively. When used in combination with Ad/PGK-GV16, HT29 was suppressed to 82.7% and the percentage of apoptotic cells was 24.6%. This result showed significantly enhanced therapeutic efficiency on HT29 and thus inhibiting of its growth ($P < 0.05$). CONCLUSION: Ad/GT-TRAIL is able to induce apoptosis of HT29 and inhibit its growth. Ad/GT-TRAIL shows significantly enhanced therapeutic efficiency for HT29 when used in combination with Ad/PGK-GV16.

He, D., et al. (2003). "Overexpression of the promyelocytic leukemia gene suppresses growth of human bladder cancer cells by inducing G1 cell cycle arrest and apoptosis." Chin Med J (Engl) **116**(9): 1394-1398.

OBJECTIVES: To examine the anti-oncogenic effects of promyelocytic leukemia (PML) on bladder cancer and to explore its molecular mechanisms of growth suppression. METHODS: Wild-type PML was transfected into bladder cancer cells (5637 cell) and expressed in a replication-deficient adenovirus-mediated gene delivery system and introduced into human bladder cancer cells (5637 cell) in vitro and in vivo. The effect and mechanisms of the PML gene in cell growth, clonogenicity, and tumorigenicity of bladder cancer cells were studied using in vitro and in vivo growth assays, soft agar colony-forming assay, cell cycle analysis, apoptosis assay and in vivo tumorigenicity assay. RESULTS: Overexpression of PML in 5637 cells significantly reduced their growth rate and clonogenicity on soft agar. PML suppressed bladder cancer cell growth by inducing G1 cell cycle arrest and apoptosis. Adenovirus-mediated PML (Ad-PML) significantly suppressed the tumorigenicity and growth of bladder cancer cells. Intratumoral injection of Ad-PML into tumors induced by 5637 cells dramatically suppressed their growth. CONCLUSIONS: The results indicated that overexpression of PML protein may promote efficient growth inhibition of human bladder cancer cells by inducing G1 cell cycle arrest and apoptosis, and adenovirus-mediated PML (Ad-PML) expression efficiently suppresses human bladder cancer growth.

He, X. H., et al. (2003). "[Expression of human novel gene CT120 in lung cancer and its effects on cell growth]." Ai Zheng **22**(2): 113-118.

BACKGROUND & OBJECTIVE: A novel membrane-associated gene CT120 was isolated from chromosome 17p13.3 locus in our laboratory. Its mRNA was not expressed in human normal lung tissues, but was abundant in human lung cancer cell line SPC-A-1. This study was designed to investigate the differential expression patterns of CT120 in different lung cancer and noncancerous tissues using immunohistochemistry and to explore the effects of ectopic expression and overexpression of CT120 on cell growth in vitro and in vivo. METHODS: A polypeptide at the C-terminus of CT120 was selected by bioinformatics, then was synthesized and conjugated to KLH (a high molecular carrier). The chicken anti-CT120 antibody IgY was prepared with the synthesized antigen and was used to determine the different expression patterns of CT120 in various tumor cell lines and in lung cancer and noncancerous tissues. The effects of ectopic expression of CT120 on NIH/3T3 cell growth were investigated through colony formation analysis. The effect of overexpression of CT120 on the cell growth of A549 was analyzed using growth curve assay and tumor formation assay of transfected cells in nude mice. RESULTS: The novel gene CT120 expressed in various tumor cell lines and expressed remarkably higher in lung cancers than in noncancerous tissues as well as normal lung tissues. Also, it promoted the proliferation of NIH/3T3 and A549 cells in vitro and in vivo. CONCLUSION: CT120 gene may be a novel candidate gene closely related to lung carcinogenesis.

Hochscheid, R., et al. (2000). "Transfection of human insulin-like growth factor-binding protein 3 gene inhibits cell growth and tumorigenicity: a cell culture model for lung cancer." J Endocrinol **166**(3): 553-563.

IGF-I and IGF-II are potent mitogens, postulated to exert autocrine/paracrine effects on growth regulation in human lung cancer. Their proliferative effects are modulated by IGF-binding proteins (IGFBPs), which are found in conditioned medium (CM) of lung cancer cell lines. The biological role of the IGFBPs, which are ontogenetically and hormonally regulated, is not fully understood. Both inhibitory and stimulatory effects on cell growth have been demonstrated. Exogenous IGFBP-3 has been consistently shown to block IGF action, inhibiting cell

growth in vitro. In order to evaluate the action of endogenously produced IGFBP-3 on cell growth in lung cancer, we stably transfected the non-small cell lung cancer cell line NCI-H23 with human IGFBP-3 cDNA (resulting in NCI-H23 pOPI3/BP-3) or with the vector pOPI3CAT as control (resulting in NCI-H23 pOPI3CAT). RT-PCR confirmed expression of IGFBP-3-specific mRNA in NCI-H23 pOPI3/BP-3, but not in N! CI-H23 or NCI-H23 pOPI3CAT. Western ligand blot and Western immunoblot analysis of CMs yielded strong signals of the characteristic 40-44 kDa human IGFBP-3 protein in NCI-H23 pOPI3/BP-3. An IGFBP-3 ELISA demonstrated a 20-fold increase in IGFBP-3 protein expression in NCI-H23 pOPI3/BP-3 as compared with NCI-H23. The growth of NCI-H23 pOPI3/BP-3 in serum-containing medium was significantly slower (1.7-fold) than that of NCI-H23 or the vector-transfected control NCI-H23 pOPI3CAT. While the proliferation rate of parental and vector-transfected cells could be stimulated by IGF-I, IGF-II, IGF-I analog Long R (3) IGF-I or insulin, that of NCI-H23 pOPI3/BP-3 could not. Xenotransplantation in nude mice resulted in marked tumor growth after the injection of NCI-H23 or NCI-H23 pOPI3CAT, but absent or minimal growth for the IGFBP-3-transfected cell line. These data suggest that IGFBP-3 is a potent inhibitor of cell growth in human lung cancer c! ell lines and may impair tumorigenicity in vivo.

Hong, L., et al. (2004). "Suppression of the cell proliferation in stomach cancer cells by the ZNRD1 gene." Biochem Biophys Res Commun **321**(3): 611-616.

Zinc ribbon domain-containing 1 (ZNRD1), a transcription-associated gene, was recently found to be downregulated in human gastric cancer tissues as compared to the matched adjacent nonneoplastic tissues. In this study, we constructed the siRNA eukaryotic expression vectors of ZNRD1 and transfected them into normal gastric epithelial cells (GES-1). We also introduced the ZNRD1 gene into gastric cancer cells that do (SGC7901) and do not (AGS) express ZNRD1 endogenously. GES-1 cells stably transfected with the ZNRD1-RNAi were found to exhibit significantly quicker proliferation than empty vector transfectants. AGS cells stably transfected with the ZNRD1 cDNA exhibited significantly decreased growth rate as compared to control vector transfectants, whereas SGC7901 cells did not. Furthermore, ZNRD1 suppresses growth of AGS cells in soft agar and tumor formation in athymic nude mice. This study clearly demonstrates that ZNRD1 may play an important role in the control of human gastric cancer development by regulating cell proliferation. These results provide new insights into the function of ZNRD1 and further validate ZNRD1 as a potential therapeutic target in gastric cancer.

Hoshida, T., et al. (2002). "Gene therapy for pancreatic cancer using an adenovirus vector encoding soluble flt-1 vascular endothelial growth factor receptor." Pancreas **25**(2): 111-121.

INTRODUCTION: Vascular endothelial growth factor (VEGF) plays an important role in tumor angiogenesis. The soluble form of flt-1 VEGF receptor inhibits VEGF activity in a dominant-negative manner. AIM: This study demonstrated the regional tumor suppression effect of adenovirus-mediated soluble flt-1 in human pancreatic cancer cells. METHODOLOGY: The VEGF expression level was examined in nine cell lines. Panc-1 and PK-8 were used as lower- and higher-VEGF-producing cell lines, respectively. The in vitro proliferation of cancer cells infected with adenovirus vectors encoding soluble flt-1 (Adsflt) and control vectors (AdLacZ) was not different. To assess the in vivo tumor growth suppression, cancer cells were inoculated subcutaneously in SCID mice. Adsflt, AdLacZ, or vehicle was injected directly into the tumors. The early process of tumor angiogenesis in a dorsal skinfold chamber was monitored by intravital microscopy. RESULTS: In both Panc-1 cells and PK-8 cells, the tumor growth of the Adsflt-treated group was significantly suppressed. The microvessel density, revealed by CD31 immunostaining, was also significantly lower in the Adsflt-treated group. Apoptosis index was higher in the Adsflt group. Immunofluorescence staining revealed the expression of VEGF not only in cancer cells but also in tumor stromal cells. Wild-type cells and AdLacZ-infected cells prompted strong tumor angiogenesis, whereas Adsflt-infected cells failed to exert such an effect. CONCLUSION: These results indicate that antiangiogenic gene therapy using soluble flt-1 might be an effective approach for pancreatic cancer treatment.

Hoshiya, Y., et al. (2003). "Mullerian inhibiting substance promotes interferon gamma-induced gene expression and apoptosis in breast cancer cells." J Biol Chem **278**(51): 51703-51712.

This report demonstrates that in addition to interferons and cytokines, members of the TGF beta superfamily such as Mullerian inhibiting substance (MIS) and activin A also regulate IRF-1 expression. MIS induced IRF-1 expression in the mammary glands of mice in vivo and in breast cancer cells in vitro and stimulation of IRF-1 by MIS was dependent on activation of the NF kappa B pathway. In the rat mammary gland, IRF-1 expression gradually decreased during pregnancy and lactation but increased at involution. In breast cancer, the IRF-1

protein was absent in 13% of tumors tested compared with matched normal glands. Consistent with its growth suppressive activity, expression of IRF-1 in breast cancer cells induced apoptosis. Treatment of breast cancer cells with MIS and interferon gamma (IFN-gamma) co-stimulated IRF-1 and CEACAM1 expression and synergistic induction of CEACAM1 by a combination of MIS and IFN-gamma was impaired by antisense IRF-1 expression. Furthermore, a combination of IFN-gamma and MIS inhibited the growth of breast cancer cells to a greater extent than either one alone. Both reagents alone significantly decreased the fraction of cells in the S-phase of the cell cycle, an effect not enhanced when they were used in combination. However, MIS promoted IFN-gamma-induced apoptosis demonstrating a functional interaction between these two classes of signaling molecules in regulation of breast cancer cell growth.

Hosoi, F., et al. (2009). "N-myc downstream regulated gene 1/Cap43 suppresses tumor growth and angiogenesis of pancreatic cancer through attenuation of inhibitor of kappaB kinase beta expression." Cancer Res **69**(12): 4983-4991.

N-myc downstream regulated gene 1 (NDRG1)/Cap43 expression is a predictive marker of good prognosis in patients with pancreatic cancer as we reported previously. In this study, NDRG1/Cap43 decreased the expression of various chemoattractants, including CXC chemokines for inflammatory cells, and the recruitment of macrophages and neutrophils with suppression of both angiogenesis and growth in mouse xenograft models. We further found that NDRG1/Cap43 induced nuclear factor-kappaB (NF-kappaB) signaling attenuation through marked decreases in inhibitor of kappaB kinase (IKK) beta expression and IkappaBalpha phosphorylation. Decreased IKKbeta expression in cells overexpressing NDRG1/Cap43 resulted in reduction of both nuclear translocation of p65 and p50 and their binding to the NF-kappaB motif. The introduction of an exogenous IKKbeta gene restored NDRG1/Cap43-suppressed expression of melanoma growth-stimulating activity alpha/CXCL1, epithelial-derived neutrophil activating protein-78/CXCL5, interleukin-8/CXCL8 and vascular endothelial growth factor-A, accompanied by increased phosphorylation of IkappaBalpha in NDRG1/Cap43-expressing cells. In patients with pancreatic cancer, NDRG1/Cap43 expression levels were also inversely correlated with the number of infiltrating macrophages in the tumor stroma. This study suggests a novel mechanism by which NDRG1/Cap43 modulates tumor angiogenesis/growth and infiltration of macrophages/neutrophils through attenuation of NF-kappaB signaling.

Hsieh, T. C. and J. M. Wu (2001). "Cell growth and gene modulatory activities of Yunzhi (Windsor Wunxi) from mushroom Trametes versicolor in androgen-dependent and androgen-insensitive human prostate cancer cells." Int J Oncol **18**(1): 81-88.

The incidence of prostate cancer varies greatly throughout the world; it is highest in African-Americans and lowest in the Asian populations of China, India, and Japan. Geographical differences in both prevalence of latent prostate cancer and mortality have been postulated to be influenced by diverse tumor-promoting and protective factors, both environmental and dietary. Prostate cancer is a tumor with an extremely long latency; the pattern of prostate tumorigenesis, in terms of the display and sequence of appearance of particular molecular or biochemical features, or morphological changes, characterizing different stages of the carcinogenic process, is expected to be heterogeneous. Some insights into tumor heterogeneity and progression can be obtained from studies using cell lines, particularly those derived from different anatomical sites. The present study aims to investigate whether hormone-responsive LNCaP and androgen-refractory JCA-1, PC-3, and DU-145 prostate cancer cells are responsive to Yunzhi (YZ), a proprietary dietary supplement prepared from extracts of Trametes versicolor, also known as Coriolus versicolor (a mushroom consumed by Chinese for its purported health benefits), and to elucidate its mechanism of action. Ethanolic extracts (70%) of YZ significantly reduced LNCaP cell growth, down-regulated the levels of secreted PSA, but had less effects on the expression of intracellular PSA and did not affect levels of the androgen receptor. In androgen-unresponsive prostate cancer cells, YZ had a much less pronounced suppressive effect on proliferation of PC-3 and DU-145 cells, compared to LNCaP, and was inactive against JCA-1 cells. Western blot analyses show that the expression of Rb, a key regulatory protein in G1/S transition, and PCNA, integrally involved in mammalian cell DNA replication, were significantly reduced by treatment with YZ in PC-3 and DU-145 cells, respectively. In contradiction, none of these biochemical parameters were affected in JCA-1 cells under identical treatment conditions. Further analysis shows that YZ increased the levels of signal transducer and activator family of transcription factors STAT 1 and STAT 3 in JCA-1 and not LNCaP cells. The greater sensitivity of LNCaP cells to this polysaccharopeptide raises the possibility that YZ may be considered as an adjuvant therapy in the treatment of hormone responsive prostate cancer; additionally, it may have chemopreventive potential to restrict prostate tumorigenic progression from the hormone-dependent to the hormone-refractory state.

Huang, A., et al. (2014). "Circadian clock gene expression regulates cancer cell growth through glutaminase." Acta Biochim Biophys Sin (Shanghai) **46**(5): 409-414.

Glutamine is an essential amino acid for malignant tumor cells. Glutaminase that metabolizes glutamine reaches a maximum expression in tumors immediately before the maximum proliferation rate. Tumor cells grow at different rates during the day. We postulated that the activity of glutaminase in tumor cells is subject to the regulation of circadian clock gene. We measured glutaminase by western blot analysis and circadian clock gene expression by real-time polymerase chain reaction in the liver and tumor cells at six equispaced time points of the day in individual mice of a 12/12 h light/dark schedule. The results showed that the tumor-bearing mice, under normal diurnal conditions, are circadianly entrained, as reflected by the normal host locomotor activity rhythms and rhythmic liver clock gene expression. The tumors within these mice are also circadianly organized, as reflected by circadian clock gene (Bmal1) expression. What is most remarkable is that kidney-type glutaminase also showed circadian rhythms in the same pattern with tumor circadian clock gene expression in liver cancer xenograft model, indicating that conditionally inhibiting glutaminase activity may provide a new target for cancer therapy.

Huang, E. Y., et al. (2001). "Genomic structure, chromosomal localization and expression profile of a novel melanoma differentiation associated (mda-7) gene with cancer specific growth suppressing and apoptosis inducing properties." Oncogene **20**(48): 7051-7063.

Abnormalities in cellular differentiation are frequent occurrences in human cancers. Treatment of human melanoma cells with recombinant fibroblast interferon (IFN-beta) and the protein kinase C activator mezerein (MEZ) results in an irreversible loss in growth potential, suppression of tumorigenic properties and induction of terminal cell differentiation. Subtraction hybridization identified melanoma differentiation associated gene-7 (mda-7), as a gene induced during these physiological changes in human melanoma cells. Ectopic expression of mda-7 by means of a replication defective adenovirus results in growth suppression and induction of apoptosis in a broad spectrum of additional cancers, including melanoma, glioblastoma multiforme, osteosarcoma and carcinomas of the breast, cervix, colon, lung, nasopharynx and prostate. In contrast, no apparent harmful effects occur when mda-7 is expressed in normal epithelial or fibroblast cells. Human clones of mda-7 were isolated and its organization resolved in terms of intron/exon structure and chromosomal localization. Hu-mda-7 encompasses seven exons and six introns and encodes a protein with a predicted size of 23.8 kDa, consisting of 206 amino acids. Hu-mda-7 mRNA is stably expressed in the thymus, spleen and peripheral blood leukocytes. De novo mda-7 mRNA expression is also detected in human melanocytes and expression is inducible in cells of melanocyte/melanoma lineage and in certain normal and cancer cell types following treatment with a combination of IFN-beta plus MEZ. Mda-7 expression is also induced during megakaryocyte differentiation induced in human hematopoietic cells by treatment with TPA (12-O-tetradecanoyl phorbol-13-acetate). In contrast, de novo expression of mda-7 is not detected nor is it inducible by IFN-beta+MEZ in a spectrum of additional normal and cancer cells. No correlation was observed between induction of mda-7 mRNA expression and growth suppression following treatment with IFN-beta+MEZ and induction of endogenous mda-7 mRNA by combination treatment did not result in significant intracellular MDA-7 protein. Radiation hybrid mapping assigned the mda-7 gene to human chromosome 1q, at 1q 32.2 to 1q41, an area containing a cluster of genes associated with the IL-10 family of cytokines. Mda-7 represents a differentiation, growth and apoptosis associated gene with potential utility for the gene-based therapy of diverse human cancers.

Huang, H. J., et al. (1988). "Suppression of the neoplastic phenotype by replacement of the RB gene in human cancer cells." Science **242**(4885): 1563-1566.

Mutational inactivation of the retinoblastoma susceptibility (RB) gene has been proposed as a crucial step in the formation of retinoblastoma and other types of human cancer. This hypothesis was tested by introducing, via retroviral-mediated gene transfer, a cloned RB gene into retinoblastoma or osteosarcoma cells that had inactivated endogenous RB genes. Expression of the exogenous RB gene affected cell morphology, growth rate, soft agar colony formation, and tumorigenicity in nude mice. This demonstration of suppression of the neoplastic phenotype by a single gene provides direct evidence for an essential role of the RB gene in tumorigenesis.

Huang, Z., et al. (2000). "Impact of liver P450 reductase suppression on cyclophosphamide activation, pharmacokinetics and antitumoral activity in a cytochrome P450-based cancer gene therapy model." Cancer Gene Ther **7**(7): 1034-1042.

The effect of the antithyroid drug methimazole (MMI) on cytochrome P450/P450 reductase-dependent activation of the anti-cancer prodrug cyclophosphamide (CPA) was investigated in a rat model of P450 prodrug activation-based cancer gene

therapy. MMI treatment decreased the expression of hepatic P450 reductase by approximately 75% but did not alter P450 reductase levels in a 9L gliosarcoma growing in vivo as a subcutaneous solid tumor. In a pharmacokinetic study, MMI treatment significantly decreased the peak plasma concentration of the active, P450-generated metabolite 4-hydroxy-CPA, from 84.1 to 57.8 microM, and substantially prolonged its apparent half-life, from 25.4 to 54.3 minutes. The area under the plasma concentration x time curve and clearance values for 4-hydroxy-CPA were largely unchanged, however, indicating that MMI decreases the rate but not the overall extent of hepatic CPA activation. MMI alleviated some of the systemic toxicities of CPA treatment, as judged by the moderation of CPA-induced body weight loss and hematuria. The impact of MMI on CPA antitumoral activity was evaluated in rats implanted with 9L tumors transduced with P450 reductase in combination with the CPA-activating P450 2B1, which confers the capacity for intratumoral prodrug activation and leads to markedly enhanced chemosensitivity. CPA given as a single, subtherapeutic dose of 75 mg/kg resulted in a 13.8 day growth delay, whereas CPA in combination with MMI increased the growth delay to 17.4 days. By contrast, a tumor growth delay of only 3.4 days was observed in animals bearing 9L wild-type tumors given the same drug combination. We conclude that the selective reduction of liver P450 reductase after MMI treatment decreases the rate of hepatic drug activation and the host toxicity of CPA without loss of the antitumoral effect, thus increasing the therapeutic index of CPA in a P450-based cancer gene therapy model, where CPA undergoes localized drug activation at its intratumoral site of action.

Hwang, J. Y., et al. (1992). "Tamoxifen stimulates human papillomavirus type 16 gene expression and cell proliferation in a cervical cancer cell line." Cancer Res **52**(24): 6848-6852.

The widely adopted use of tamoxifen as a chemotherapeutic agent is primarily based on its inhibition of cancer cell growth. However, we report that tamoxifen at low concentrations (10(-9) and 10(-11) M) causes stimulation of cell proliferation in a cervical cancer cell line, SFR. The facts that SFR cells do not contain estrogen receptors and are estrogen nonresponsive imply the existence of an antiestrogen-specific binding protein and suggest that the effect of tamoxifen is possibly mediated through a pathway other than estrogen receptors. Tamoxifen at low concentrations stimulated human papillomavirus type 16 (HPV-16) gene transcription and E7 protein production. Levels of HPV-16 mRNA and E7 protein reached a peak at approximately 2-4 h after tamoxifen treatment, persisted for several hours, and subsequently decreased to their prestimulation levels by about 24 h after treatment. Our results indicate for the first time that tamoxifen stimulates cell proliferation of cervical cancer cells, and we suggest that the enhanced HPV-16 mRNA and E7 protein levels are probably responsible.

Hwang, R. F., et al. (1998). "Gene therapy for primary and metastatic pancreatic cancer with intraperitoneal retroviral vector bearing the wild-type p53 gene." Surgery **124**(2): 143-150; discussion 150-141.

BACKGROUND: Metastatic pancreatic cancer is uniformly fatal because no effective chemotherapy is available. Mutations in the p53 tumor suppressor gene are found in up to 70% of pancreatic adenocarcinomas. We examined the efficacy of a retroviral vector containing the wild-type p53 gene on metastatic pancreatic cancer in a nude mouse model. METHODS: Bxpc3 human pancreatic cancer cells were transduced with either a retroviral p53 vector or an LXSN empty vector. Cells were examined for incorporation of tritiated thymidine to determine the effect of p53 retroviral transduction on DNA synthesis, and a TACS2 assay for apoptosis was performed. The functional activity of p53 in transduced cells was assessed by Western blot analysis with an antibody to WAF1/p21. In vivo effects of intraperitoneal injections of the p53 vector were examined in a nude mouse model of peritoneal carcinomatosis. RESULTS: Cells treated with the p53 vector exhibited a 59% to 85.5% reduction in cell number compared with the control cells (P <.05). p53-treated cells demonstrated decreased incorporation of tritiated thymidine (12.7% +/- 0.7% vs 17.5% +/- 1.4%; P =.002), increased staining for apoptosis, and increased expression of the WAF1/p21 protein. Treatment of nude mice with the retroviral p53 vector resulted in a significant inhibition of growth of the primary pancreatic tumor, as well as the peritoneal tumor deposits, compared with the LXSN control vector. CONCLUSIONS: Intraperitoneal delivery of a retroviral p53 vector may provide a novel treatment approach for peritoneal carcinomatosis from pancreatic cancer.

Iejima, D., et al. (2010). "FRS2beta, a potential prognostic gene for non-small cell lung cancer, encodes a feedback inhibitor of EGF receptor family members by ERK binding." Oncogene **29**(21): 3087-3099.

An adaptor protein FRS2beta inhibits epidermal growth factor-receptor (EGFR) tyrosine kinase without being phosphorylated at tyrosine residues after EGF stimulation. Although binding to ERK appears to be important for this inhibition, the precise molecular mechanisms and the role of FRS2beta in signal

transduction mediated by other EGFR family members, as well as its role in human cancer, remain unclear. In this study, we demonstrate that FRS2beta inhibits anchorage-independent cell growth induced by oncogenic ErbB2, another member of EGFR family, and that it inhibits heterodimer formation between EGFR and ErbB2. We mapped the residues important for the FRS2beta and ERK interaction to two docking (D) domain-like sequences on FRS2beta and two aspartic acid residues in the common docking (CD) domain of ERK. Moreover, in response to EGF, ERK translocated to the plasma membrane in cells expressing FRS2beta but not an FRS2beta mutant in which four arginine residues in the D domains were replaced with alanines, suggesting that FRS2beta serves as a plasma membrane anchor for activated ERK. Finally, a low mRNA expression level of FRS2beta was significantly correlated with poor prognosis in a cohort of 60 non-small cell lung cancer patients. Therefore, we have identified the molecular mechanisms by which FRS2beta acts as a feedback inhibitor of EGFR family members and suggest a role for FRS2beta as a tumor suppressor.

Inaba, Y., et al. (2003). "Gene transfer of alpha1,3-fucosyltransferase increases tumor growth of the PC-3 human prostate cancer cell line through enhanced adhesion to prostatic stromal cells." Int J Cancer **107**(6): 949-957.

Elevated expression of sialyl Lewis X has been postulated to be a prognostic indicator of prostate cancer. However, direct evidence for the relationship between increased expression of sialyl Lewis X and malignancy of prostate cancer is still lacking. To determine whether increased levels of sialyl Lewis X leads to malignancy in prostate tumor, we transfected the human prostate cancer cell line PC-3 with alpha1,3-fucosyltransferase III (FTIII) to obtain stable transfectants, PC-3-FTIII lines, that highly express sialyl Lewis X. When inoculated in the prostate of nude mice, PC-3-FTIII cells produced large prostate tumors, while mock-transfected PC-3 cells, which are negative for sialyl Lewis X antigen, produced small prostate tumors. The aggressive tumor formation by PC-3-FTIII cells was inhibited by preincubation of the tumor cells with anti-sialyl Lewis X antibody, by the presence of sialyl Lewis X oligosaccharide or by selectin ligand mimic peptide but not by control peptide. PC-3-FTIII cells and mock-transfected PC-3 cells exhibited no significant difference in cell numbers when cultured in vitro. Remarkably, PC-3-FTIII adhered to prostatic stromal cells in vitro with higher affinity than mock-transfected PC-3. Such adhesion was inhibited by preincubation of PC-3-FTIII cells with antisialyl Lewis X antibody, by the addition of sialyl Lewis X oligosaccharide or by selectin ligand mimic peptide. However, anti-E-selectin, anti-P-selectin or anti-L-selectin antibodies did not inhibit the adhesion of PC-3-FTIII cells to the stromal cells. These results suggest that prostate cancer cells gain aggressiveness through adhesive interaction with prostatic stromal cells by a novel mechanism involving sialyl Lewis X.

Inoue, K., et al. (2000). "Gene therapy of human bladder cancer with adenovirus-mediated antisense basic fibroblast growth factor." Clin Cancer Res **6**(11): 4422-4431.

We previously investigated the role of basic fibroblast growth factor (bFGF) as a mediator of angiogenesis, tumorigenicity, and metastasis of transitional cell carcinoma (TCC) of the bladder. In the present study, we determined whether adenoviral-mediated antisense bFGF gene transfer therapy (Ad bFGF-AS) would inhibit TCCs growing in the subcutis of nude mice. In vitro, Ad bFGF-AS inhibited endothelial cell proliferation and enhanced apoptosis. The highly metastatic human TCC cell line 253J-BV (R) was implanted ectopically in the subcutis of athymic nude mice, and therapy was begun when the tumors reached a diameter between 5 and 7 mm. Intralesional therapy with Ad bFGF-AS decreased the in vivo expression of bFGF and matrix metalloproteinase type 9 mRNA and protein, and reduced microvessel density and enhanced endothelial cell apoptosis. Tumor growth was significantly inhibited by Ad bFGF-AS (mean, 58 mg) compared with controls [saline (mean, 562 mg), beta-galactosidase adenovirus (mean, 586 mg), and sense bFGF adenoviral therapy (Ad bFGF-S; mean, 3012 mg)]. These results suggest that Ad bFGF-AS therapy affects endothelial cells directly and tumor cells indirectly through down-regulation of bFGF and matrix metalloproteinase type 9, resulting in endothelial cell apoptosis and significant tumor growth inhibition. Furthermore, these studies confirm that bFGF expression is a valid target for the therapy of bladder cancer.

Inoue, K., et al. (2001). "Adenoviral-mediated gene therapy of human bladder cancer with antisense interleukin-8." Oncol Rep **8**(5): 955-964.

We previously demonstrated the importance of interleukin-8 (IL-8) as a mediator of angiogenesis, tumorigenicity, and metastasis of transitional cell carcinoma (TCC) of the bladder. In the present study, we evaluated the feasibility of adenoviral mediated antisense IL-8 gene transfer (Ad IL-8-AS) as therapy for established TCC. In vitro, Ad IL-8-AS inhibited endothelial cell proliferation and enhanced endothelial cell apoptosis. The highly metastatic human TCC cell line 253J B-V (R) was implanted into the subcutis of

athymic nude mice, and intralesional therapy with Ad IL-8-AS commenced when the tumors reached a diameter between 5 and 7 mm. Tumor growth was significantly inhibited compared with therapy in controls (saline and beta-galactosidase adenovirus). Ad IL-8-AS therapy decreased the in vivo expression of IL-8 and matrix metalloproteinase type 9 (MMP-9), reduced microvessel density, and enhanced endothelial cell apoptosis. These results indicate that Ad IL-8-AS therapy targets both tumor cells and host endothelial cells resulting in endothelial cell apoptosis and significant inhibition of tumor growth.

Inoue, R., et al. (2006). "Gefitinib-related gene signature in bladder cancer cells identified by a cDNA microarray." Anticancer Res **26**(6B): 4195-4202.

BACKGROUND: The aim of this study was to identify key genes linked to the molecular action of gefitinib, a promising anticancer agent on human bladder cancer cell lines. MATERIALS AND METHODS: cDNA microarrays were used to profile feature genes in 5637 and T24 cells before and after treatment with gefitinib. PCR-based direct sequencing and Western blot analysis were performed to examine the mutation status and protein levels of EGFR in the cell lines. RESULTS: Gefitinib significantly inhibited the proliferation of 5637 cells, while showing little inhibitory effect on T24 cells. Theses effects were independent of the mutation status and protein levels of EGFR. cDNA microarray analysis identified 15 feature genes classified as a cell cycle, apoptotic pathway and transcription. Notably, levels of expression of the cell invasion-related genes, YY1 and E-cadherin, were increased in 5637 cells sensitive to gefitinib. CONCLUSION: Unique genes involved in the action of gefitinib were identified. Particularly, the upregulation of YY1 and E-cadherin may account for the efficacy of gefitinib in bladder cancer.

Inui, M., et al. (1996). "Enhanced gene expression of transforming growth factor-alpha and c-met in rat urinary bladder cancer." Urol Res **24**(1): 55-60.

To investigate the roles of growth factors in bladder cancer, changes in the expression of messenger RNAs (mRNAs) for several growth factors and their receptors were examined during rat bladder carcinogenesis induced with N-butyl-N-(4-hydroxybutyl)-nitrosamine (BBN). Northern blot analysis showed that the contents of mRNAs for transforming growth factor-alpha (TGF-alpha) and c-met/hepatocyte growth factor (HGF) receptor increased with BBN treatment. Epidermal growth factor (EGF) receptor mRNA was hardly affected by the treatment; while mRNA for fibroblast growth factor (FGF) receptor 1 and transforming growth factor-beta (TGF-beta) type II receptor decreased with BBN treatment. A rat bladder tumor cell line, NBT-II, expressed both TGF-alpha and c-met mRNAs, and HGF showed apparent scattering and growth-stimulating effects on the cells. These results indicate the possibility that TGF-alpha produced by a bladder cancer, in addition to urinary EGF, plays a role in the development of bladder cancer, and that enhanced cell motility due to activation of the c-met/HGF receptor participates in the invasion and metastasis of the cancer cells.

Ishii, H., et al. (2001). "Effect of adenoviral transduction of the fragile histidine triad gene into esophageal cancer cells." Cancer Res **61**(4): 1578-1584.

Reintroduction of a tumor suppressor gene product in cancer cells is a promising strategy for cancer gene therapy. The fragile histidine triad (FHIT) gene has been identified in a region at chromosome 3p14.2, which is deleted in many tumors, including esophageal cancer. Previous studies have shown frequent biallelic alterations of the FHIT gene in numerous tumors, and have demonstrated a tumor suppressor function of Fhit. We have studied the biological effects of adenoviral-FHIT transduction in esophageal cancer cell lines. Results showed suppression of cell growth in vitro in three of seven esophageal cancer cell lines, all seven of which showed abundant expression of the transgene. Adenoviral-FHIT expression, but not control adenoviral infections, induced caspase-dependent apoptosis in two esophageal cancer cell lines, TE14 and TE4, which express no or very little Fhit, respectively. Treatment of TE14 cells with adenoviral-FHIT vectors resulted in abrogation of tumorigenicity in nude mice. A third esophageal cancer cell line, TE12, without detectable endogenous Fhit, showed accumulation of cells at S to G2-M and a small apoptotic cell fraction after adenoviral-FHIT transduction. Thus, adenoviral-FHIT expression can inhibit the growth of esophageal cancer cells, at least in part through caspase-dependent apoptosis, suggesting that adenoviral-FHIT infection should be explored as a therapeutic strategy.

Ishii, H., et al. (2001). "FEZ1/LZTS1 gene at 8p22 suppresses cancer cell growth and regulates mitosis." Proc Natl Acad Sci U S A **98**(18): 10374-10379.

The FEZ1/LZTS1 gene maps to chromosome 8p22, a region that is frequently deleted in human tumors. Alterations in FEZ1/LZTS1 expression have been observed in esophageal, breast, and prostate cancers. Here, we show that introduction of FEZ1/LZTS1 into Fez1/Lzts1-negative cancer cells

results in suppression of tumorigenicity and reduced cell growth with accumulation of cells at late S-G (2)/M stage of the cell cycle. Fez1/Lzts1 protein is hyperphosphorylated by cAMP-dependent kinase during cell-cycle progression. We found that Fez1/Lzts1 is associated with microtubule components and interacts with p34(cdc2) at late S-G (2)/M stage in vivo. Present data show that FEZ1/LZTS1 inhibits cancer cell growth through regulation of mitosis, and that its alterations result in abnormal cell growth.

Itoh, Y., et al. (1995). "Characterization of tumor-necrosis-factor-gene-transduced tumor-infiltrating lymphocytes from ascitic fluid of cancer patients: analysis of cytolytic activity, growth rate, adhesion molecule expression and cytokine production." Cancer Immunol Immunother **40**(2): 95-102.

We characterized tumor-infiltrating lymphocytes (TIL) from ascites of patients with ovarian or pancreatic cancer in which the human tumor necrosis factor (TNF) gene was successfully transduced with retrovirus vector. The TNF-gene-transduced TIL (TNF-TIL) from these patients showed a higher level of TNF production and higher cytotoxic activity against K562 and Daudi cells than did neomycin-phosphotransferase-gene-transduced TIL (neo-TIL). Of these TIL preparations, only that from pancreatic cancer was further characterized since it was collected in a relatively large amount. In spite of the fact that the autologous tumor cells showed resistance to soluble TNF, the TNF-TIL clearly demonstrated enhanced cytotoxicity against them as compared with neo-TIL. The enhanced cytotoxicity was ascribed to autocrine effects of secreted TNF on TIL, which included augmentation of adhesion molecule (CD2 and CD11a) and interleukin-2 receptor expression, and elevation of production of interferon gamma, lymphotoxin and granulocyte/macrophage-colony-stimulating factor and its paracrine effect on target cells to facilitate them to be more susceptible to TIL.

Jeng, M. H., et al. (1994). "Paradoxical regulation of estrogen-dependent growth factor gene expression in estrogen receptor (ER)-negative human breast cancer cells stably expressing ER." Cancer Lett **82**(2): 123-128.

We have previously demonstrated that transfection of estrogen receptor (ER)-negative human breast cancer MDA-MB-231 (clone 10A) cells with a sense constitutive wildtype ER expression vector regains hormonal responsiveness (Jiang and Jordan, J. Natl. Cancer Inst., 84 (1992) 580-591). We have therefore undertaken studies using stable transfectant S30 cells to determine the function of ER in the regulation of the levels of growth factor mRNAs, an event believed to be mediated via the ER and is important for the paracrine and autocrine regulation of breast cancer cell proliferation. Northern blot analysis demonstrated that 17 beta-estradiol (E2) increased the level of TGF alpha mRNA and decreased the level of TGF beta 2 mRNA. TGF beta 1 and TGF beta 3 mRNA levels were not affected by ER in S30 cells. The addition of anti-estrogen ICI 164,384 blocked the regulation of the mRNA levels of TGF alpha and TGF beta 2 by E2. The expression of these growth factor mRNAs was not affected by E2 or ICI 164,384 in the parental MDA-MB-231 10A and antisense ER transfectant AS23 cells. We demonstrated that the expression of ER in previously ER-negative human breast cancer cells can restore the regulation of growth factor mRNA expression by E2. An increase in TGF alpha and a decrease in TGF beta 2 is associated with an increase in growth of hormone responsive cells. Paradoxically the transfected cells have decreased growth in response to estrogen. Furthermore, these data suggest that other factors in addition to ER are required for TGF beta 1 and TGF beta 3 gene regulation by E2.

Jiang, H., et al. (1996). "The melanoma differentiation associated gene mda-7 suppresses cancer cell growth." Proc Natl Acad Sci U S A **93**(17): 9160-9165.

Cancer is a disease characterized by defects in growth control, and tumor cells often display abnormal patterns of cellular differentiation. The combination of recombinant human fibroblast interferon and the antileukemic agent mezerein corrects these abnormalities in cultured human melanoma cells resulting in irreversible growth arrest and terminal differentiation. Subtraction hybridization identifies a melanoma differentiation associated gene (mda-7) with elevated expression in growth arrested and terminally differentiated human melanoma cells. Colony formation decreases when mda-7 is transfected into human tumor cells of diverse origin and with multiple genetic defects. In contrast, the effects of mda-7 on growth and colony formation in transient transfection assays with normal cells, including human mammary epithelial, human skin fibroblast, and rat embryo fibroblast, is quantitatively less than that found with cancer cells. Tumor cells expressing elevated mda-7 display suppression in monolayer growth and anchorage independence. Infection with a recombinant type 5 adenovirus expressing antisense mda-7 eliminates mda-7 suppression of the in vitro growth and transformed phenotype. The ability of mda-7 to suppress growth in cancer cells not expressing or containing defects in both the retinoblastoma (RB) and p53 genes indicates a lack of involvement of these critical tumor suppressor

elements in mediating mda-7-induced growth inhibition. The lack of protein homology of mda-7 with previously described growth suppressing genes and the differential effect of this gene on normal versus cancer cells suggests that mda-7 may represent a new class of cancer growth suppressing genes with antitumor activity.

Jin, C., et al. (2016). "Safe engineering of CAR T cells for adoptive cell therapy of cancer using long-term episomal gene transfer." EMBO Mol Med **8**(7): 702-711.

Chimeric antigen receptor (CAR) T-cell therapy is a new successful treatment for refractory B-cell leukemia. Successful therapeutic outcome depends on long-term expression of CAR transgene in T cells, which is achieved by delivering transgene using integrating gamma retrovirus (RV) or lentivirus (LV). However, uncontrolled RV/LV integration in host cell genomes has the potential risk of causing insertional mutagenesis. Herein, we describe a novel episomal long-term cell engineering method using non-integrating lentiviral (NILV) vector containing a scaffold/matrix attachment region (S/MAR) element, for either expression of transgenes or silencing of target genes. The insertional events of this vector into the genome of host cells are below detection level. CD19 CAR T cells engineered with a NILV-S/MAR vector have similar levels of CAR expression as T cells engineered with an integrating LV vector, even after numerous rounds of cell division. NILV-S/MAR-engineered CD19 CAR T cells exhibited similar cytotoxic capacity upon CD19(+) target cell recognition as LV-engineered T cells and are as effective in controlling tumor growth in vivo We propose that NILV-S/MAR vectors are superior to current options as they enable long-term transgene expression without the risk of insertional mutagenesis and genotoxicity.

Johnson, M. D., et al. (1989). "Oestrogenic activity of tamoxifen and its metabolites on gene regulation and cell proliferation in MCF-7 breast cancer cells." Br J Cancer **59**(5): 727-738.

The effects of tamoxifen, three of its in vivo metabolites and 3-hydroxytamoxifen on cellular proliferation and the induction of four oestrogen-regulated RNAs (pNR-1, pNR-2, pNR-25 and cathepsin D) have been measured in MCF-7 breast cancer cells in phenol red-free culture medium. Tamoxifen and 3-hydroxytamoxifen acted as partial oestrogens to stimulate cell growth and the levels of the pNR-2 and pNR-25 RNAs. They were full oestrogens for the induction of cathepsin D RNA and induced the pNR-1 RNA above the level found in oestrogen-treated cells. N-Desmethyltamoxifen and 4-hydroxytamoxifen behaved like tamoxifen except that N-desmethyltamoxifen did not induce the pNR-2 RNA and was only a partial oestrogen for the induction of cathepsin D RNA, and 4-hydroxytamoxifen did not induce the pNR-2 or pNR-25 RNAs. In the presence of oestradiol, the four anti-oestrogens prevented the stimulation of growth and reduced (pNR-2 and pNR-25) or increased (pNR-1) the RNA levels to those present in MCF-7 cells treated with the anti-oestrogen alone. In contrast, for cathepsin D RNA levels there was a synergistic effect of the anti-oestrogens and oestradiol. The concentration at which each anti-oestrogen was effective was related to its affinity for the oestrogen receptor. Metabolite E was a full oestrogen for the induction of cell proliferation and the oestrogen-regulated RNAs. pNR-25 and pNR-2 RNA levels correlated most closely with effects on cell proliferation. These RNAs are therefore potentially the most useful for predicting the response of breast cancer patients to tamoxifen therapy.

Joshi, U. S., et al. (1998). "Inhibition of tumor cell growth by p21WAF1 adenoviral gene transfer in lung cancer." Cancer Gene Ther **5**(3): 183-191.

Gene replacement therapy is potentially a very powerful tool, targeting specific molecular mediators of cancer development and progression. p21WAF1 (p21) is a cyclin-dependent kinase inhibitor that is induced by p53 upon DNA damage or p53 overexpression, resulting in cell cycle arrest at the G1 checkpoint and inhibition of further cell proliferation. Using a replication-deficient recombinant adenovirus (AdV) ((rAd)-p21) as a p21 gene delivery system, we have evaluated the effect of p21 introduction in lung cancer cells in vitro as well as in vivo. In in vitro experiments, two human non-small cell lung cancer (NSCLC) cell lines, NCI-H460 and NCI-H322, showed dose-dependent p21 induction and concomitant cell growth inhibition following rAd/p21 infection. Flow cytometric analysis of the cell cycle revealed a significant increase in the percentage of NCI-H460 cells in G0/G1 following rAd/p21 infection compared with untreated cells, suggesting that p21-induced growth inhibition was due to G0/G1 arrest. We also tested the therapeutic efficacy of rAd/p21 in an in vivo human NSCLC model in SCID mice. Tumor-bearing mice were established by subcutaneous injections of NCI-H460 cells and treated by repeated intratumoral injections of rAd/p21. Intratumoral delivery of rAd/p21 significantly suppressed tumor growth and prolonged survival in rAd/p21-treated mice. Our in vitro and in vivo results provide strong preliminary evidence for the growth inhibition of NSCLC by rAd/p21. Collectively, these results justify further studies to evaluate the efficacy of rAd/p21 for gene therapy in human lung cancer.

Jounaidi, Y. and D. J. Waxman (2000). "Combination of the bioreductive drug tirapazamine with the chemotherapeutic prodrug cyclophosphamide for P450/P450-reductase-based cancer gene therapy." Cancer Res **60**(14): 3761-3769.

Tirapazamine (TPZ) is a bioreductive drug that exhibits greatly enhanced cytotoxicity in hypoxic tumor cells, which are frequently radiation-resistant and chemoresistant. TPZ exhibits particularly good activity when combined with alkylating agents such as cyclophosphamide (CPA). The present study examines the potential of combining TPZ with CPA in a cytochrome P450-based prodrug activation gene therapy strategy. Recombinant retroviruses were used to transduce 9L gliosarcoma cells with the genes encoding P450 2B6 and NADPH-P450 reductase. Intratumoral coexpression of P450 2B6 with P450 reductase sensitized 9L tumor cells to CPA equally well under normoxic (19.6% O2) and hypoxic (1% O2) conditions. The P450 2B6/P450 reductase combination also sensitized 9L tumor cells to TPZ under both culture conditions. Interestingly, bystander cytotoxic effects were observed for both CPA and TPZ under hypoxia. Furthermore, TPZ exerted a striking growth-inhibitory effect on CPA-treated 9L/2B6/P450 reductase cells under both normoxia and hypoxia, which suggests the utility of this drug combination for P450-based gene therapy. To evaluate this possibility, 9L tumor cells were transduced in culture with P450 2B6 and P450 reductase and grown as solid tumors in severe combined immune deficient mice in vivo. Although these tumors showed little response to TPZ treatment alone, tumor growth was significantly delayed, by up to approximately four doubling times, when TPZ was combined with CPA. Some toxicity from the drug combination was apparent, however, as indicated by body weight profiles. These findings suggest the potential benefit of incorporating TPZ, and perhaps other bioreductive drugs, into a P450/P450 reductase-based gene therapy strategy for cancer treatment.

Ju, Y. H., et al. (2000). "Estrogenic effects of extracts from cabbage, fermented cabbage, and acidified brussels sprouts on growth and gene expression of estrogen-dependent human breast cancer (MCF-7) cells." J Agric Food Chem **48**(10): 4628-4634.

Cruciferous vegetable extracts from freeze-dried cabbage (FDC), freeze-dried fermented cabbage (FDS), and acidified Brussels sprouts (ABS) were prepared by exhaustive extraction with ethyl acetate. Estrogenic and antiestrogenic effects of these extracts were analyzed. To identify whether the extracts are potential estrogen receptor (ER) ligands that can act as agonists or antagonists, the binding affinity of extracts for the ER was measured using a competitive radiometric binding assay. The extracts bound with low affinity to the ER, and the relative binding affinity is estradiol > FDS > FDC > ABS. These extracts were evaluated for their estrogenic and antiestrogenic activities in estrogen-dependent human breast cancer (MCF-7) cells using as endpoints proliferation and induction of estrogen-responsive pS2 gene expression, which was analyzed using Northern blot assay. At low concentrations (5-25 ng/mL) all of the extracts reduced 1 nM estradiol-induced MCF-7 cell proliferation. Extracts at 25 ng/mL also inhibited estradiol-induced pS2 mRNA expression. At higher extract concentrations (50 ng/mL-25 microg/mL), however, increased proliferation in MCF-7 cells was observed. Similarly, expression of the pS2 gene was induced by higher extract concentrations (0.25-25 microg/mL). The pure estrogen antagonist, ICI 182,780, suppressed the cell proliferation induced by the extracts as well as by estradiol and also the induction of pS2 expression by the extracts. The ER subtype-selective activities of FDC and FDS were analyzed using a transfection assay in human endometrial adenocarcinoma (HEC-1) cells. FDS acted as an ERalpha-selective agonist while FDC fully activated both ER-alpha and ER-beta. Growth of the ER-negative MDA-231 cells was not affected by the extracts or by estradiol. This study demonstrates that cruciferous vegetable extracts act bifunctionally, like an antiestrogen at low concentrations and an estrogen agonist at high concentrations.

Kagawa, S., et al. (1999). "Overexpression of the p21 sdi1 gene induces senescence-like state in human cancer cells: implication for senescence-directed molecular therapy for cancer." Cell Death Differ **6**(8): 765-772.

Normal cells in a culture enter a nondividing state after a finite number of population doubling, which is termed replicative senescence, whereas cancer cells have unlimited proliferative potential and are thought to exhibit an immmortal phenotype by escaping from senescence. The p21 gene (also known as sdi1), which encodes the cyclin-dependent kinase inhibitor, is expressed at high levels in senescent cells and contributes to the growth arrest. To examine if the p21sdi1 gene transfer could induce senescence in human cancer cells, we utilized an adenoviral vector-based expression system and four human cancer cell lines differing in their p53 status. Transient overexpression of p21sdi1 on cancer cells induced quiescence by arresting the cell cycle at the G1 phase and exhibited morphological changes, such as enlarged nuclei as well as a flattened cellular shape, specific to the senescence phenotype. We also showed that

p21sdi1-transduced cancer cells expressed beta-galactosidase activity at pH 6.0, which is known to be a marker of senescence. Moreover, the polymerase chain reaction-based assay demonstrated that levels of telomerase activity were significantly lower in p21sdi1-expressing cells compared to parental cancer cells. These observations provide the evidence that p21sdi1 overexpression could induce a senescence-like state and reduce telomerase activity in human cancer cells, suggesting that these novel p21sdi1 functions may have important implications for anticancer therapy.

Kaneko, H., et al. (1998). "Involvement of apoptosis and cyclin D1 gene repression in growth inhibition of T-47D human breast cancer cells by methylglyoxal bis (cyclopentylamidinohydrazone)." Int J Mol Med **1**(6): 931-936.

Polyamines are considered to be important intracellular molecules for the proliferation of the cancer cells. In this study, effects of methylglyoxal bis (cyclopentylamidinohydrazone) (MGBCP), a potent inhibitor of the polyamine biosynthetic pathway, on the growth and cell cycle of T-47D human breast cancer cells were investigated. MGBCP dose-dependently inhibited the growth of T-47D cells, in which the contents of spermine, spermidine and putrescine decreased concomitantly. The gene expression of cyclin D1 was also repressed by the MGBCP treatment. The MGBCP-treated cells clearly exhibited morphological changes indicating the blebbing and chromatin condensation which are characteristic of apoptosis. Flow cytometric analysis showed hypo-diploid subpopulations due to apoptotic cells, and characteristic oligonucleosomal-sized DNA fragments were clearly observed for MGBCP-treated cells as the concentration of the drug was increased. These findings suggest that the inhibition of polyamine synthesis results in the repressions of cyclin D1 expression and cell cycle progression, eventually inducing apoptosis in these human breast cancer cells.

Kanemitsu, N., et al. (2001). "Correlation between induction of the mac25 gene and anti-proliferative effects of 1alpha,25(OH)2-D3 on breast cancer and leukemic cells." Int J Mol Med **7**(5): 515-520.

In the differentiation of a myelomonocytic cell line U937 treated with 1alpha,25-dihydroxyvitamin D3 [1alpha,25(OH)2-D3], transient proliferation was observed prior to cell growth arrest. The expression of the p21 and p27 genes increased transiently and decreased quickly in the proliferation, suggesting that other genes may contribute to the growth arrest of the cell line after reduction of the p21 and p27 genes. The mac25 gene was isolated as a gene associated with cellular senescence and growth suppression. Despite a previous report that retinoic acid (RA) induced the mac25 gene, the mac25 gene did not increase in U937 cells treated with RA but did increase in the cells treated with 1alpha,25(OH)2-D3. The high level of the expression of the mac25 gene was detected for four days after the 1alpha,25(OH)2-D3 treatment. Therefore, mac25 may contribute to the growth arrest of U937 cells treated with 1,25-D3. The growth responses to 1alpha,25(OH)2-D3 and the expression of the mac25 gene of three other cancer cell lines (Saos-2, U2OS and MCF7) were studied. Although the growth suppression was observed in MCF7 cells treated with 1alpha,25(OH)2-D3 dose-dependently (1-100 nM of 1alpha,25(OH)2-D3), the treatment of 100 nM of 1alpha,25(OH)2-D3 had no effect on the growth of Saos-2 and U2OS cells. The expression of the mac25 gene was up-regulated in MCF7 cells treated with 100 nM of 1alpha,25(OH)2-D3, whereas no transcript of the mac25 gene was detected in Saos-2 and U2OS cells even when they were treated with 100 nM of 1alpha,25(OH)2-D3. These results suggest that the cellular response to 1alpha,25(OH)2-D3 may depend on the induction of the mac25 gene.

Kang, S. K., et al. (2001). "Estradiol regulates gonadotropin-releasing hormone (GnRH) and its receptor gene expression and antagonizes the growth inhibitory effects of GnRH in human ovarian surface epithelial and ovarian cancer cells." Endocrinology **142**(2): 580-588.

In the present study, we investigated the expression of estrogen receptors (ERalpha and ERbeta) in human ovarian surface epithelial (hOSE) cells and the ovarian cancer cell line, OVCAR-3, and provided novel evidence that estrogen may have a growth regulatory effect in these cells. Expression levels of ERalpha messenger RNA (mRNA) were 1.5-fold higher in OVCAR-3 cells than in hOSE cells, as revealed by semiquantitative RT-PCR and Southern blot analysis. A significant increase (3.3-fold) in ERss mRNA levels was observed in OVCAR-3 cells compared with hOSE cells. In parallel with mRNA levels, expression levels of ERalpha and ERbeta proteins were also higher in OVCAR-3 cells compared with hOSE cells. We recently proposed that GnRH and its receptor may have an autocrine role in hOSE and ovarian cancer cells. To determine whether estrogen regulates GnRH and GnRH receptor (GnRHR), hOSE and OVCAR-3 cells were treated with various concentrations of 17beta-estradiol for 24 h. Expression levels of GnRH and GnRHR mRNA were examined using quantitative and competitive RT-PCR, respectively. Treatment with 17beta-estradiol induced a significant down-regulation of GnRH mRNA in OVCAR-3 cells, but not in hOSE cells and of GnRHR

mRNA in both hOSE and OVCAR-3 cells. Tamoxifen, an estrogen antagonist, prevented the effects of 17ssestradiol, suggesting that estradiol action is mediated via the ER. Finally, the effect of estrogen on the growth of hOSE and OVCAR-3 cells was investigated. The cells were treated with various concentrations of 17ss-estradiol, and the proliferative index of cells was measured using [(3)H]thymidine incorporation and DNA fluorometric assays. 17beta-Estradiol stimulated the growth of OVCAR-3 cells in a dose- and time-dependent manner. In contrast, 17beta-estradiol failed to stimulate the growth of hOSE cells. As estrogen down-regulated GnRH and GnRHR mRNA, we investigated whether estrogen treatment blocks the growth inhibitory effect of a GnRH agonist in OVCAR-3 and hOSE cells. Cells were treated with 17beta-estradiol (10(-7) M) together with (D-Ala (6))-GnRH (10(-7) M), and the proliferative index of cells was measured. Pre- or cotreatment of cells with 17beta-estradiol significantly attenuated the growth inhibitory effect of the GnRH agonist in OVCAR-3 cells, whereas no effect of 17ss-estradiol treatment was observed in hOSE cells. To our knowledge, these results provide the first demonstration of a potential interaction between the estradiol/ER and GnRH/GnRHR systems, which may be important in the growth regulation of normal and neoplastic hOSE cells.

Kawabe, S., et al. (2001). "Adenovirus-mediated wild-type p53 gene expression radiosensitizes non-small cell lung cancer cells but not normal lung fibroblasts." Int J Radiat Biol **77**(2): 185-194.

PURPOSE: We compared the ability of adenoviral-mediated wild-type p53 RPR/INGN201(Ad5/CMV/p53) to radiosensitize non-small cell lung carcinoma (NSCLC) and normal lung fibroblast cells. MATERIALS AND METHODS: NSCLC cell lines (A549 and H322) and human lung fibroblast cells (MRC-9 and CCD-16) were used in this study. Radiosensitivity was determined by clonogenic assay and tumor growth delay. Expression of p53, Bax, and p21WAF1 protein were evaluated by immunoblot. A FITC conjugate of annexin V was used for flow cytometric detection of apoptosis. RESULTS: Clonogenic and apoptotic assays indicated that Ad5/CMV/p53 enhanced the radiosensitivity of both NSCLC cell lines. On the other hand, the two normal human fibroblast cell lines appeared to be resistant to the cytotoxic effects of Ad5/CMV/p53 and were not radiosensitized compared to the NSCLC cells. According to immunoblot analysis, Bax expression was increased in the NSCLC cells treated with the combination therapy; Bax expression, however, was unchanged in normal cells. In in vivo studies, tumor growth suppression was enhanced by this combination strategy in xenograft tumors growing in nude mice compared to Ad5/CMV/p53 or radiation therapy when used alone. CONCLUSIONS: Our data indicate that therapy using Ad5/CMV/p53 and irradiation in combination is more effective than either treatment when used alone on NSCLC cells, is not limited to cells with defective endogenous p53, and does not enhance the radiosensitivity of normal cells.

Kawakami, Y., et al. (2001). "Adenovirus-mediated p16 gene transfer changes the sensitivity to taxanes and Vinca alkaloids of human ovarian cancer cells." Anticancer Res **21**(4A): 2537-2545.

BACKGROUND: Deletions and point mutations of the p16 gene are detectable in more than 50% of ovarian cancer cells. In this study, we examined the effect of p16 gene transduction on the growth of ovarian cancer cells and on the effect of anti-cancer agents. MATERIALS AND METHODS: p16-null human ovarian cancer cell lines, SKOV-3 and OVCAR-5, were used in this study. We transduced the full-length human p16 gene using recombinant adenovirus (AxCA-hp16). RESULTS: The spontaneous growth of these cells was significantly inhibited by hp16 transduction. MTT assay revealed that AxCA-hp16 infection induced chemoresistance in both cell lines. Flow cytometric analysis revealed that only hp16 -transduced SKOV-3, were arrested at the G1-phase for 3 days whereas those infected with AxCA-mock and OVCAR-5 infected with both recombinant viruses did not. Western blot analysis showed increased microtubule-associated proteins 4 (MAP4) in both cell lines. CONCLUSION: These results suggest that in SKOV-3 cells, G1-arrest induced by p16-transduction prevents paclitaxel- and vindesine-induced cell death, and in OVCAR-5 cells, the other unknown mechanisms play a role of chemoresistance.

Kazmi, S. M., et al. (1996). "Comparison of N-(4-hydroxyphenyl)retinamide and all-trans-retinoic acid in the regulation of retinoid receptor-mediated gene expression in human breast cancer cell lines." Cancer Res **56**(5): 1056-1062.

The activities of N-(4-hydroxyphenyl)retinamide [(4-HPR), Fenretinide] and all-trans-retinoic acid (RA) were determined for (a) the inhibition of cell proliferation; (b) the activation of human retinoid receptor-mediated target gene expression; (c) the inhibition of estradiol- and progesterone-induced gene activation in breast cancer cell lines; and (d) the regulation of the expression of tumor suppressor retinoblastoma protein. Similar to RA, both 4-HPR and its active metabolite N-(4-methoxyphenyl)retinamide (4-MPR) effectively impeded the growth of MCF7 and T-47D human

breast cancer cell lines, except that 4-HPR also inhibited the proliferation of RA-resistant BT-20 cells. However, when tested in human recombinant retinoic acid receptor (RAR-alpha, RAR-beta, and RAR-gamma)-induced reporter gene assays, RA was much more potent (>100-fold) than either 4-HPR or 4-MPR. 4-HPR induced transcriptional activation through all three RAR subtypes at 1-10microM, while RA showed comparable activity at 10-100microM. Despite the apparent weak interaction at the RAR level, 4-HPR was comparable to RA in the inhibition of both estrogen receptor- and progesterone receptor-mediated transcriptional activation in MCF7 and T-47D cells, respectively. Moreover, similar to RA, 4-HPR and 4-MPR caused marked up-regulation of tumor suppressor retinoblastoma protein in both MCF7 and T-47D cells. Since RA and 4-HPR showed comparable activity in the inhibition of estrogen recptor- and progesterone receptor-induced gene transcription and in the stimulation of retinoblastoma protein expression in MCF7 and T-47D cells, the reduced RAR activation by 4-HPR may result in the lack of hepatic toxicity and therefore the improved therapeutic efficacy relative to RA.

Kebebew, E., et al. (2004). "Id1 gene expression is up-regulated in hyperplastic and neoplastic thyroid tissue and regulates growth and differentiation in thyroid cancer cells." J Clin Endocrinol Metab **89**(12): 6105-6111.

The Id (inhibitor of DNA binding) proteins are a family of helix-loop-helix (HLH) proteins (Id1, Id2, Id3, and Id4) that lack the basic domain necessary for DNA binding. The Id1 protein enhances cell proliferation and inhibits cellular differentiation in a variety of cell types. We have previously demonstrated that the Id1 gene is up-regulated in papillary and medullary thyroid cancers. In this study we characterized the expression and distribution of the Id1 protein in normal, hyperplastic, and neoplastic human thyroid tissue. We also evaluated the effect of the Id1 gene on thyroid cancer cell growth and markers of thyroid cell differentiation. We used semiquantitative immunohistochemistry to characterize Id1 protein expression in normal, hyperplastic (multinodular goiter and Graves' disease), and neoplastic thyroid tissue from 103 patients. Normal thyroid tissue had the lowest level of Id1 protein expression ($P < 0.0001$). Anaplastic thyroid cancer had the highest level (vs. benign and malignant thyroid tissues, $P < 0.01$). Id1 protein expression was higher in malignant thyroid tissue than in hyperplastic thyroid tissue ($P < 0.02$). We found no significant association between the level of Id1 protein expression and patient age, sex, tumor-node-metastasis stage, tumor size, primary tumor vs. lymph node metastasis, primary tumor vs. recurrent tumors, and extent of tumor differentiation. Inhibiting Id1 mRNA expression in thyroid cancer cell lines using Id1 antisense oligonucleotides resulted in growth inhibition ($P < 0.03$) and decreased thyroglobulin and sodium-iodine symporter mRNA expression ($P < 0.02$). In conclusion, Id1 is overexpressed in hyperplastic and neoplastic thyroid tissue and directly regulates the growth of thyroid cancer cells of follicular cell origin, but is not a marker of aggressive phenotype in differentiated thyroid cancer.

Kelley, J. R., et al. (2000). "The cancer-associated Sm-like oncogene: a novel target for the gene therapy of pancreatic cancer." Surgery **128**(2): 353-360.

BACKGROUND: The prognosis for pancreatic cancer (PC) remains dismal, providing a clear need for the development of novel therapies. We have previously shown that the cancer-associated Sm-like (CaSm) oncogene is overexpressed in the great majority of pancreatic tumors and is required to maintain the transformed phenotype. The purpose of this study was to determine whether the application of CaSm antisense gene therapy would generate a significant antitumor effect against PC. METHODS: An adenoviral vector (Ad-alphaCaSm) expressing a 900-base pair antisense RNA to CaSm was created. The PC cell lines AsPC-1 and Capan-1 were infected with this vector and examined for changes in in vitro proliferation by using methyl thiazol tetrazolium and soft agar assays. SCID-Bg mice bearing subcutaneous AsPC-1 tumors were treated with Ad-alphaCaSm (1 x 10(9) plaque-forming units) as a single intratumor injection with tumor growth and survival monitored. RESULTS: AsPC-1 and Capan-1 cells showed decreased in vitro proliferation (93%, P =.0041, and 70%, P =. 0038, respectively) and anchorage independent growth (55%, P =.02, and 45%, P =.03, respectively) after treatment. Ad-alphaCaSm reduced in vivo AsPC-1 tumor growth by 40% (n = 10), extending median survival time from 35 to 60 days. CONCLUSIONS: Ad-alphaCaSm demonstrates a significant antitumor effect against pancreatic cancer both in vitro and in vivo. These results support the role of CaSm as a significant gene involved in the neoplastic transformation of pancreatic tumors. Thus CaSm represents a novel gene target in PC and holds potential as a new treatment approach either alone or in combination with existing therapies.

Kelley, J. R., et al. (2001). "CaSm/gemcitabine chemo-gene therapy leads to prolonged survival in a murine model of pancreatic cancer." Surgery **130**(2): 280-288.

BACKGROUND: CaSm, the cancer-associated Sm-like oncogene, is overexpressed in greater than 80%

of pancreatic tumors. We previously reported that an adenovirus expressing antisense RNA to CaSm (Ad-alpha CaSm) can decrease pancreatic tumor growth in vivo but is not curative. In the current study we investigated the mechanism of Ad-alpha CaSm's antitumor effect to rationally approach combinatorial therapy for improved efficacy. METHODS: AsPC-1 and Panc-1 human pancreatic cancer cells were treated with Ad-alpha CaSm and examined by MTT assay for in vitro proliferation changes. Flow cytometry determined the effect of CaSm down-regulation on the cell cycle, and then cells treated with Ad-alpha CaSm in combination with cisplatin, etoposide, or gemcitabine chemotherapies were reexamined by MTT assay. SCID-Bg mice bearing subcutaneous AsPC-1 tumors were treated with Ad-alpha CaSm, gemcitabine, or the combination and monitored for tumor growth and survival. RESULTS: Treatment with Ad-alpha CaSm reduced the proliferation of AsPC-1 and Panc-1 cells (59% and 44%, respectively; P <.05). The cell cycle revealed a cytostatic block with decreased G (1) phase and increased DNA content in treated cells. The combination of Ad-alpha CaSm with gemcitabine significantly reduced in vitro proliferation (66% vs 39% and 48% for controls), decreased in vivo AsPC-1 tumor growth by 71% (n = 10), and extended survival time from 57 to 100 days. CONCLUSIONS: Down-regulation of CaSm reduces the growth of pancreatic cancer cells by altering the cell cycle in a cytostatic manner. The combination of Ad-alpha CaSm with gemcitabine is more effective than either agent used separately.

Khalighinejad, N., et al. (2008). "Adenoviral gene therapy in gastric cancer: a review." World J Gastroenterol **14**(2): 180-184.

Gastric cancer is one of the most common malignancies worldwide. With current therapeutic approaches the prognosis of gastric cancer is very poor, as gastric cancer accounts for the second most common cause of death in cancer related deaths. Gastric cancer like almost all other cancers has a molecular genetic basis which relies on disruption in normal cellular regulatory mechanisms regarding cell growth, apoptosis and cell division. Thus novel therapeutic approaches such as gene therapy promise to become the alternative choice of treatment in gastric cancer. In gene therapy, suicide genes, tumor suppressor genes and anti-angiogenesis genes among many others are introduced to cancer cells via vectors. Some of the vectors widely used in gene therapy are Adenoviral vectors. This review provides an update of the new developments in adenoviral cancer gene therapy including strategies for inducing apoptosis, inhibiting metastasis and targeting the cancer cells.

Kim, C. K., et al. (2003). "Enhanced p53 gene transfer to human ovarian cancer cells using the cationic nonviral vector, DDC." Gynecol Oncol **90**(2): 265-272.

OBJECTIVE: Previously we have formulated a new cationic liposome, DDC, composed of dioleoyltrimethylamino propane (DOTAP), 1,2-dioeoyl-3-phosphophatidylethanolamine (DOPE), and cholesterol (Chol), and it efficiently delivered plasmid DNA into ovarian cancer cells. Mutations in the p53 tumor suppressor gene are the most common molecular genetic abnormalities to be described in ovarian cancer. However, there has been so far no report of nonviral vector-mediated p53 gene deliveries in ovarian cancer. In this study, wild-type p53 DNA was transfected into the ovarian cancer cells, using the DDC as a nonviral vector and the expression and activity of p53 gene were evaluated both in vitro and in vivo. METHOD: DDC liposomes were prepared by mixing DOTAP:DOPE:Chol in a 1:0.7:0.3 molar ratio using the extrusion method. Plasmid DNA (pp53-EGFP) and DDC complexes were transfected into ovarian carcinoma cells (OVCAR-3 cells) and gene expression was determined by reverse transcription-polymerase chain reaction and Western blot analysis. The cellular growth inhibition and apoptosis of DDC-mediated p53 transfection were assessed by trypan blue exclusion assay and annexin-V staining, respectively. The OVCAR-3 cells treated with DDC/pp53-EGFP complexes were inoculated into female balb/c nude mice and tumor growth was observed. RESULTS: The transfection of liposome-complexed p53 gene resulted in a high level of wild-type p53 mRNA and protein expressions in OVCAR-3 cells. In vitro cell growth assay showed growth inhibition of cancer cells transfected with DDC/pp53-EGFP complexes compared with the control cells. The reestablishment of wild-type p53 function in ovarian cancer cells restored the apoptotic pathway. Following the inoculation of DDC/pp53-EGFP complexes, the volumes of tumors in nude mice were significantly reduced more than 60% compared to the control group. CONCLUSION: The DDC-mediated p53 DNA delivery may have the potential for clinical application as nonviral vector-mediated ovarian cancer therapy due to its effective induction of apoptosis and tumor growth inhibition.

Kim, I. Y., et al. (1996). "Genetic change in transforming growth factor beta (TGF-beta) receptor type I gene correlates with insensitivity to TGF-beta 1 in human prostate cancer cells." Cancer Res **56**(1): 44-48.

Transforming growth factor beta 1 (TGF-beta 1), a potential regulator of growth of prostate cancer cells, exerts its effects through interaction with membrane

receptors. In the present study, an attempt was made to establish a correlation between TGF-beta 1 sensitivity and TGF-beta receptor expression in three prostate cancer cell lines (PC3, DU145, and LNCaP). In a dose-dependent manner, TGF-beta 1 inhibited the proliferation of PC3 and DU145 cells but not LNCaP cells. Since TGF-beta signals through a heteromeric complex composed of TGF-beta receptors type II and type I, the expression of these receptors was investigated by Western blot analysis and reverse transcriptase-PCR. These studies demonstrated that all three prostate cancer cell lines express type II receptor. In contrast, type I receptor was detected only in the TGF-beta 1-sensitive PC3 and DU145 cells but not in the TGF-beta 1-insensitive LNCaP cells. To investigate the possibility that the undetectable expression of type I receptor in LNCaP cells is due to a change in the respective gene, Southern blot analysis was performed. The result demonstrated that there was a genetic change in type I receptor gene in these cells. Subsequently, when LNCaP cells were transiently transfected with T beta R-I cDNA, sensitivity to TGF-beta 1 was restored. These observations indicate that LNCaP cells contain a defective T beta R-I gene which rendered these cells insensitive to the action of TGF-beta 1.

Kim, M. H., et al. (2009). "C/EBPbeta regulates metastatic gene expression and confers TNF-alpha resistance to prostate cancer cells." Prostate **69**(13): 1435-1447.

BACKGROUND: CCAAT/enhancer-binding protein beta (C/EBPbeta) is a transcription factor and consists of three isoforms, transcription-activating A/B (C/EBPbeta-AB) and transcription inhibitory C (C/EBPbeta-C). We previously reported that C/EBPbeta-C was predominantly expressed in hormone-dependent LNCaP cells, while C/EBPbeta-AB forms were predominant in hormone-independent prostate cancer (HI-PCa) cells. METHODS: Reporter gene analysis was performed to investigate transcriptional activity of C/EBPbeta on metastatic gene expression upon TNF-alpha treatment. NF-kappaB activation and C/EBPbeta protein upregulation were determined by immunoblotting. WST assay was used to determine the role of C/EBPbeta in TNF-alpha-induced cell death. RESULTS: We first determined that the C/EBPbeta-C overexpression or siRNA-mediated C/EBPbeta depletion decreased TNF-alpha-induced promoter activities of Bfl-1, IL-6, and IL-8 genes. IL-6 and IL-8 are autocrine growth factors of HI-PCa cells and Bfl-1 is an anti-apoptotic protein whose function in prostate cancer is yet to be determined. Secondly, we determined differential regulation of C/EBPbeta by TNF-alpha. In DU-145 cells, C/EBPbeta was upregulated by TNF-alpha, but downregulated in LNCaP cells, although NF-kappaB was activated in both cells. This result suggested cell-type specific activation of signaling pathways leading to C/EBPbeta upregulation, which was distinct from that leading to NF-kappaB activation. Most importantly, C/EBPbeta depletion decreased cell growth and sensitized DU-145 cells to TNF-alpha-induced cell death. Conversely, overexpression of C/EBPbeta-A in LNCaP cells increased resistance to TNF-induced cell death and TNF-induced promoter activities of IL-6 and Bfl-1. CONCLUSION: Our study, for the first time, demonstrated that C/EBPbeta regulated cell growth and conferred TNF-alpha resistance to PCa cells, in part, via regulation of metastatic gene expression. Prostate 69: 1435-1447, 2009. (c) 2009 Wiley-Liss, Inc.

Kim, S., et al. (2004). "A screen for genes that suppress loss of contact inhibition: identification of ING4 as a candidate tumor suppressor gene in human cancer." Proc Natl Acad Sci U S A **101**(46): 16251-16256.

We have devised a screen for genes that suppress the loss of contact inhibition elicited by overexpression of the protooncogene MYCN. The initial application of this screen detected nine distinctive suppressors within a representative human cDNA library. One of these genes was ING4, a potential tumor suppressor gene that maps to human chromosome 12p13. Ectopic expression of ING4 suppressed the loss of contact inhibition elicited by either MYCN or MYC but had no direct effect on cellular proliferation. Pursuing the possibility that ING4 might be a tumor suppressor gene, we found inactivating mutations in ING4 transcripts from various human cancer cell lines. In addition, we used comparative genomic hybridization to detect deletion of the ING4 locus in 10-20% of human breast cancer cell lines and primary breast tumors. Ectopic expression of ING4 attenuated the growth of T47D human breast cancer cells in soft agar. We conclude that ING4 is a strong candidate as a tumor suppressor gene.

Kim, S. B., et al. (2001). "Growth inhibition and chemosensitivity of poorly differentiated human thyroid cancer cell line (NPA) transfected with p53 gene." Head Neck **23**(3): 223-229.

BACKGROUND: We investigated whether retroviral p53 transfection could enhance growth inhibition and chemosensitivity in a p53 mutant papillary thyroid cancer cell line (NPA). METHODS: NPA cells were transfected with either LXSN/p53 or mock infection in the presence of Adriamycin. Gene expression was confirmed by western blotting. Nude mice were injected subcutaneously with NPA cells

after transfection with either LXSN/p53 or mock infection on opposite sides, and the tumor growth was compared. RESULTS: There was a dose-dependent inhibition of tumor growth with LXSN/p53 transfection. Tumor growth was inhibited more by p53 gene transfection relative to mock transfection in the presence of Adriamycin. CONCLUSION: These treatment modalities could be beneficial in the treatment of p53 mutant positive thyroid cancers.

Kimura, M., et al. (1998). "Loss of tumorigenicity of human pancreatic carcinoma cells engineered to produce interleukin-2 or interleukin-4 in nude mice: a potentiality for cancer gene therapy." Cancer Lett **128**(1): 47-53.

To examine the possibility of cytokine gene therapy in relation to pancreatic cancer, we evaluated the antitumor effect of human pancreatic carcinoma cells (AsPC-1) which were retrovirally-transduced with several kinds of cytokine genes. These cells were inoculated into BALB/c nude mice and their tumor volumes were assessed. The in vitro growth rate of the transduced cells was not different from that of a parental cell line. Among the transduced cells, human interleukin (IL)-6-transduced AsPC-1 and mouse granulocyte macrophage colony-stimulating factor-transduced AsPC-1 cells showed a significant retardation of tumor growth compared with a parental cell line. In the cases of AsPC-1 cells transduced with the human IL-2 or mouse IL-4 gene, small tumors were generated but thereafter they regressed completely. Histological examinations showed monocytic cell infiltration around the tumors of IL-2- or IL-4-producing cells. These data suggest that secretion of IL-2 or IL-4 from tumor cells can induce an antitumor effect even in the defective condition of mature T cells.

Kito, M., et al. (1999). "Induction of apoptosis in cultured colon cancer cells by transfection with human interferon beta gene." Biochem Biophys Res Commun **257**(3): 771-776.

The growth of SW480 colon cancer cells following the transfection with the human interferon beta (hIFNbeta) gene entrapped in cationic multilamellar liposomes was effectively inhibited, but not that of the cells transfected with the gene from which the secretion signal sequence of hIFNbeta had been deleted. The amount of hIFNbeta secreted in the medium from SW480 cells transfected with hIFNbeta gradually increased and became maximum 3 days after the transfection, but no hIFNbeta was detected in the medium of the cells transfected with the secretion signal-deleted hIFNbeta. These findings indicate that the growth inhibition of SW480 cells after the transfection with hIFNbeta was caused by hIFNbeta secreted from the transfected cells. At that time, SW480 cells were induced to undergo apoptosis, which was identified by morphological aspects, viz., chromatin condensation, nuclear segmentation, and nucleosomal DNA fragmentation. The hIFNbeta-induced apoptosis was found to be linked to the activation of caspases 3 and 8 as evidenced by immunoblot, enzymological, and cell death inhibition analyses.

Kleinerman, D. I., et al. (1996). "Suppression of human bladder cancer growth by increased expression of C-CAM1 gene in an orthotopic model." Cancer Res **56**(15): 3431-3435.

Recently, we demonstrated that an immunoglobulin-like cell adhesion molecule, C-CAM, acts as a tumor suppressor in prostate cancer. It is known that C-CAM is expressed in many epithelial cell types. In this study, we tested the possibility that C-CAM may also suppress bladder cancer progression. We used an orthotopic tumor model, which provides a relevant organ condition for examining the interaction between primary tumor cells and their microenvironment; this interaction has a critical impact on the behavior of carcinoma. We constructed a recombinant adenovirus expressing C-CAM1 (an isoform of C-CAM) and infected the 253J B-V cell line, a tumorigenic human bladder carcinoma subline. In vitro, C-CAM1 protein was detected in C-CAM1 adenovirus-infected cells but not in antisense control virus-infected cells, and the levels of expression showed dose dependency. When these cells were injected orthotopically in nude mice, we found that the increased expression of C-CAM1 in the 253J B-V cells repressed the growth of 253J B-V-induced tumors. Taken together, these data indicate that C-CAM1 is a potent tumor suppressor in human bladder cancer.

Kloth, J. N., et al. (2005). "Substantial changes in gene expression of Wnt, MAPK and TNFalpha pathways induced by TGF-beta1 in cervical cancer cell lines." Carcinogenesis **26**(9): 1493-1502.

Transforming growth factor-beta 1 (TGF-beta1) is a potent inhibitor of epithelial cell proliferation. During the development of cervical carcinoma however, an increase in production of TGF-beta1 is accompanied by decreased sensitivity for the growth-limiting effect of TGF-beta1. TGF-beta1 has an anti-proliferative effect on cells of the immune system and thus can be advantageous for tumor progression. The aim of the present study was to determine the effect of TGF-beta1 on mRNA expression profile of genes in pathways involved in cell growth and cell death, in cervical carcinoma cell lines with different sensitivity to TGF-beta1. For this purpose, we have investigated

changes in gene expression in TGF-beta1 stimulated cervical cancer cell lines with high (CC10B), intermediate (SiHa) and low (HeLa) sensitivity to the anti-proliferative effect of TGF-beta1, at timepoints 0, 6, 12 and 24 h. Microarray analysis, using Affymetrics focus arrays, representing 8973 genes, was used to measure gene expression. In our study novel target genes involved in tumor necrosis factor alpha (TNFalpha), mitogen-activated protein kinase (MAPK) and wingless type (Wnt) pathways in response to TGF-beta1 were found. Substantial differences in gene expression between TGF-beta1 sensitive and insensitive cell lines were observed involving genes in TNFalpha, MAPK, Wnt and Smad pathways. Since these pathways are implicated in cell proliferation and cell death, these pathways may play a role in determining the overall sensitivity of a cell to TGF-beta1 induced cell growth inhibition. The results were subsequently validated by quantitative real-time PCR. Increased resistance to TGF-beta1 induced cell growth inhibition was correlated with an elevated production of TGF-beta1 by the cell lines, as measured by enzyme linked immunosorbent assay. TGF-beta1 production did not inhibit cell growth, since blocking TGF-beta1 protein by anti-TGF-beta had no effect on cell proliferation. TGF-beta1 excretion by tumor cells more likely contributes to paracrine stimulation of tumor development.

Kochetkova, M., et al. (2002). "CBFA2T3 (MTG16) is a putative breast tumor suppressor gene from the breast cancer loss of heterozygosity region at 16q24.3." Cancer Res **62**(16): 4599-4604.

Numerous cytogenetic and molecular studies of breast cancer have identified frequent loss of heterozygosity (LOH) of the long arm of human chromosome 16. On the basis of these data, the likely locations of breast cancer tumor suppressor genes are bands 16q22.1 and 16q24.3. We have mapped the CBFA2T3 (MTG16) gene, previously cloned as a fusion partner of the AML1 protein from a rare (16;21) leukemia translocation, to the 16q24.3 breast cancer LOH region. The expression of CBFA2T3 was significantly reduced in a number of breast cancer cell lines and in primary breast tumors, including early ductal carcinomas in situ, when compared with nontransformed breast epithelial cell lines and normal breast tissue. Reintroduction of CBFA2T3 into different breast tumor derived cell lines with decreased expression of this gene reduced colony growth on plastic and in soft agar. CBFA2T3 was shown to function as a transcriptional repressor when tethered to the GAL4 DNA-binding domain in a reporter gene assay and, therefore, has the potential to be a transcriptional repressor in normal breast epithelial cells. Taken together, these findings suggest that CBFA2T3 is a likely candidate for the breast cancer tumor suppressor gene that is the target for the frequent 16q24 LOH in breast neoplasms.

Kojima, A., et al. (1998). "Reversal of CPT-11 resistance of lung cancer cells by adenovirus-mediated gene transfer of the human carboxylesterase cDNA." Cancer Res **58**(19): 4368-4374.

To evaluate the concept that transfer of the human carboxylesterase (CE) gene will overcome the drug resistance of a solid tumor to CPT-11 (irinotecan), we used an adenovirus vector (AdCMV.CE) carrying human CE cDNA to infect CPT-11-resistant A549 human adenocarcinoma cells (A549/CPT) in vitro and in vivo and evaluated cell growth over time. The A549/CPT cells, selected by stepwise and continuous exposure of parental A549 cells to CPT-11 over 10 months, had a 6-fold resistance to CPT-11 and 42% CE activity in comparison with parental A549 cells. AdCMV.CE infection resulted in an increase in functional CE protein in resistant cells in vitro that was sufficient to convert CPT-11 to its active metabolite, SN-38, and effectively suppressed resistant cell growth in vitro in the presence of CPT-11. When AdCMV.CE was directly injected into established s.c. resistant A549-based tumors in nude mice receiving CPT-11, there was a 1.8-fold reduction in tumor size at day 20 compared to that of controls ($P < 0.05$). These observations suggest that adenovirus-mediated gene transfer of the human CE gene and concomitant administration of CPT-11 may have potential as a strategy for local control of acquired CPT-11 resistance of solid tumors.

Kondo, M., et al. (2001). "Overexpression of candidate tumor suppressor gene FUS1 isolated from the 3p21.3 homozygous deletion region leads to G1 arrest and growth inhibition of lung cancer cells." Oncogene **20**(43): 6258-6262.

Recently we identified FUS1 as a candidate tumor suppressor gene (TSG) in the 120 kb 3p21.3 critical region contained in nested lung and breast cancer homozygous deletions. Mutation of FUS1 is infrequent in lung cancers which we have confirmed in 40 other primary lung cancers. In addition, we found no evidence for FUS1 promoter region methylation. Because haploinsufficiency or low expression of Fus1 may play a role in lung tumorigenesis, we tested the effect of exogenously induced overexpression of Fus1 protein and found 60-80% inhibition of colony formation for non-small cell lung cancer lines NCI-H1299 (showing allele loss for FUS1) and NCI-H322 (containing only a mutated FUS1 allele) in vitro. By contrast, a similar level of expression of a tumor-acquired mutant form of FUS1 protein did not significantly suppress colony formation. Also, induced

expression of Fus1 under the control of an Ecdysone regulated promoter decreased colony formation 75%, increased the doubling time twofold, and arrested H1299 cells in G1. In conclusion, our data are consistent with the hypothesis that FUS1 may function as a 3p21.3 TSG, warranting further studies of its function in the pathogenesis of human cancers.

Kresty, L. A., et al. (2011). "Cranberry proanthocyanidins mediate growth arrest of lung cancer cells through modulation of gene expression and rapid induction of apoptosis." Molecules **16**(3): 2375-2390.

Cranberries are rich in bioactive constituents purported to enhance immune function, improve urinary tract health, reduce cardiovascular disease and more recently, inhibit cancer in preclinical models. However, identification of the cranberry constituents with the strongest cancer inhibitory potential and the mechanism associated with cancer inhibition by cranberries remains to be elucidated. This study investigated the ability of a proanthocyanidin rich cranberry fraction (PAC) to alter gene expression, induce apoptosis and impact the cell cycle machinery of human NCI-H460 lung cancer cells. Lung cancer is the leading cause of cancer-related deaths in the United States and five year survival rates remain poor at 16%. Thus, assessing potential inhibitors of lung cancer-linked signaling pathways is an active area of investigation.

Krishnan, A. V., et al. (2003). "Inhibition of prostate cancer growth by vitamin D: Regulation of target gene expression." J Cell Biochem **88**(2): 363-371.

Prostate cancer (PCa) cells express vitamin D receptors (VDR) and 1,25-dihydroxyvitamin D (3) (1,25(OH) (2)D (3)) inhibits the growth of epithelial cells derived from normal, benign prostate hyperplasia, and PCa as well as established PCa cell lines. The growth inhibitory effects of 1,25(OH) (2)D (3) in cell cultures are modulated tissue by the presence and activities of the enzymes 25-hydroxyvitamin D (3) 24-hydroxylase which initiates the inactivation of 1,25(OH) (2)D (3) and 25-hydroxyvitamin D (3) 1alpha-hydroxylase which catalyses its synthesis. In LNCaP human PCa cells 1,25(OH) (2)D (3) exerts antiproliferative activity predominantly by cell cycle arrest through the induction of IGF binding protein-3 (IGFBP-3) expression which in turn increases the levels of the cell cycle inhibitor p21 leading to growth arrest. cDNA microarray analyses of primary prostatic epithelial and PCa cells reveal that 1,25(OH) (2)D (3) regulates many target genes expanding the possible mechanisms of its anticancer activity and raising new potential therapeutic targets. Some of these target genes are involved in growth regulation, protection from oxidative stress, and cell-cell and cell-matrix interactions. A small clinical trial has shown that 1,25(OH) (2)D (3) can slow the rate of prostate specific antigen (PSA) rise in PCa patients demonstrating proof of concept that 1,25(OH) (2)D (3) exhibits therapeutic activity in men with PCa. Further investigation of the role of calcitriol and its analogs for the therapy or chemoprevention of PCa is currently being pursued.

Krishnan, A. V., et al. (2004). "Analysis of vitamin D-regulated gene expression in LNCaP human prostate cancer cells using cDNA microarrays." Prostate **59**(3): 243-251.

BACKGROUND: 1,25-dihydroxyvitamin D (3) [1,25(OH)2D3] exerts growth inhibitory, pro-differentiating, and pro-apoptotic effects on prostate cells. To better understand the molecular mechanisms underlying these actions, we employed cDNA microarrays to study 1,25(OH)2D3-regulated gene expression in the LNCaP human prostate cancer cells. METHODS: mRNA isolated from LNCaP cells treated with vehicle or 50 nM 1,25(OH)2D3 for various lengths of time were hybridized to microarrays carrying approximately 23,000 genes. Some of the putative target genes revealed by the microarray analysis were verified by real-time PCR assays. RESULTS: 1,25(OH)2D3 most substantially increased the expression of the insulin-like growth factor binding protein-3 (IGFBP-3) gene. Our analysis also revealed several novel 1,25(OH)2D3-responsive genes. Interestingly, some of the key genes regulated by 1,25(OH)2D3 are also androgen-responsive genes. 1,25(OH)2D3 also down-regulated genes that mediate androgen catabolism. CONCLUSIONS: The putative 1,25(OH)2D3 target genes appear to be involved in a variety of cellular functions including growth regulation, differentiation, membrane transport, cell-cell and cell-matrix interactions, DNA repair, and inhibition of metastasis. The up-regulation of IGFBP-3 gene has been shown to be crucial in 1,25(OH)2D3-mediated inhibition of LNCaP cell growth. 1,25(OH)2D3 regulation of androgen-responsive genes as well as genes involved in androgen catabolism suggests that there are interactions between 1,25(OH)2D3 and androgen signaling pathways in LNCaP cells. Further studies on the role of these genes and others in mediating the anti-cancer effects of 1,25(OH)2D3 may lead to better approaches to the prevention and treatment of prostate cancer.

Kruzelock, R. P., et al. (2000). "Functional evidence for an ovarian cancer tumor suppressor gene on chromosome 22 by microcell-mediated chromosome transfer." Oncogene **19**(54): 6277-6285.

The identity of many tumor suppressor genes important in epithelial ovarian cancer tumorigenesis remains unknown. In an effort to localize a novel tumor suppressor on chromosome 22, a psv2neo tagged human chromosome 22 was transferred into the malignant epithelial ovarian cancer cell line, SKOv-3, by microcell-mediated chromosome transfer. Complete suppression of the transformed phenotype was observed in 16 of 18 individual microcell hybrid clones as evidenced by the complete abrogation of cell growth under anchorage-independent conditions. In vitro doubling times were also dramatically reduced, as was the ability to form subcutaneous tumors in CD1 nu/nu mice. Only one polymorphic marker, D22S429, segregated with decreased transformation and tumorigenic potential, suggesting that an unrecognized tumor suppressor may localize to chromosome 22q11-q12. These data provide functional support for the presence of a novel tumor suppressor locus (or loci) on chromosome 22 that is important in ovarian cancer tumorigenesis.

Kubota, Y., et al. (1991). "[Study of c-myc gene transfected T-24 human bladder cancer cells]." Nihon Hinyokika Gakkai Zasshi **82**(4): 593-599.

To investigate the roll of c-myc protooncogene in human bladder cancer, c-myc gene was transfected into T-24 human bladder cancer cells and the changes of cell characteristics were studied. C-myc gene transfection was performed, using the electroporation method described previously (J.J. Urology, 80, 1989). After electroporation, c-myc gene was transfected and neo cells were cloned in a neomycin containing medium. One typical cloned cell (myc-cl3) was obtained. And this cell clone was shown to contain more than 3 extra-copies of c-myc gene by Southern blotting analysis. Morphology and growth speed of the myc-cl3 cells were not significantly different from those of original T-24 cells. However, they easily made overlapped cell-layers in the confluent growth phase. In the soft-agarose semi-solid medium, myc-cl3 cells formed about 35 times more numerous colonies than T-24 cells. Myc-cl3 cells also formed tumors on nude-mice at a significantly higher rate than T-24 cells did. These results suggest that c-myc gene plays a key roll in clonal growth and tumor formation in human bladder cancer.

Kuhn, H., et al. (2002). "Adenovirus-mediated E2F-1 gene transfer in nonsmall-cell lung cancer induces cell growth arrest and apoptosis." Eur Respir J **20**(3): 703-709.

Since overexpression of E2F-1 has been shown to induce apoptosis, the ability of adenovirus-mediated transfer of E2F-1 to inhibit tumour growth in nonsmall-cell lung cancer cell lines was investigated. Three cell lines with various genomic status were infected with AdE2F. Cell proliferation and viability were determined by trypan blue exclusion. Apoptosis induction was assessed by flow cytometry and poly-adenosine diphosphate-ribose-polymerase cleavage assay. In vivo, the effect of E2F-1 on tumour growth was determined in severe combined immunodeficiency (SCID) mice. The current experiments showed that overexpression of E2F-1 suppressed tumour cell growth. The population of apoptotic cells was dramatically increased 96 h after infection with AdE2F. Inhibition of cell growth and induction of apoptosis was not dependent on genomic status. Moreover, treatment of implanted tumours in SCID mice with AdE2F inhibited tumour growth. These data suggest that adenovirus-mediated E2F-1 gene therapy may be effective in the treatment of nonsmall-cell lung cancer.

Kumagai, T., et al. (1996). "Eradication of Myc-overexpressing small cell lung cancer cells transfected with herpes simplex virus thymidine kinase gene containing Myc-Max response elements." Cancer Res **56**(2): 354-358.

Herpes simplex virus thymidine kinase (HSV-TK) gene was ligated with four repeats of the Myc-Max response elements (a core nucleotide sequence CACGTG), and its utility for gene therapy was examined by the treatment of either c-, L- or N-myc-overexpressing the small cell lung cancer (SCLC) cell line with ganciclovir (GCV). The chloramphenicol acetyltransferase assay demonstrated that the overexpression of any myc genes activated transcription from the CAT gene depending on the Myc-Max binding sites. The transduction of the HSV-TK gene ligated with the CACGTG core rendered all three SCLC lines to be more sensitive to GCV than parental ones in vitro. In addition, the growth of c- or L-myc-overexpressing SCLC cells containing the hybrid HSV-TK gene were significantly suppressed by GCV in vivo. When parental SCLC cells were mixed with HSV-TK-expressing tumor cells at a ratio of 1:3, GCV treatment inhibited tumor growth by 90% compared with parental cells only, indicating the existence of the "bystander effect." These data suggest that the CACGTG-driven HSV-TK gene may be useful for the treatment of SCLC overexpressing any type of myc family oncogenes.

Kusumoto, M., et al. (1999). "Adenovirus-mediated p53 gene transduction inhibits telomerase activity independent of its effects on cell cycle arrest and apoptosis in human pancreatic cancer cells." Clin Cancer Res **5**(8): 2140-2147.

Evidence for a relationship between overexpression of wild-type p53 and telomerase activity remains controversial. We investigated

whether p53 gene transduction could cause telomerase inhibition in pancreatic cancer cell lines, focusing on the relation of transduction to growth arrest, cell cycle arrest, and apoptotic cell death. The cells were infected with recombinant adenovirus expressing wild-type p53 or p21WAF1 at a multiplicity of infection of 100 or were continuously exposed to 10 microM VP-16, which is well known to induce apoptosis. Adenovirus-mediated p53 gene transduction caused G1 cell cycle arrest, apoptosis, and resultant growth inhibition in MIA PaCa-2 cells; the cell number 2 days after infection was 50% of preinfection value, and 13% of the cells were dead. Moreover, the transduction resulted in complete depression of telomerase activity through down-regulation of hTERT mRNA expression. In contrast, p21WAF1 gene transduction only arrested cell growth and cell cycle at G1 phase, and VP-16 treatment inhibited cell growth with G2-M arrest and apoptosis; after treatment, the cell number was 73% of pretreatment, and 12% of the cells were dead. Neither p21WAF1 gene transduction nor VP-16 treatment caused telomerase inhibition. Similar results were obtained in two other pancreatic cancer cell lines, SUIT-2 and AsPC-1. Thus, our results demonstrate that the p53 gene transduction directly inhibits telomerase activity, independent of its effects on cell growth arrest, cell cycle arrest, and apoptosis.

Lambert, J. R., et al. (2006). "Prostate derived factor in human prostate cancer cells: gene induction by vitamin D via a p53-dependent mechanism and inhibition of prostate cancer cell growth." J Cell Physiol **208**(3): 566-574.

The secosteroid hormone 1alpha, 25-dihydroxyvitamin D3 (1,25D) has been shown to regulate the growth and differentiation of human prostate cancer (PCa) cells, although the precise molecular mechanisms mediating these effects have not been defined. Previous studies in our laboratory demonstrated that the antiproliferative effects of 1,25D on PCa cells are mediated through the nuclear vitamin D receptor (VDR). In the present study, we performed gene profiling of LNCaP human PCa cells following 1,25D treatment and identified the antitumorigenic gene, prostate derived factor (PDF), as being highly induced by 1,25D. PDF is a member of the TGF-beta superfamily and has been implicated in a variety of functions directly related totumorigenicity including antiproliferative and pro-apoptotic effects. Gene expression studies using 1,25D analogs and a VDR antagonist demonstrate that 1,25D-mediated induction of PDF message and protein in PCa cells is dependent on VDR action. PDF is a transcriptional target of the tumor suppressor, p53. Here we show that the expression of PDF in nine PCa cell lines is dependent on functional p53. Additionally, transfection of p53-null ALVA-31 PCa cells with a p53 expression plasmid, and expression of dominant negative p53 in LNCaP PCa cells, show that the ability of VDR to induce PDF requires functional p53. Importantly, forced PDF expression in PC-3 cells results in decreased cell proliferation, soft agar cloning, and xenograft tumor size. These data demonstrate that PDF exerts antitumorigenic properties on PCa cells and its regulation by 1,25D may provide insights into the action of 1,25D in PCa.

Lebedeva, I. V., et al. (2002). "The cancer growth suppressing gene mda-7 induces apoptosis selectively in human melanoma cells." Oncogene **21**(5): 708-718.

Human melanoma cells growth arrest irreversibly, lose tumorigenic potential and terminally differentiate after treatment with a combination of fibroblast interferon (IFN-beta) and the protein kinase C activator mezerein (MEZ). Applying subtraction hybridization to this model differentiation system permitted cloning of melanoma differentiation associated gene-7, mda-7. Expression of mda-7 inversely correlates with melanoma development and progression, with elevated expression in normal melanocytes and nevi and increasingly reduced expression in radial growth phase, vertical growth phase and metastatic melanoma. When expressed by means of a replication incompetent adenovirus (Ad.mda-7) growth of melanoma, but not normal early passage or immortal human melanocytes, is dramatically suppressed and cells undergo programmed cell death (apoptosis). Infection of metastatic melanoma cells with Ad.mda-7 results in an increase in cells in the G (2)/M phase of the cell cycle and changes in the ratio of pro-apoptotic (BAX, BAK) to anti-apoptotic (BCL-2, BCL-XL) proteins. Ad.mda-7 infection results in a temporal increase in mda-7 mRNA and intracellular MDA-7 protein in most of the melanocyte/melanoma cell lines and secretion of MDA-7 protein is readily detected following Ad.mda-7 infection of both melanocytes and melanoma cells. The present studies document a differential response of melanocytes versus melanoma cells to ectopic expression of mda-7 and support future applications of mda-7 for the gene-based therapy of metastatic melanoma.

Lee, A. V., et al. (1997). "Activation of estrogen receptor-mediated gene transcription by IGF-I in human breast cancer cells." J Endocrinol **152**(1): 39-47.

Estrogen and IGF-I are potent mitogens for most breast cancer cell lines, and although their signaling pathways contrast, there is considerable interaction between them. Recent evidence indicating that IGF-I can alter estrogen receptor (ER) action led us to investigate whether an inhibitor of IGF-I action. IGF-

binding protein-1 (IGFBP-1), could affect transcriptional activation of ER. First, we confirmed that tamoxifen (TAM) could inhibit IGF-I-mediated proliferation of MCF-7 cells. Although TAM can increase IGFBP-3 expression in MCF-7 cells, and this binding protein has been shown to be able to inhibit IGF action, TAM had no effect on IGF-I-stimulated tyrosine phosphorylation of IGF-I receptor or the downstream signaling molecule, insulin receptor substrate-1. Therefore, to confirm that IGF-I was affecting transcriptional activation of ER, we utilized a gene reporter assay using a single consensus estrogen response element (ERE-tk-luc) upstream of luciferase. As expected, estradiol (E2; 1nM) increased transcriptional activation three- to fivefold from the ERE in three ER-positive breast cancer cell lines (MCF-7, ZR-75 and T47D). A 2.5-to 4-fold increase was also seen with IGF-I (5 nM). TAM (1 microM) effectively blocked activation by E2 and IGF-I, indicating disruption of ER-mediated transcription. As expected, human recombinant IGFBP-1 (80 nM) completely inhibited IGF-I-mediated activation of ER, however, IGFBP-1 also caused a significant decrease in E2-mediated activation. We also noticed that IGF-I increased the activity of all plasmids that we cotransfected including TATA-luc, SV40-luc and pGL Basic. This effect was post-transcriptional, as it was not affected by actinomycin D (2 micrograms/ml), while we were able to completely inhibit E2-mediated transcriptional activation of ERE-tk-luc. Unlike the complete inhibition of ER-mediated transcriptional activation by actinomycin D, IGF-I-mediated transactivation was reduced by only 50%, indicating that the activation by IGF-I represented both transcriptional and post-transcriptional effects. This study confirmed that IGF-I can cause transcriptional activation of endogenous ER in human breast cancel cells, and inhibition of ER action by IGFBP-1 suggests that IGF-1 signaling may be necessary for maximal ER activation.

Lee, C. H., et al. (1996). "Prostate-specific antigen promoter driven gene therapy targeting DNA polymerase-alpha and topoisomerase II alpha in prostate cancer." Anticancer Res **16**(4A): 1805-1811.

Attainment of cell type-specific cytotoxicity with minimal side effects is the ultimate goal of cancer therapy. By employing the prostate-specific antigen promoter (PSAP), we investigated (1) whether PSAP-driven antisense genetic constructs targeting DNA polymerase-alpha and topoisomerase II alpha (Top II alpha), designated PSAP-antipol and PSAP-antitop respectively, could induce death of prostate cancer cells, and (2) whether the cytotoxicity is restricted to cells of prostate origin. A PSAP-driven beta-galactosidase gene, PSAP-LacZ, was also used to estimate the expression of the PSAP-driven transcripts. Lipofection-mediated gene transfers were performed with these 3 constructs and a control plasmid, pCDNA3, in 3 human prostate cancer cell lines (LNCaP, DU-145, PC-3) and 5 other cell lines (Cos-1 [monkey kidney], HL-60 [human myeloid leukemia], Hep G2 [human hepatoma], NCI H460 [human lung cancer] and SW 480 [human colon cancer]). On transfection with PSAP-LacZ, LNCaP, DU-145, and PC-3 showed a 10.8, 1.8, and 1.6 fold increase in beta-galactosidase activity, respectively. The remaining 5 cell lines showed no changes after transfection. Corresponding to the levels of the induced beta-galactosidase activity, LNCaP showed the strongest growth inhibition by the antisense constructs: 36% by PSAP-antipol, 39% by PSAP-antitop and 80% by PSAP-antipol+PSAP-antitop. DU-145 and PC-3 had minimal growth inhibition with PSAP-antipol alone or PSAP-antitop alone. However, when cotransfected with PSAP-antipol and PSAP-antitop, DU-145 and PC-3 displayed 42% and 55% growth inhibition, respectively. In contrast, no cytotoxicity was observed in the remaining 5 cell lines when transfected with PSAP-antipol, PSAP-antitop or both. Therefore, PSAP-driven antisense gene therapy targeting DNA polymerase-alpha and Top II alpha inhibits the growth of human prostate cancer cells and the cytotoxic effect is restricted in cells of prostate origin.

Lee, J. H., et al. (1998). "The inhibitory effect of adenovirus-mediated p16INK4a gene transfer on the proliferation of lung cancer cell line." Anticancer Res **18**(5A): 3257-3261.

Abnormalities in the p16INK4a tumor suppressor gene are found in many lung cancer cell lines and primary lung cancer tissue. To examine its tumor suppressor function and potential adequacy in cancer gene replacement therapy, wild-type p16INK4a gene was inserted in an adenovirus derived gene delivery system and introduced into lung cancer cell lines (NCI-H441 and NCI-H157) that did not express p16INK4a. Western blot assay and immunocytochemistry demonstrated production of wild-type p16 protein in these cell lines. The biological function of exogenous p16 protein was confirmed by the inhibition of pRB phosphorylation. The expression of exogenous p16 protein via recombinant adenovirus significantly inhibited cancer cell growth and colony formation in vitro of NSCLC that can not express endogenous p16. The flow cytometric analysis showed these results correlated with G1 cell cycle arrest. These observations suggest the value of adenovirally-mediated p16INK4a gene replacement therapy for lung cancer.

Lee, W. H. and E. Y. Lee (1997). "[The retinoblastoma gene: from its basic understanding as a signal mediator for growth and differentiation to its use in the treatment of cancer]." Gan To Kagaku Ryoho **24**(11): 1368-1380.

The retinoblastoma gene (RB) plays important roles in the genesis of human cancers. Several pieces of evidence have shown that the retinoblastoma protein (Rb) has dual roles in gating cell cycle progression and promoting cellular differentiation. The molecular mechanisms involved in these roles have been elucidated in some biological systems: Rb sequesters the transcription factors of E2F-1 to regulate entry of cell cycle, but enhances the activities of transcription factors such as C/ EBPs to initiate terminal differentiation. Thus, the Rb protein can serve as a mediator of extracellular signals for growth or differentiation. RB also has a tumor suppression function. Introduction of wild-type RB into human tumor cells deficient for RB suppresses their tumorigenicity in nude mice. Similarly, mice with RB+/- genotypes created by gene knockout methods spontaneously develop melanotrophic tumors with complete penetrance. Immune-competent RB+/- mice benefited significantly from adenovirus-mediated RB gene therapy. The fundamental knowledge of Rb function will allow us to take the next steps toward the use of tumor suppressor genes in the treatment of human cancer.

Leung, W. K., et al. (2004). "Effect of peroxisome proliferator activated receptor gamma ligands on growth and gene expression profiles of gastric cancer cells." Gut **53**(3): 331-338.

BACKGROUND AND AIMS: Although peroxisome proliferator activated receptor gamma (PPARgamma) agonists have been implicated in differentiation and growth inhibition of cancer cells, the potential therapeutic and chemopreventive effects on gastric cancer are poorly defined. We examined the in vitro and in vivo effects of PPARgamma ligands on growth of gastric cancer, and the effect of PPARgamma activation on expression of cyclooxygenase 2 (COX-2) and cancer related genes. METHODS: Gastric cell lines (MKN28 and MKN45) were treated with two specific PPARgamma ligands: ciglitazone and 15-deoxy-Delta (12,) (14)-prostaglandin J (2). Cell growth was determined by bromodeoxyuridine incorporation assay and apoptosis was measured by DNA fragmentation. Expression of COX-2 was determined by western blot and real time quantitative polymerase chain reaction (PCR). Expression profiles of cancer related genes were screened with cDNA array. In vivo growth of implanted MKN45 cells in nude mice was monitored after oral treatment with rosiglitazone. RESULTS: PPARgamma ligands suppressed the in vitro growth of MKN45 cells in a dose dependent manner whereas prostacyclin, a PPARdelta agonist, had no growth inhibitory effect. Growth inhibition was more pronounced in MKN45 cells, which was accompanied by DNA fragmentation and downregulation of COX-2. Screening by cDNA microarray showed that PPARgamma ligand treatment was associated with upregulation of bad and p53, and downregulation of bcl-2, bcl-xl, and cyclin E1 in MKN45 cells, which was confirmed by quantitative real time PCR. In contrast, MKN28 cells with lower PPARgamma and COX-2 expression levels had lower growth inhibitory responses to PPARgamma ligands. Microarray experiments only showed induction of the bad gene in MKN28 cells. In vivo growth of MKN45 cells in nude mice was retarded by rosiglitazone. Mean tumour volume in rosiglitazone treated mice was significantly lower than controls at six weeks ($p = 0.019$) and seven weeks ($p = 0.001$) after treatment. CONCLUSIONS: PPARgamma ligands suppress both in vitro and in vivo growth of gastric cancer and may play a major role in cancer therapy and prevention.

Lilja, J. F., et al. (2001). "Growth suppression activity of the PTEN tumor suppressor gene in human endometrial cancer cells." Anticancer Res **21**(3B): 1969-1974.

The AKT proteins are constitutively activated in several types of human cancers, which may play a role in carcinogenesis. In this study, we examined the activation of AKT in a panel of human endometrial cancer cell lines and tumor samples in this study. Two endometrial cancer cell lines, Ishikawa (ISK) and RL-95 and several tumor samples showed elevated levels of phosphorylated AKT PTEN, which is mutated in 45% of endometrial cancers, is a negative regulator of AKT. We examined the growth suppression activity of PTEN in ISK and KLE endometrial cancer cells. Expression of PTEN significantly suppressed the growth of both cell clines. In primary rat embryo fibroblasts, PTEN also inhibited malignant transformation mediated by ras and c-myc oncogenes. These two oncogenes are commonly mutated or amplified in endometrial cancer. These results suggest that PTEN may be a potent therapeutic agent for endometrial cancer.

Lindner, D. J., et al. (1997). "Tamoxifen enhances interferon-regulated gene expression in breast cancer cells." Mol Cell Biochem **167**(1-2): 169-177.

The molecular basis for the enhanced growth inhibition of MCF-7 human breast cancer xenografts by a combination of human interferon-beta (IFN-beta) and tamoxifen was investigated. Treatment of MCF-7,

MDA-MB-231, and BT-20 cells with the combination of IFN-beta and tamoxifen resulted in enhanced antiproliferative effects in vitro. Treatment with the combination of IFN-beta and tamoxifen enhanced the expression of several IFN-beta-inducible genes in human breast carcinoma cell lines relative to levels induced by IFN-beta alone. Tamoxifen alone did not induce transcription of IFN-stimulated genes (ISGs). Augmentation of ISG expression by the combination of IFN-beta and tamoxifen was noted in breast tumor cell lines irrespective of their functional estrogen receptor (ER) status or their dependence on estradiol for growth, suggesting that upregulation of ISGs was independent of ER status. Enhancement of IFN-stimulated gene expression by tamoxifen occurred at the transcriptional level. Expression of transfected reporter genes under the control of IFN-alpha/beta regulated promoters was also enhanced in IFN-beta and tamoxifen-treated cells. Similarly, transcriptional induction of chimeric reporter plasmids driven by an IFN-gamma inducible promoter (GAS; IFN-gamma activated site) was also enhanced by the combination of IFN-gamma and tamoxifen. In tamoxifen treated cells, IFN-beta and IFN-gamma readily activated transcription factors ISGF-3 and GAF, respectively. Therefore, augmentation of ISG expression by tamoxifen is an early event in the antitumoral activity of this drug combination.

Lipponen, P. K. and T. J. Liukkonen (1995). "Reduced expression of retinoblastoma (Rb) gene protein is related to cell proliferation and prognosis in transitional-cell bladder cancer." J Cancer Res Clin Oncol **121**(1): 44-50.

Archival biopsy specimens from transitional-cell bladder cancers (n = 222) were analysed immunohistochemically for expression of retinoblastoma (Rb) gene protein. The intensity of staining for Rb protein and the fraction of positive nuclei were analysed and related to several other prognostic factors and survival. Six per cent of the tumours were totally negative for Rb protein and abnormal (weak) expression was found in 40% of cases. The fraction of positive nuclei and abnormal expression (weak) were highly significantly interrelated (P < 0.0001). A low value for the fraction of Rb-protein-positive nuclei was related to a large fraction in S phase (P = 0.001), high mitotic index (P = 0.016) and overexpression of epidermal growth factor receptor (P = 0.034) and p53 protein (P = 0.019). A normal Rb protein expression pattern was related to low S-phase values (P = 0.0001) whereas over-expression of p53 was related to high S-phase values (P = 0.0077). Morphometrically measured nuclear atypia and the fraction of Rb-protein-positive nuclei were negatively correlated (P < 0.05). In univariate survival analysis altered expression of Rb protein (P = 0.07) and low frequency (< or = 50%) of Rb-protein-positive nuclei (P = 0.0128) predicted a poor outcome. In a multivariate analysis, reduced expression of Rb protein had no independent prognostic value over T category, papillary status and the size of the S-phase fraction. The results show that tumor-suppressor genes Rb and p53 participate in the growth regulation of human bladder cancer cells in vivo and accordingly modify the prognosis.

Liu, J. C., et al. (2012). "Seventeen-gene signature from enriched Her2/Neu mammary tumor-initiating cells predicts clinical outcome for human HER2+:ERalpha- breast cancer." Proc Natl Acad Sci U S A **109**(15): 5832-5837.

Human Epidermal Growth Factor Receptor 2-positive (HER2(+)) breast cancer (BC) is a highly aggressive disease commonly treated with chemotherapy and anti-HER2 drugs, including trastuzumab. There is currently no way to predict which HER2(+) BC patients will benefit from these treatments. Previous prognostic signatures for HER2(+) BC were developed irrespective of the subtype or the hierarchical organization of cancer in which only a fraction of cells, tumor-initiating cells (TICs), can sustain tumor growth. Here, we used serial dilution and single-cell transplantation assays to identify MMTV-Her2/Neu mouse mammary TICs as CD24(+):JAG1(-) at a frequency of 2-4.5%. A 17-gene Her2-TIC-enriched signature (HTICS), generated on the basis of differentially expressed genes in TIC versus non-TIC fractions and trained on one HER2(+) BC cohort, predicted clinical outcome on multiple independent HER2(+) cohorts. HTICS included up-regulated genes involved in S/G2/M transition and down-regulated genes involved in immune response. Its prognostic power was independent of other predictors, stratified lymph node (+) HER2(+) BC into low and high-risk subgroups, and was specific for HER2(+):estrogen receptor alpha-negative (ERalpha (-)) patients (10-y overall survival of 83.6% for HTICS (-) and 24.0% for HTICS (+) tumors; hazard ratio = 5.57; P = 0.002). Whereas HTICS was specific to HER2(+):ERalpha (-) tumors, a previously reported stroma-derived signature was predictive for HER2(+):ERalpha (+) BC. Retrospective analyses revealed that patients with HTICS (+) HER2(+):ERalpha (-) tumors resisted chemotherapy but responded to chemotherapy plus trastuzumab. HTICS is, therefore, a powerful prognostic signature for HER2(+):ERalpha (-) BC that can be used to identify high risk patients that would benefit from anti-HER2 therapy.

Lopez-Lazaro, M. (2018). "Cancer etiology: Variation in cancer risk among tissues is poorly

explained by the number of gene mutations." Genes Chromosomes Cancer **57**(6): 281-293.

Recent evidence indicates that the risk of being diagnosed with cancer in a tissue is strongly correlated (0.80) with the number of stem cell divisions accumulated by the tissue. Since cell division can generate random mutations during DNA replication, this correlation has been used to propose that cancer is largely caused by the accumulation of unavoidable mutations in driver genes. However, no correlation between the number of gene mutations and cancer risk across tissues has been reported. Because many somatic mutations in cancers originate prior to tumor initiation and the number of cell divisions occurring during tumor growth is similar among tissues, I use whole genome sequencing information from 22 086 cancer samples and incidence data from the largest cancer registry in each continent to study the relationship between the number of gene mutations and the risk of cancer across 33 tissue types. Results show a weak positive correlation (mean = 0.14) between these 2 parameters in each of the 5 cancer registries. The correlation became stronger (mean = 0.50) when gender-related cancers were excluded. Results also show that 1003 samples from 29 cancer types have zero mutations in genes. These data suggest that cancer etiology can be better explained by the accumulation of stem cell divisions than by the accumulation of gene mutations. Possible mechanisms by which the accumulation of cell divisions in stem cells increases the risk of cancer are discussed.

Luparello, C., et al. (2003). "T47-D cells and type V collagen: a model for the study of apoptotic gene expression by breast cancer cells." Biol Chem **384**(6): 965-975.

We have previously reported that type V collagen is a poorly adhesive, anti-proliferative and motility-inhibitory substrate for the 8701-BC breast cancer cell line, which also triggers DNA fragmentation and impairs survival of the same cell line. In the present work we have extended to other breast cancer cell lines (T47-D, MDA-MB231, Hs578T) our investigation of type V collagen influence on the DNA status and cell survival, also examining whether adhesion and growth of cells on this collagen substrate could exert some effect on the expression level of selected apoptosis-related genes. We report here that, among the cell lines tested, only T47-D is responsive to the death-promoting influence of type V collagen. In addition, the latter induces changes in gene expression by up-regulating p53, Waf-1, Cas, Dap kinase and caspases 1, -5 and -14 and down-regulating Bcl-2. Our data validate the T47-D line as a suitable in vitro model for further and more detailed studies on the molecular mechanisms of the death response induced by type V collagen on mammary tumor cells.

Maemondo, M., et al. (2004). "Gene therapy with secretory leukoprotease inhibitor promoter-controlled replication-competent adenovirus for non-small cell lung cancer." Cancer Res **64**(13): 4611-4620.

Secretory leukoprotease inhibitor (SLPI) is highly expressed in almost all non-small cell lung cancers (NSCLCs), but not in the majority of other tumor types. In an attempt to create a specific gene therapy for NSCLC, we constructed AdSLPI.E1AdB, an adenovirus vector with a double expression cassette consisting of E1A driven by the SLPI promoter gene followed by E1B-19K under the control of the cytomegalovirus (CMV) promoter that can selectively replicate only in NSCLC cells. Infection with AdSLPI.E1AdB yielded E1A protein expression and adenovirus replication resulting in a >100-fold increase of the virus titers only in SLPI-producing NSCLC cells (A549, H358, and HS24 cells). In contrast, neither E1A protein nor replication was detected in non-SLPI-producing HepG2 cells. Treatment with AdSLPI.E1AdB significantly inhibited the proliferation of NSCLC cells in vitro in a dose-dependent manner, whereas the cell growth of HepG2 or normal human bronchial epithelial cells was not affected by AdSLPI.E1AdB infection. Direct injection of AdSLPI.E1AdB into A549 and H358 tumors in nude mice resulted in a marked reduction in tumor growth compared with controls (A549, 57%, $P < 0.02$; H358, 67%, $P < 0.03$). Histological examination revealed the replication of AdSLPI.E1AdB and strong induction of necrosis and apoptosis. In addition, we evaluated the combination of AdSLPI.E1AdB and AdCMV.NK4 encoding NK4 protein, which has strong antiangiogenic activity. E1A expressed by AdSLPI.E1AdB trans-acts on the replication of AdCMV.NK4 and thus increases the expression of NK4. Injection of these two vectors into H358 tumors resulted in a more striking reduction of tumor growth compared with single injection of each vector. These results suggest that AdSLPI.E1AdB could provide a selective therapeutic modality for NSCLC and that the combination of AdSLPI.E1AdB and AdCMV.NK4 may be a more effective gene therapy for NSCLC.

Manabe, T., et al. (2003). "Cell-based protein delivery system for the inhibition of the growth of pancreatic cancer: NK4 gene-transduced oral mucosal epithelial cell sheet." Clin Cancer Res **9**(8): 3158-3166.

PURPOSE: Pancreatic resection for pancreatic cancer is the only curative modality, but the high incidence of local recurrence after surgery results in a very poor prognosis. This study aims to develop a new therapeutic tool that could inhibit the growth of

remnant cancer cells, which is based on local delivery of NK4 (hepatocyte growth factor antagonist) secreted from an NK4 gene-transduced oral mucosal epithelial cell (OMEC) sheet (NK4-sheet), which is adhered to the resected surface. EXPERIMENTAL DESIGN: OMECs, harvested and cultured according to 3T3 feeder layer technique, were seeded on a collagen mesh-overlayered, biodegradable VICRYL mesh to produce an OMEC sheet. NK4 gene transduction was mediated by recombinant adenovirus (Ad-NK4). Applicability of OMECs for cell-based NK4 delivery was examined. An experimental model using nude mice was established to determine the effect of an NK4-sheet on both tumor growth and angiogenesis. RESULTS: NK4 secreted from Ad-NK4-transduced OMECs suppressed MRC-5-induced invasion of pancreatic cancer cell lines. Heterotopically implanted gene-transduced OMECs remained for >/==" BORDER="0">10 days while gradually decreasing. NK4-sheets inhibited both angiogenesis and tumor growth in vivo. CONCLUSION: Autologous OMEC was found to be suited to this purpose because of no secretion of hepatocyte growth factor, ease in harvesting from a patient, reasonably high proliferation potential, and no immune reaction. Although NK4-sheets under development exhibited a low level and short period of NK4 secretion, it is expected that this system may have a great potentiality of protein delivery system to target tissue at clinical situations when it is loaded with multilayered OMECs.

Manjeshwar, S., et al. (2003). "Tumor suppression by the prohibitin gene 3'untranslated region RNA in human breast cancer." Cancer Res **63**(17): 5251-5256.

Prohibitin is a candidate tumor suppressor gene located on human chromosome 17q21, a region of frequent loss of heterozygosity in breast cancers. We showed previously that microinjection of RNA encoded by the prohibitin gene 3'untranslated region (3'UTR) blocks the G (1)-S transition causing cell cycle arrest in several human cancer cell lines, including MCF7. Two allelic forms (C versus T) of the prohibitin 3'UTR exist, and carriers of the less common variant (Tallele) with a family history of breast cancer exhibited an increased risk of breast cancer. In the present study, we examined the tumor suppressor activity of the prohibitin 3'UTR in human breast cancer cells. Stable clones of MCF7 cells expressing either the C allele or the T allele RNA under the control of the cytomegalovirus promoter were isolated and compared with empty vector clones. Clones expressing the C allele RNA (UTR/C) exhibited significant suppression of growth in cell proliferation assays, inhibition of colony formation in soft agar assays, and suppression of xenograft tumor growth when implanted on nude mice, compared with either T allele expressing or empty vector clones. Immunohistochemical analyses with Ki67 staining confirmed a significant reduction in proliferation of UTR/C tumors. Thus, the C allele of prohibitin 3'UTR produces a functional RNA, whereas a single nucleotide polymorphism creates a null allele (T allele) of which the RNA product has lost activity. Our data demonstrate for the first time that an RNA molecule functions as a tumor suppressor in human breast cancer.

Margueron, R., et al. (2003). "Oestrogen receptor alpha increases p21(WAF1/CIP1) gene expression and the antiproliferative activity of histone deacetylase inhibitors in human breast cancer cells." J Endocrinol **179**(1): 41-53.

We analysed the antiproliferative activity of various histone deacetylase (HDAC) inhibitors such as trichostatin A (TSA) on human breast cancer cells. We observed a lower sensitivity to HDAC inhibition for oestrogen receptor negative (ER-) versus positive (ER+) cell lines. This differential response was associated neither with a modification of drug efflux via the multidrug resistance system nor with a global modification of histone acetyltransferase (HAT)/HDAC activities. In contrast, we demonstrated that in ER+ breast cancer cells the p21(WAF1/CIP1) gene was more sensitive to TSA regulation and was expressed at higher levels. These differences were observed both in transient transfection experiments and on the endogenous p21(WAF1/CIP1) gene. The Sp1 transcription factor, which was shown to interact in vitro with both class I and class II HDACs, is sufficient to confer the differential sensitivity to TSA and participated in the control of p21(WAF1/CIP1) basal expression. Finally, re-expression of ERalpha following adenoviral infection of ER- breast cancer cells increased both p21(WAF1/CIP1) protein accumulation and the growth inhibitory activity of TSA. Altogether, our results highlight the key role of ERalpha and p21(WAF1/CIP1) gene expression in the sensitivity of breast cancer cells to hyperacetylating agents.

Markowitz, S. D., et al. (1994). "A benign cultured colon adenoma bears three genetically altered colon cancer oncogenes, but progresses to tumorigenicity and transforming growth factor-beta independence without inactivating the p53 tumor suppressor gene." J Clin Invest **93**(3): 1005-1013.

We describe the spontaneous progression of a colon adenoma cell line to tumorigenicity and growth factor independence. This system allows direct comparison of biologic stages of malignant progression with alterations of colon cancer suppressor

genes and oncogenes. VACO-235, a human colon adenoma cell line, is at early passages nontumorigenic in the nude mouse, unable to grow in soft agar, growth stimulated by serum and EGF, and growth inhibited by TGF-beta. VACO-235 daughter passages 93 and higher have in culture spontaneously progressed to being weakly tumorigenic, but retain all other growth characteristics of VACO-235 early passages. A mouse xenograft from late passage VACO-235 was reestablished in culture as the granddaughter cell line, VACO-411. VACO-411 is highly tumorigenic, clones in soft agar, and is unresponsive to serum, EGF, and TGF-beta. Early passage VACO-235 bears a mutant K-ras allele, bears only mutant APC alleles, expresses no DCC transcripts, and expresses only wild type p53 transcripts. VACO-411 retains the identical genotype, still expressing only wild type p53. Colonic cells after ras mutation, APC mutation, and DCC inactivation remain nontumorigenic and growth factor dependent. Malignant progression involves at least two additional steps, and in VACO-411 can proceed by a novel pathway not requiring p53 inactivation.

Matozaki, T., et al. (1992). "p53 gene mutations in human gastric cancer: wild-type p53 but not mutant p53 suppresses growth of human gastric cancer cells." Cancer Res **52**(16): 4335-4341.

To further investigate the role of p53 gene inactivation in gastric tumorigenesis, the mutational status of the p53 gene in primary human gastric cancer samples was examined. Reverse transcriptase polymerase chain reaction and subsequent direct sequencing of the p53 gene from gastric cancer samples revealed frequent point mutations of the p53 gene: some of these coincided with those previously identified in gastric cancer cell lines. In addition, both allelic deletion analysis using pYNZ 22 and polymerase chain reaction-restriction fragment length polymorphism analysis demonstrated an allelic deletion of the p53 gene in cancer tissue which contained a point mutation of the p53 gene in the remaining allele. Transfection of the wild-type or mutant p53 genes into gastric cancer cells showed that the wild-type but none of the mutated p53 genes suppressed the colony formation of gastric cancer cells. Furthermore, the incorporation of thymidine into DNA was reduced in cancer cells expressing the wild-type p53 gene. The glutathione S-transferase-wild type p53 fusion protein bound to simian virus 40 large T antigen in COS-1 cell lysate. None of the p53 fusion proteins containing mutations at codons 143, 175, 248, or 273 bound to simian virus 40 large T antigen. By contrast, two different mutant p53 fusion proteins containing mutations specifically observed in gastric cancer bound to simian virus 40 large T antigen. These results indicate that inactivation of the p53 gene through mutations and the allelic deletion may play an important role in gastric tumorigenesis. These mutations may cause a conformational change in the p53 protein resulting in the loss of the suppression by p53 of the growth of gastric cells, partly through disruption of the association of p53 protein with a cellular component.

Matsushima-Nishiu, M., et al. (2001). "Growth and gene expression profile analyses of endometrial cancer cells expressing exogenous PTEN." Cancer Res **61**(9): 3741-3749.

The PTEN tumor suppressor gene encodes a multifunctional phosphatase that plays an important role in inhibiting the phosphatidylinositol-3-kinase pathway and downstream functions that include activation of Akt/protein kinase B, cell survival, and cell proliferation. Enforced expression of PTEN in various cancer cell lines decreases cell proliferation through arrest of the cell cycle, accompanied in some cases by induction of apoptosis. We used cDNA microarrays containing 4009 cDNAs to examine changes in gene-expression profiles when exogenous PTEN was induced in PTEN-defective cells. The microarrays and subsequent semi-quantitative reverse transcription-PCR analysis revealed transcriptional stimulation of 99 genes and repression of 72 genes. Some of the differentially expressed genes already had been implicated in cell proliferation, differentiation, apoptosis, or cell cycle control, e.g., overexpression of PTEN-induced transactivation of cyclin-dependent inhibitor 1B (p27Kip1) and 2B (p15INK4B), members of the TNF receptor family, tumor necrosis factor-associated genes, and members of the Notch-signaling and Mad families. To our knowledge this is the first report of transactivation of those genes by PTEN. The genes differentially expressed in our experiments also included many whose correlation with cancer development had not been recognized before. Our data should contribute to a greater understanding of the broad spectrum of ways in which PTEN affects intracellular signaling pathways. Analysis of expression profiles with microarrays appears to be a powerful approach for identifying anticancer genes and/or disease-specific targets for cancer therapy.

Maurice-Duelli, A., et al. (2004). "Enhanced cell growth inhibition following PTEN nonviral gene transfer using polyethylenimine and photochemical internalization in endometrial cancer cells." Technol Cancer Res Treat **3**(5): 459-465.

PTEN is a tumor suppressor gene mapped on chromosome 10q23.3 and encodes a dual specificity phosphatase. PTEN has major implication in PI3 kinase (PI3K) signal transduction pathway and negatively controls PI3 phosphorylation. It has been

reported to be implicated in cell cycle progression and cell death control through inhibition of PI3K-Akt signal transduction pathway and in the control of cell migration and spreading through its interaction with focal adhesion kinase. Somatic mutations of PTEN are frequently detected in several cancer types including brain, prostate and endometrium with more than 30% of tumor tissue specimens bearing PTEN mutations and/or deletions. Because of its high frequency of mutations and its important function as tumor suppressor gene, PTEN is a good candidate for gene therapy. Inducible expression of PTEN has been also reported. In cancer cells bearing PTEN abnormalities, the reversion of PTEN function by external gene transfer becomes more and more investigated in cancer treatment research. Several technologies including the photochemical internalization (PCI) and aiming at improving the transfection efficiency have been reported. PCI is an innovative procedure based on light-induced delivery of macromolecules such as DNA, proteins and other therapeutic molecules from endocytic vesicles to the cytosol of target cells. PCI has been reported to enhance the gene delivery potential of viral and nonviral vectors. The present study was designed to evaluate the influence of photochemical internalization on polyethylenimine (PEI)-mediated PTEN gene transfer and its effects on the cellular viability in Ishikawa endometrial cancer cells bearing PTEN abnormalities. PCI was found to significantly ($P < 0.01$) enhance PTEN mRNA expression (4.2 fold increase). Subsequently, following PEI-mediated PTEN gene transfer, the restoration of the PTEN protein expression was observed. As a consequence, significant cell growth inhibition (44%) was observed in Ishikawa endometrial cells. Using PCI for PEI-mediated PTEN gene transfer was found to further enhance PTEN mRNA and protein expression as well as PTEN-related cell growth inhibition reaching 89%.

Mhashilkar, A. M., et al. (2001). "Melanoma differentiation associated gene-7 (mda-7): a novel anti-tumor gene for cancer gene therapy." Mol Med **7**(4): 271-282.

BACKGROUND: The mda-7 gene (melanoma differentiation associated gene-7) is a novel tumor suppressor gene. The anti-proliferative activity of MDA-7 has been previously reported. In this report, we analyze the anti-tumor efficacy of Ad-mda7 in a broad spectrum of cancer lines. MATERIALS AND METHODS: Ad-mda7-transduced cancer or normal cell lines were assayed for cell proliferation (tritiated thymidine incorporation assay, Alamar blue assay, and trypan-blue exclusion assay), apoptosis (TUNEL, and Annexin V staining visualized by fluorescent microscopy or FACs analysis), and cell cycle regulation (Propidium Iodide staining and FACs analysis). RESULTS: Ad-mda7 treatment of tumor cells resulted in growth inhibition and apoptosis in a temporal and dose-dependent manner. The anti-tumor effects were independent of the genomic status of p53, RB, p16, ras, bax, and caspase 3 in these cells. In addition, normal cell lines did not show inhibition of proliferation or apoptotic response to Ad-mda7. Moreover, Ad-mda7-transduced cancer cells secreted a soluble form of MDA-7 protein. Thus, Ad-mda7 may represent a novel gene-therapeutic agent for the treatment of a variety of cancers. CONCLUSIONS: The potent and selective killing activity of Ad-mda7 in cancer cells but not in normal cells makes this vector a potential candidate for cancer gene therapy.

Miki, K., et al. (2001). "Demethylation by 5-aza-2'-deoxycytidine (5-azadC) of p16INK4A gene results in downregulation of vascular endothelial growth factor expression in human lung cancer cell lines." Oncol Res **12**(8): 335-342.

Vascular endothelial growth factor (VEGF) plays a pivotal role in tumor progression via angiogenesis. Recently, gene transduction of wild-type p16INK4A, tumor suppressor gene, has been shown to result in downregulation of VEGF expression in p16INK4A-deleted glioma cells. Because expression of p16INK4A is regulated by methylation of the p16INK4A gene, we examined whether demethylation of the p16INK4A gene by 5-aza-2'-deoxycytidine (5-azadC) could cause the protein expression of VEGF as well as of p16INK4A in human lung cancer cells. For this, five different lung cancer cell lines with or without loss of p16 activity were used. H841 and Ma-10 cells had the methylated p16INK4A gene without expression of p16INK4A protein, whereas Ma-1 and H209 cells had the unmethylated p16INK4A gene with constitutive expression of p16INK4A protein. Neither the p16INK4A gene nor p16INK4A protein was detected in A549 cells. Treatment with 5-azadC caused demethylation of the p16INK4A gene with reexpression of p16INK4A protein in H841 and Ma-10 (methylated p16INK4A gene dominant) cell, but not in other cell lines such as Ma-1, H209 (unmethylated p16INK4A gene dominant), or A549 (p16INK4A gene deleted). In a parallel experiment, 5-azadC inhibited production of VEGF protein by H841 and Ma-10 cells, especially in the later hypermethylated cells, but not Ma-1, H209, or A549 cells. RT-PCR analysis showed that Ma-10 cells expressed VEGF isoforms 121, 165, and 189, all of which were inhibited by 5-azadC. These findings indicate that the methylation status of the p16INK4A gene plays an important role in the regulation of angiogenesis associated with progression of lung cancer, through regulation of VEGF expression.

Minaguchi, T., et al. (1999). "Growth suppression of human ovarian cancer cells by adenovirus-mediated transfer of the PTEN gene." Cancer Res **59**(24): 6063-6067.

A tumor suppressor gene on chromosome 10q23, PTEN, encodes a phosphatidylinositol phosphatase that antagonizes activation of the phosphatidylinositol 3'-kinase-mediated pathway involved in cell growth. A gene encoding the catalytic subunit of phosphatidylinositol 3'-kinase (PIK3CA) is frequently activated in ovarian cancers; therefore, overexpression of the PTEN product through gene transfer might be an effective strategy for treating ovarian cancers. To test the potential for this type of gene therapy, we constructed a recombinant adenovirus encoding wild-type PTEN and examined its effects on nine cell lines derived from human ovarian carcinomas. Transduction of the PTEN gene significantly inhibited growth of six of these cell lines compared with infection with virus alone, and the degree of inhibition correlated with the efficiency of gene transfer as determined by beta-galactosidase assay. Results of flow cytometry suggested that the observed effects were mediated by two mechanisms, apoptosis and/or arrest in the G1 phase of the cell cycle, and that high adenoviral transduction efficiency of cells was associated with induction of apoptosis. We also found that the level of transcription of Integrin alpha (v) in ovarian cancer cells correlated with the efficiency of transduction (P = 0.014) and with the degree of growth inhibition after PTEN gene transfer (P = 0.009). These findings carry significant implications for adenovirus vector-based PTEN gene therapies for ovarian cancers.

Mittal, R. D., et al. (2007). "Role of an androgen receptor gene polymorphism in development of hormone refractory prostate cancer in Indian population." Asian Pac J Cancer Prev **8**(2): 275-278.

BACKGROUND: Androgen receptors play critical roles in the development of primary as well as advanced hormone-refractory prostate cancers. Since the growth of prostate cancer is androgen-sensitive, metastatic disease has been treated by hormonal therapy in the form of androgen ablation. Prostate cancer cells rely on androgen receptor (AR) for proliferation and survival. AIM: To evaluate the prognostic significance of androgen receptor polymorphism in patients under hormonal therapy in any form. METHODS: Complete follow up data were available for 87 patients out of 130 patients enrolled for study. DNA was extracted from blood samples using salting out method and then subjected to PCR Genscan for CAG and GGN genotyping. The mean follow up was 10.12+/-8.83 months. RESULTS: Out of 87 patients, 64 experienced clinical as well as biochemical recurrence. The overall hormone refractory rates were 73.4% after one year. We observed a significant shorter median CAG repeats in HRPC patients (20 vs 22). The hazard ratio for HRPCs with the < or =20 CAG repeat genotype was 0.602 (0.33-1.08, p=0.09). Kaplan-Meier analysis showed that HRPC rates were not significantly associated with CAG repeat (p=0.06) but a trend was observed with short CAG repeats. No significant association was observed with AR-GGN repeats. CONCLUSIONS: A trend for association of AR-CAG repeats with HRPC patients in north Indian population was observed, suggesting this to be a prognostic factor for determining the therapeutic regimen.

Miyake, H., et al. (2000). "Synergistic chemosensitization and inhibition of tumor growth and metastasis by adenovirus-mediated P53 gene transfer in human bladder cancer model." Urology **56**(2): 332-336.

OBJECTIVES: To determine whether an adenovirus-mediated p53 gene (Ad5CMV-p53) transfer enhances cisplatin cytotoxicity in vitro and whether Ad5CMV-p53 and cisplatin synergistically inhibit growth and metastasis in vivo using human bladder cancer KoTCC-1 cells. METHODS: MTT assays and DNA fragmentation assays were used to examine the effects of treatment with Ad5CMV-p53 and/or cisplatin on growth inhibition and induction of apoptosis, respectively, in KoTCC-1 cells. The efficacies of combined Ad5CMV-p53 and/or cisplatin therapy against growth and metastasis of KoTCC-1 tumors were assessed using subcutaneous and orthotopic tumor cell injection models. RESULTS: Ad5CMV-p53 substantially enhanced cisplatin chemosensitivity in a dose-dependent manner, reducing the median IC (50) by more than 50%. Characteristic apoptotic DNA laddering was induced by the combination of sublethal doses of Ad5CMV-p53 and cisplatin, but not by either agent alone. Furthermore, combined Ad5CMV-p53 and cisplatin therapy synergistically inhibited growth of subcutaneous KoTCC-1 tumors and the incidence of metastasis after orthotopic injection. CONCLUSIONS: These findings illustrate that combined treatment with Ad5CMV-p53 and cisplatin could be an attractive strategy for inhibiting progression of bladder cancer through effective induction of apoptosis.

Morioka, C. Y., et al. (2005). "Suppression of invasion of a hamster pancreatic cancer cell line by antisense oligonucleotides mutation-matched to K-ras gene." In Vivo **19**(3): 535-538.

The anti-invasive activity of antisense oligonucleotides (ASO) specific to the K-ras gene in hamster pancreatic cancer was investigated. HaP-T1, a

cell culture derived from BHP-induced hamster pancreatic cancer, was used. After liposome-mediated transfection with mutation-matched and mutation-mismatched ASO in different concentrations, cell proliferation was studied by MTT and MTT-agarose methods. In vitro chemoinvasion assay with the reconstitution of a matrix of a basement membrane onto a filter in a Boyden chamber was performed. Mutation-matched ASO inhibited the tumor growth and invasiveness of HaP-T1 in a dose-dependent manner, while mutation-mismatched ASO were not effective in inhibiting invasion. The present study suggests that antisense oligonucleotides mutation-matched to the K-ras gene may be a new anticancer strategy for pancreatic cancer since they inhibited not only tumor growth but also invasiveness in vitro.

Mu, Y. M., et al. (2003). "Human pituitary tumor transforming gene (hPTTG) inhibits human lung cancer A549 cell growth through activation of p21(WAF1/CIP1)." Endocr J **50**(6): 771-781.

Pituitary tumor transforming gene (PTTG) is a proto-oncogene cloned from rat GH4 cells. This gene was able to induce cell transformation in vitro and is also associated with p53-dependent and -independent apoptosis. In this study, we cloned human PTTG (hPTTG) from a pituitary tumor and then stably transfected the hPTTG into HeLa and A549 cells. An overexpression of hPTTG significantly inhibited cell growth, which was determined by the adherent cell growth properties, colony formation in soft agar and [3H] thymidine incorporation, respectively, in HeLa and A549 cells. The inhibitory effect on cell growth was associated with the activation of p21WAF1/CIP1 in A549 cells, but not in HeLa cells. The hPTTG overexpression increased both the p21WAF1/CIP1 mRNA and protein expression levels as determined by both Northern and Western blot analysis, respectively, in A549 cells. The increased expression of p21WAF1/CIP1 mRNA was regulated at the transcription level and was independent on p53 expression because the luciferase activity increased after the co-transfection of hPTTG and p21WAF1/CIP1 promoter fragments with and without a p53 binding sequence. The subcellular distribution of hPTTG was dependent on cell type, and was predominantly in the nucleus in HeLa, Cos-7 and DU145 cells, but showed a diffuse distribution in both the nucleus and cytoplasm in A549, DLD-1 and NIH3T3 cells. These results indicate that an overexpression of hPTTG inhibits the cell growth due to different mechanisms, which are p21WAF1/CIP1 -dependent and -independent.

Muramaki, M., et al. (2003). "Introduction of midkine gene into human bladder cancer cells enhances their malignant phenotype but increases their sensitivity to antiangiogenic therapy." Clin Cancer Res **9**(14): 5152-5160.

PURPOSE: Midkine (MK) is a member of a family of heparin-binding growth factors, which was reported to have an important role in angiogenesis. Although MK was reported to be associated with bladder cancer progression, the functional significance of MK expression in bladder cancer progression has not been elucidated. The objectives of this study were to determine whether overexpression of MK in bladder cancer cells enhances their malignant potential and to evaluate the inhibitory effect of the antiangiogenic agent TNP-470 on the growth of MK-overexpressing bladder cancer cells in vivo. EXPERIMENTAL DESIGN: We introduced the MK gene into human bladder cancer UM-UC-3 cells that do not secrete a detectable level of MK protein and generated the MK-overexpressing cell line UM-UC-3/MK. The biological activity of secreted MK was evaluated using a human umbilical vein endothelial cell proliferation assay. To investigate the in vivo effects of MK overexpression on tumor growth, each cell line was injected s.c. and orthotopically into nude mice. To evaluate the therapeutic effects of the antiangiogenic agent, mice were given TNP-470 after s.c. injection of each cell line. The microvessel density of tumors was quantitated by immunohistochemistry of CD31. RESULTS: The heparin affinity-purified conditioned media of UM-UC-3/MK cells significantly enhanced human umbilical vein endothelial cell proliferation. MK expression had no effect on in vitro growth but conferred a growth advantage on both s.c. and orthotopic tumors in vivo. Furthermore, enhanced tumor growth was closely associated with increased microvessel density. Significant inhibition of tumor growth by TNP-470 treatment was observed only in UM-UC-3/MK tumors and not in control tumors. CONCLUSIONS: We demonstrated that overexpression of the MK gene causes an increase in the angiogenic activity of cells through vascular endothelial cell growth, resulting in enhanced malignant potential of human bladder cancer cells. Moreover, the present findings suggest that TNP-470 could be used as a novel therapeutic adjunct to conventional agents for patients with advanced bladder cancer overexpressing MK.

Murphy, L. C., et al. (1994). "Regulation of gene expression in T-47D human breast cancer cells by progestins and antiprogestins." Hum Reprod **9 Suppl 1**: 174-180.

The molecular mechanisms by which progestins and antiprogestins inhibit human breast cancer cell growth are essentially unknown. The mechanisms by which they mediate growth inhibition in human breast

cancer cells and the expression of the putative autocrine/paracrine growth factors, epidermal growth factor and transforming growth factors alpha and beta-1 were studied under conditions in which progestin and antiprogestin inhibit the growth of T-47D human breast cancer cells in culture. Under the same conditions, the expression of genes such as c-myc, c-jun and c-fos, which are known to have important roles in growth and differentiation, has been measured. The results indicate that progestins and antiprogestins differentially regulate expression of these genes. The data are consistent with the conclusion that the mechanism of growth inhibition of these two agents differs, although an initial interaction with the progesterone receptor is a necessary first step in initiating the as yet ill-defined cascade of events leading to growth inhibition.

Nakano, K., et al. (1997). "Butyrate activates the WAF1/Cip1 gene promoter through Sp1 sites in a p53-negative human colon cancer cell line." J Biol Chem **272**(35): 22199-22206.

Butyrate is a well known colonic luminal short chain fatty acid, which arrests cell growth and induces differentiation in various cell types. We examined the effect of butyrate on the expression of WAF1/Cip1, a potent inhibitor of cyclin-dependent kinases, and its relation to growth arrest in a p53-mutated human colon cancer cell line WiDr. Five millimolar butyrate completely inhibited the growth of WiDr and caused G1-phase arrest. WAF1/Cip1 mRNA was rapidly induced within 3 h by treatment with 5.0 mM butyrate, and drastic WAF1/Cip1 protein induction was detected. Using several mutant WAF1/Cip1 promoter fragments, we found that the butyrate-responsive elements are two Sp1 sites at -82 and -69 relative to the transcription start site. We also found that a TATA element at -46 and two overlapping consensus Sp1 sites at -60 and -55 are essential for the basal promoter activity of WAF1/Cip1. These findings suggest that butyrate arrests the growth of WiDr by activating the WAF1/Cip1 promoter through specific Sp1 sites in a p53-independent fashion.

Naruse, I., et al. (1998). "High concentrations of recombinant adenovirus expressing p16 gene induces apoptosis in lung cancer cell lines." Anticancer Res **18**(6A): 4275-4282.

In this study, we discussed the effects of treatment with recombinant adenovirus expressing p16 (AX-p16) on cell growth and cell death. Ax-p16 at 10 m.o.i. groups showed growth inhibition 3 days after gene transfection, but the cells regrew and did not undergo cell death. On the other hand, Ax-p16 at 300 m.o.i. groups showed complete cell growth inhibition leading to cell death which was apparent 7 days after p16 gene transfection. In the high m.o.i. Ax-mock groups, cell death was marked just after infection, but had diminished by 7 days after infection. Downregulation of pRB was detected only in Ax-p16 at 300 m.o.i. groups. These data suggest that a) high m.o.i. condition of Ax-p16 gives therapeutic benefits due to the combined effects of adenovirus and high expression of p16; and b) the cell killing mechanism of the p16 transgene is different from that of high m.o.i. adenoviral infection.

Nawa, A., et al. (2000). "Tumor metastasis-associated human MTA1 gene: its deduced protein sequence, localization, and association with breast cancer cell proliferation using antisense phosphorothioate oligonucleotides." J Cell Biochem **79**(2): 202-212.

Using differential cDNA library screening techniques based on metastatic and nonmetastatic rat mammary adenocarcinoma cell lines we previously cloned and sequenced the metastasis-associated gene mta1. Using homology to the rat MTA1 gene we cloned the human MTA1 gene and found it to be overexpressed in a variety of human cell lines. We found a close similarity between the human MTA1 and rat MTA1 genes, as shown by 88% and 96% identities of the nucleotide and predicted amino acid sequences, respectively. Both genes encode novel proteins that contain a proline-rich region (SH3 binding motif), a putative zinc finger motif, a leucine zipper motif, and five copies of the SPXX motif often found in gene regulatory proteins. Using Southern blot analysis, the MTA1 gene was found to be highly conserved among all species examined; and using Northern blot analysis, MTA1 transcripts were found in virtually all cell lines of human origin that were analyzed, including melanoma and breast, cervix and ovarian carcinoma cells and normal breast epithelial cells. However, the expression level of the MTA1 gene in a normal breast epithelial cell was approximately 50% of that found in rapidly growing breast adenocarcinoma cell lines and an atypical mammary cell line. Experimental inhibition of MTA1 protein expression using antisense phosphorothioate oligonucleotides resulted in growth inhibition of human MDA-MB-231 breast cancer cells with relatively high expression of the MTA1 gene. Furthermore, the MTA1 protein was localized in the nuclei of cells transfected using a mammalian expression vector containing the full-length MTA1 gene. The results suggest that the MTA1 protein may function in cellular signaling processes important in the progression and growth of cancer cells, possibly as a nuclear regulatory factor.

Ndisang, D., et al. (1999). "The Brn-3a transcription factor plays a critical role in regulating human papilloma virus gene expression and determining the growth characteristics of cervical cancer cells." J Biol Chem **274**(40): 28521-28527.

The Brn-3a POU family transcription factor has previously been shown to activate the human papilloma virus type 16 (HPV-16) promoter driving the expression of the E6- and E7-transforming proteins. Moreover, Brn-3a is overexpressed approximately 300-fold in cervical biopsies from women with cervical intra-epithelial neoplasia type 3 (CIN3) compared with normal cervical material. To test the role of Brn-3a in cervical neoplasia we have manipulated its expression in cervical carcinoma-derived cell lines with or without endogenous HPV genes. In HPV-expressing cells, reduction in Brn-3a expression specifically reduces HPV gene expression, growth rate, saturation density and anchorage-independent growth, whereas these effects are not observed when Brn-3a expression is reduced in cervical cells lacking HPV genomes. Together with our previous observations, these findings indicate a critical role for Brn-3a in regulating HPV gene expression and thereby in controlling the growth/transformation of cervical cells.

Nesaretnam, K., et al. (2000). "Effect of a carotene concentrate on the growth of human breast cancer cells and pS2 gene expression." Toxicology **151**(1-3): 117-126.

Breast cancer is the most common cancer in women worldwide. The growth of breast cancer cells is either hormone-dependent or hormone-independent. Both types are represented in vitro by the estrogen-receptor positive (ER+) MCF-7 and the estrogen-receptor negative (ER-) MDA-MB-231 cell lines, respectively. The pS2 gene is an estrogen-regulated gene and serves as a marker for the ER+ tumours. Carotenoids are pigments with anti-cancer properties besides having pro-vitamin A, antioxidant and free-radical quenching effects. This study was designed firstly, to compare the effect of palm oil carotene concentrate with retinoic acid on the growth of the ER+ MCF-7 and the ER- MDA-MB-231 cells; and secondly to evaluate the effect of the palm oil carotene concentrate on the regulation of pS2 mRNA. The growth experiments were performed with monolayer cells seeded in phenol red free RPMI 1640 culture media and subsequently treated with varying concentrations of either retinoic acid or palm oil carotenoids. The cell numbers were determined at the start of each experiment and then at successive time intervals. The results showed that the palm oil carotene concentrate caused dose-dependent inhibition of estradiol-stimulated growth of MCF-7 cells but did not affect the proliferation of MDA-MB-231 cells. Retinoic acid caused similar, albeit more potent effects, as significant inhibition was observed at lower concentrations than the palm oil carotenoids. In the pS2 gene expression experiment, cell monolayers were treated with the carotene concentrate (10(-6) M), either with or without supplemented estradiol (10(-8) M), and subsequently the RNA was extracted. Northern blotting was performed and the regulation of pS2 mRNA determined using a 32P-labelled pS2 cDNA probe. The results showed that the palm oil carotene concentrate did not affect the expression of pS2 mRNA and are therefore independent of the estrogen-regulated pathway.

Nguyen, D. M., et al. (1997). "Delivery of the p53 tumor suppressor gene into lung cancer cells by an adenovirus/DNA complex." Cancer Gene Ther **4**(3): 191-198.

An adenovirus/DNA complex was constructed by chemically linking poly-L-lysine to the capsid of the replication-defective adenovirus dl312, allowing for coupling with plasmid DNA by an ionic interaction. We have previously demonstrated that this adenovirus/DNA complex can efficiently transduce malignant cells with a plasmid expressing the beta-galactosidase gene both in vitro and in vivo. In this report, we show that this system can deliver a therapeutic gene that encodes for the tumor suppressor protein p53 to lung cancer cells, both in vitro and in vivo, leading to significant biological effects. Transfection of the p53-negative human lung cancer cell line H1299 with the adenovirus/DNA complex carrying a plasmid expressing the p53 gene resulted in high levels of p53 protein and induction of apoptosis. Injection of the complex carrying the p53 gene to subcutaneous tumor sites 5 days after tumor cell implantation resulted in a significant inhibition of tumorigenicity as measured by the number and size of tumors that developed 21 days after treatment. Three and six injections of the complex carrying the p53 gene into H1299 subcutaneous tumor nodules led to significant dose-related tumor growth suppression 18 days after the first injection compared with control-treated tumors. This adenovirus/DNA complex, therefore, is capable of efficiently delivering the p53 gene into malignant cells in vitro and in vivo and now provides a general gene delivery vector that is simple to construct and capable of testing therapeutic genes in malignant cells.

Nicolson, G. L., et al. (2003). "Tumor metastasis-associated human MTA1 gene and its MTA1 protein product: role in epithelial cancer cell invasion, proliferation and nuclear regulation." Clin Exp Metastasis **20**(1): 19-24.

Using differential cDNA library screening techniques based on metastatic and nonmetastatic rat mammary adenocarcinoma cell lines, we previously cloned and sequenced the metastasis-associated gene mta1. Using homology to the rat mta1 gene, we cloned the human MTA1 gene and found it to be over-expressed in a variety of human cell lines (breast, ovarian, lung, gastric and colorectal cancer but not melanoma or sarcoma) and cancerous tissues (breast, esophageal, colorectal, gastric and pancreatic cancer). We found a close similarity between the human MTA1 and rat mta1 genes (88% and 96% identities of the nucleotide and predicted amino acid sequences, respectively). Both genes encode novel proteins that contain a proline rich region (SH3-binding motif), a putative zinc finger motif, a leucine zipper motif and 5 copies of the SPXX motif found in gene regulatory proteins. Using Southern blot analysis the MTA1 gene was highly conserved, and using Northern blot analysis MTA1 transcripts were found in virtually all human cell lines (melanoma, breast, cervix and ovarian carcinoma cells and normal breast epithelial cells). However, the expression level of the MTA1 gene in normal breast epithelial cells was approximately 50% of that found in rapidly growing adenocarcinoma and atypical epithelial cell lines. Experimental inhibition of MTA1 protein expression using antisense phosphorothioate oligonucleotides resulted in inhibition of growth and invasion of human MDA-MB-231 breast cancer cells with relatively high MTA1 expression. Furthermore, the MTA1 protein was localized in the nuclei of cells transfected with a mammalian expression vector containing a full-length MTA1 gene. Although some MTA1 protein was found in the cytoplasm, the vast majority of MTA1 protein was localized in the nucleus. Examination of recombinate MTA1 and related MTA2 proteins suggests that MTA1 protein is a histone deacetylase. It also appears to behave like a GATA-element transcription factor, since transfection of a GATA-element reporter into MTA1-expressing cells resulted in 10-20-fold increase in reporter expression over poorly MTA1-expressing cells. Since it was reported that nucleosome remodeling histone deacetylase complex (NuRD complex) involved in chromatin remodeling contains MTA1 protein and a MTA1-related protein (MTA2), we examined NuRD complexes for the presence of MTA1 protein and found an association of this protein with histone deacetylase. The results suggest that the MTA1 protein may serve multiple functions in cellular signaling, chromosome remodeling and transcription processes that are important in the progression, invasion and growth of metastatic epithelial cells.

Nielsen, L. L., et al. (1997). "Efficacy of p53 adenovirus-mediated gene therapy against human breast cancer xenografts." Cancer Gene Ther **4**(2): 129-138.

In response to DNA damage, p53 protein accumulates in the cell nucleus causing cells to undergo DNA repair or apoptosis, programmed cell death. Reintroduction of wild-type p53 into tumors with null or mutant p53 offers a novel strategy for controlling tumor growth, by inducing apoptotic death in neoplastic cells. The efficacy of a replication-deficient p53 adenovirus construct was tested against three human breast cancer cell lines expressing mutant p53, MDA-MB-231, -468, and -435. 231 and 468 cells were both highly transduced at a multiplicity of infection of 10. By contrast, 435 cells were rarely transduced. p53 adenovirus-mediated gene therapy was highly effective against 231 and 468 tumor xenografts in nude mice. At a total dose of 2.2 x 10(9) cellular infectious units (CIU), inhibition of 231 tumor growth was 86% (P < or =.01). Thirty-seven percent of that growth inhibition was due to p53, while 49% was adenovirus-specific. Inhibition of 468 tumor growth was 74% (P < or =.001). Forty-five percent of that inhibition was p53-specific, while 28% was adenovirus-specific. The ED50 values for 231 tumors and 468 tumor growth inhibition were 3 x 10(8) CIU and 2 x 10(8) CIU, respectively. Injection of p53 Ad into 231 or 468 tumors induced apoptosis. By contrast, growth inhibition in 435 tumors treated with p53 adenovirus was not significant, probably due to low adenovirus transduction. 231 and 435 cells both expressed high levels of alpha v, beta 1, beta 3, and beta 5 integrin subunits, ruling out lack of the appropriate integrins as the reason for the low infection rate in 435 cells. Our results demonstrate the ability of wild-type p53 to curtail cancerous cell growth in vivo in tumors expressing mutant p53. The ability of beta-gal Ad to infect tumor cells in vitro was generally predictive of in vivo p53 Ad efficacy.

Nishida, K., et al. (1996). "Introduction of the c-kit gene leads to growth suppression of a breast cancer cell line, MCF-7." Anticancer Res **16**(6B): 3397-3402.

Normal ductal cells of the breast are exceptional on that their epithelial cells abundantly express the c-kit receptor. Loss of c-kit expression has been reported in 80-90% of breast cancer specimens, suggesting a possible role in the development of tumors. In the present study, we introduced a c-kit expression vector into a breast cancer cell line. MCF-7, which does not express c-kit but does express its ligand, stem cell factor (SCF). Anchorage dependent and independent growth was found to be inhibited in bulk cultures of the c-kit transfectants, although this suppression appeared to be incomplete, allowing a considerable

fraction to tolerate c-kit expression. Heterogeneous sensitivity to the suppressive effects mediated by the c-kit receptor was also observed among individual clones isolated from the bulk cultures. These results suggest that c-kit can mediate inhibitory signals for the growth of breast cancer cells, but that cellular heterogeneity exists regarding the response. Further studies are warranted to elucidate the molecular basis for the inhibitory effects of c-kit.

Nishino, K., et al. (2001). "Adenovirus-mediated gene therapy specific for small cell lung cancer cells using a Myc-Max binding motif." Int J Cancer **91**(6): 851-856.

Recent clinical trials of gene therapy for patients with thoracic cancers have shown that these treatments were well tolerated with minimal side effects and that we need to further enhance specificity as well as efficiency of gene transfer to target cancer cells. We previously reported that myc-overexpressing SCLC cell lines became selectively sensitive to ganciclovir (GCV) by transducing the herpes simplex virus thymidine kinase (HSV-TK) gene under the control of the Myc-Max response elements (a core nucleotide sequence, CACGTG) and that this construct (MycTK) could be utilized to develop a novel treatment against chemo-radio-resistant SCLC. We report here in vivo antitumor effects and safety of a replication-deficient adenoviral vector containing the Myc-Max binding motif (AdMycTK) on SCLC cells. In vitro infection with AdMycTK selectively rendered myc-overexpressing SCLC cell lines 63- to 307-fold more sensitive to GCV. In vivo injections with AdMycTK followed by GCV administration markedly suppressed the growth of myc-overexpressing tumors established in the subcutis or in the peritoneal cavity of athymic mice. On the other hand, infection with AdMycTK did not significantly affect either in vitro GCV sensitivity of the cells expressing very low levels of the myc genes or the growth of their subcutaneous tumors. Moreover, we observed no apparent side effects of this treatment including body weight loss or biochemical abnormalities in contrast to the treatment with AdCATK that conferred strong but nonspecific expression of the HSV-TK gene. These results suggested that AdMycTK/GCV therapy is effective on SCLC patients whose tumors overexpress myc family oncogenes.

Nishioka, M., et al. (2002). "MYO18B, a candidate tumor suppressor gene at chromosome 22q12.1, deleted, mutated, and methylated in human lung cancer." Proc Natl Acad Sci U S A **99**(19): 12269-12274.

Loss of heterozygosity on chromosome 22q has been detected in approximately 60% of advanced nonsmall cell lung carcinoma (NSCLC) as well as small cell lung carcinoma (SCLC), suggesting the presence of a tumor suppressor gene on 22q that is involved in lung cancer progression. Here, we isolated a myosin family gene, MYO18B, located at chromosome 22q12.1 and found that it is frequently deleted, mutated, and hypermethylated in lung cancers. Somatic MYO18B mutations were detected in 19% (14/75) of lung cancer cell lines and 13% (6/46) of primary lung cancers of both SCLC and NSCLC types. MYO18B expression was reduced in 88% (30/34) of NSCLC and 47% (8/17) of SCLC cell lines. Its expression was restored by treatment with 5-aza-2'-deoxycytidine in 11 of 14 cell lines with reduced MYO18B expression, and the promoter CpG island of the MYO18B gene was methylated in 17% (8/47) of lung cancer cell lines and 35% (14/40) of primary lung cancers. Furthermore, restoration of MYO18B expression in lung carcinoma cells suppressed anchorage-independent growth. These results indicate that the MYO18B gene is a strong candidate for a novel tumor suppressor gene whose inactivation is involved in lung cancer progression.

Nishizaki, M., et al. (2001). "Synergistic inhibition of human lung cancer cell growth by adenovirus-mediated wild-type p53 gene transfer in combination with docetaxel and radiation therapeutics in vitro and in vivo." Clin Cancer Res **7**(9): 2887-2897.

Chemotherapy given sequentially or concurrently with external beam radiation therapy has emerged as a standard for the treatment of locally advanced lung cancer. Gene therapy by adenovirus-mediated wild-type p53 gene transfer has been shown to inhibit lung cancer growth in vitro, in animal models, and in human clinical trials. However, no information is available on the combined effects of p53 gene transfer, chemotherapy, and radiation therapy on lung cancer growth in vitro and in vivo. Therefore, we developed two-dimensional and three-dimensional isobologram modeling and statistical methods to evaluate the synergistic, additive, or antagonistic efficacy among these therapeutic agents in human non-small cell lung cancer cell lines A549, H460, H322, and H1299, at the ID50 and ID80 levels. The combination of these three therapeutic agents exhibited synergistic inhibitory effects on tumor cell growth in all four cell lines at both the ID50 and the ID80 levels in vitro. In mouse models with H1299 and A549 xenografts, combined treatment synergistically inhibited tumor growth in the absence of any apparent increase in toxicity, when compared with other treatment and control groups. Together, our findings suggest that a combination of gene therapy, chemotherapy, and radiation therapy may be an effective strategy for human cancer treatment.

Noda, D., et al. (2006). "ELAC2, a putative prostate cancer susceptibility gene product, potentiates TGF-beta/Smad-induced growth arrest of prostate cells." Oncogene **25**(41): 5591-5600.

Transforming growth factor-beta (TGF-beta) elicits a potent growth inhibitory effect on many normal cells by binding to specific serine/threonine kinase receptors and activating specific Smad proteins, which regulate the expression of cell cycle genes, including the p21 cyclin-dependent kinase (CDK) inhibitor gene. Interestingly, cancer cells are often insensitive to the anti-mitogenic effects of TGF-beta for which the molecular mechanisms are not well understood. In this study, we found that the candidate prostate cancer susceptibility gene ELAC2 potentiates TGF-beta/Smad-induced transcriptional responses. ELAC2 associates with activated Smad2; the C-terminal MH2 domain of Smad2 interacts with the N-terminal region of ELAC2. Small interfering siRNA-mediated knock-down of ELAC2 in prostate cells suppressed TGF-beta-induced growth arrest. Moreover, ELAC2 was shown to specifically associate with the nuclear Smad2 partner, FAST-1 and to potentiate the interaction of activated Smad2 with transcription factor Sp1. Furthermore, activation of the p21 CDK inhibitor promoter by TGF-beta is potentiated by ELAC2. Taken together our data indicate an important transcriptional scaffold function for ELAC2 in TGF-beta/Smad signaling mediated growth arrest.

Oakley, R., et al. (2002). "A preclinical model of minimal residual cancer in the muscle highlights challenges associated with adenovirus-mediated p53 gene transfer." Clin Cancer Res **8**(6): 1984-1994.

PURPOSE: Clinical studies have revealed that tumors may recur at the operative site if radioresistant p53 mutation-positive residual disease remains in the body after treatment. Destruction of these remaining malignant cells, which can be present in both mucosal and deep muscle margins, may be achieved using p53-mediated gene transfer techniques. Most preclinical studies designed to assess the feasibility of harnessing this approach have used s.c. tumor models in nude mice, but it is anticipated that transduction of tumor cells in the muscle in immune-competent hosts may be more difficult. EXPERIMENTAL DESIGN: To address this point a new rodent model of residual cancer was established implanting PDVC57B tumor cells to create multiple tumor tracts in the muscle of syngeneic immune-competent C57Bl/6 mice. s.c. tumors and a s.c. model of residual disease were used as comparators. RESULTS: In the s.c. model of residual disease a single administration of 5 x 10(10) viral particles of Ad5CMV-p53 suppressed the growth of encapsulated tumor at the treatment site in six of six animals, but two of these animals had viable nests of tumor outside of the encapsulated zone. However, Ad5CMV-p53 had no apparent effect on tumor cell progression in the model of residual cancer in the muscle. Creating the muscle model of residual cancer with a lower number of cells in the initial inoculum showed that immune-mediated effects, as well as those attributable to the transgene, are important in preventing tumor outgrowth. The frequency of transduction of tumor cells in the muscle, as determined after administration of Ad-beta-galactosidase, was typically <3% and markedly different from the 20% transduction observed for the s.c. tumor model. CONCLUSIONS: These studies highlight the need to devise strategies to improve delivery of adenovirus-mediated gene transfer to nests of tumor in muscle before this modality is used to treat residual cancer at this site. These may involve approaches such as intravascular delivery, strategies to improve vector diffusion, or combination with chemotherapy or radiotherapy to enhance gene delivery at these less accessible sites of disease.

O'Flanagan, C. H., et al. (2015). "The Parkinson's gene PINK1 regulates cell cycle progression and promotes cancer-associated phenotypes." Oncogene **34**(11): 1363-1374.

PINK1 (phosphatase and tensin homolog deleted on chromosome 10 (PTEN)-induced kinase 1), a Parkinson's disease-associated gene, was identified originally because of its induction by the tumor-suppressor PTEN. PINK1 promotes cell survival and potentially metastatic functions and protects against cell stressors including chemotherapeutic agents. However, the mechanisms underlying PINK1 function in cancer cell biology are unclear. Here, using several model systems, we show that PINK1 deletion significantly reduced cancer-associated phenotypes including cell proliferation, colony formation and invasiveness, which were restored by human PINK1 overexpression. Results show that PINK1 deletion causes major defects in cell cycle progression in immortalized mouse embryonic fibroblasts (MEFs) from PINK1(-/-) mice, and in BE (2)-M17 cells stably transduced with short hairpin RNA against PINK1. Detailed cell cycle analyses of MEF cell lines from several PINK1(-/-) mice demonstrate an increased proportion of cells in G2/M and decreased number of cells in G1 following release from nocodazole block. This was concomitant with increased double and multi-nucleated cells, a reduced ability to undergo cytokinesis and to re-enter G1, and significant alterations in cell cycle markers, including failure to increase cyclin D1, all indicative of mitotic arrest. PINK1(-/-) cells also demonstrated ineffective cell cycle exit following serum deprivation. Cell cycle

defects associated with PINK1 deficiency occur at points critical for cell division, growth and stress resistance in cancer cells were rescued by ectopic expression of human PINK1 and demonstrated PINK1 kinase dependence. The importance of PINK1 for cell cycle control is further supported by results showing that cell cycle deficits induced by PINK1 deletion were linked mechanistically to aberrant mitochondrial fission and its regulation by dynamin-related protein-1 (Drp1), known to be critical for progression of mitosis. Our data indicate that PINK1 has tumor-promoting properties and demonstrates a new function for PINK1 as a regulator of the cell cycle.

Oh, J. J., et al. (2002). "A candidate tumor suppressor gene, H37, from the human lung cancer tumor suppressor locus 3p21.3." Cancer Res **62**(11): 3207-3213.

Frequent allelic loss and homozygous deletions within chromosome 3p in human lung cancers have suggested that the 3p21.3 (370-kb) region contains a critical tumor suppressor gene (s) (TSG). With the exact identity/characteristics of such a gene (s) still unconfirmed, a lack of inactivating structural mutations in the expressed genes contained within this region may indicate that the 3p TSG (s) do not fit into the classical "two-mutation" model. This report characterizes a candidate 3p TSG, H37, located within the 370-kb region. Reduced expression of the H37 transcript was found in 9 of 11 (82%) of primary non-small cell lung cancers (NSCLCs) when compared with adjacent normal tissues. Generation of an H37 antibody followed by immunohistochemical analysis of primary NSCLC specimens demonstrated that 46 of 62 (73%) of these cancers contain reduced levels of H37 protein when compared with adjacent normal bronchial cells. Moreover, introduction of the H37 cDNA into human breast cancer cells deleted of 3p21-22 reduced both anchorage-dependent and -independent cell growth in vitro. Subsequent transfection of H37 cDNA into one of the human lung cancer cell lines homozygously deleted in this region resulted in a very low yield of H37-expressing clones. H37 also suppressed anchorage-dependent and -independent growth of A9 mouse fibrosarcoma cells and inhibited tumor formation in nude mice. These data indicate a potential role for H37 as one of the 3p TSGs in human lung cancer.

Oki, T., et al. (2004). "Genistein induces Gadd45 gene and G2/M cell cycle arrest in the DU145 human prostate cancer cell line." FEBS Lett **577**(1-2): 55-59.

Genistein is the most abundant isoflavone of soybeans and has been shown to cause growth arrest in various human cancer cell lines. However, the precise mechanism for this is still unclear. We report here that the growth arrest and DNA damage-inducible gene 45 (gadd45) gene is induced by genistein via its promoter in a DU145 human prostate cancer cell line. The binding of transcription factor nuclear factor-Y to the CCAAT site of the gadd45 promoter appears to be important for this activation by genistein.

Pagliuca, A., et al. (2013). "Analysis of the combined action of miR-143 and miR-145 on oncogenic pathways in colorectal cancer cells reveals a coordinate program of gene repression." Oncogene **32**(40): 4806-4813.

MicroRNAs (miRNAs) from the gene cluster miR-143-145 are diminished in cells of colorectal tumor origin when compared with normal colon epithelia. Until now, no report has addressed the coordinate action of these miRNAs in colorectal cancer (CRC). In this study, we performed a comprehensive molecular and functional analysis of the miRNA cluster regulatory network. First, we evaluated proliferation, migration, anchorage-independent growth and chemoresistance in the colon tumor cell lines after miR-143 and miR-145 restoration. Then, we assessed the contribution of single genes targeted by miR-143 and miR-145 by reinforcing their expression and checking functional recovery. Restoring miR-143 and miR-145 in colon cancer cells decreases proliferation, migration and chemoresistance. We identified cluster of differentiation 44 (CD44), Kruppel-like factor 5 (KLF5), Kirsten rat sarcoma 2 viral oncogene homolog (KRAS) and v-Raf murine sarcoma viral oncogene homolog B1 (BRAF) as proteins targeted by miR-143 and miR-145. Their re-expression can partially revert a decrease in transformation properties caused by the overexpression of miR-143 and miR-145. In addition, we determined a set of mRNAs that are diminished after reinforcing miR-143 and miR-145 expression. The whole transcriptome analysis ascertained that downregulated transcripts are enriched in predicted target genes in a statistically significant manner. A number of additional genes, whose expression decreases as a direct or indirect consequence of miR-143 and miR-145, reveals a complex regulatory network that affects cell signaling pathways involved in transformation. In conclusion, we identified a coordinated program of gene repression by miR-143 and miR-145, in CRC, where either of the two miRNAs share a target transcript, or where the target transcripts share a common signaling pathway. Major mediators of the oncosuppression by miR-143 and miR-145 are genes belonging to the growth factor receptor-mitogen-activated protein kinase network and to the p53 signaling pathway.

Park, J., et al. (2001). "Msx1 gene overexpression induces G1 phase cell arrest in human ovarian cancer cell line OVCAR3." Biochem Biophys Res Commun **281**(5): 1234-1240.

Recent evidence suggested an involvement of homeobox genes in tumorigenesis. Here we investigated whether one of homeobox-containing genes, Msx1, might be involved in the regulation of cell proliferation and cell cycle using Msx1 overexpressing human ovarian cancer cell line, OVCAR3. Overexpression of Msx1 in OVCAR3 cells inhibited cell proliferation by markedly increasing the length of the G1 phase of the cell cycle over control cells. Consistent with this result, dramatic suppression of cyclins D1, D3, E, cyclin-dependent kinase 4, c-Jun, and Rb was observed. Elevated expression of genes involved in the growth arrest and apoptosis (GADD153 and apoptotic cystein protease MCH4) and suppression of proliferation associated protein gene (PAG) in Msx1-overexpressing cells by cDNA expression array analysis provide further evidence for a potential repressor function of Msx1 in cell cycle progression.

Pei, L. J., et al. (2017). "[Effect of triptolide on human oral cancer cell proliferation and PTEN gene mRNA expression in oral cancer]." Zhonghua Kou Qiang Yi Xue Za Zhi **52**(1): 44-47.

Objective: To investigate the effect of triptolide on human oral cancer cell (HB) proliferation and phosphates and tensin homologue deleted on chromosome ten gene (PTEN) mRNA expression in oral cancer. Methods: The cancer cells were cultured in the medium containing triptolide of different concentrations for 24, 48 and 72 h. Methyl thiazolyl tetrazolium (MTT) method was used to test the rate of growth inhibition of cancer cells, flow cytometer to detect the change of cell cycle and reveres transcription-PCR (RT-PCR) to examine the expression of PTEN mRNA. The expression of PTEN protein was examined by Western blotting. Results: The rate of growth inhibition was (26.92 +/- 0.14)%, (38.67 +/- 0.11)%, (72.62 +/- 0.89)% and (90.42 +/- 0.28)%, respectively. The corresponding expression of PTEN mRNA was (3.59+/-0.21)%, (5.27+/-0.40)%, (7.18+/-0.44)% and (9.16+/-0.50)%, respectively and the corresponding A value of PTEN protein was 0.135+/-0.007, 0.410+/-0.020, 0.447+/-0.017 and 0.884+/-0.066, respectively. The proportion of G1 phase cells increased from (58.78+/-0.98)% to (84.13+/-0.47)%, but the proportion of S phase cells decreased from (25.40+/-0.43)% to (9.41+/-0.73)%. Conclusions: The triptolide not only had inhibitory effect on the HB proliferation, but also affected the cell cycle.

Peterson, G. and S. Barnes (1991). "Genistein inhibition of the growth of human breast cancer cells: independence from estrogen receptors and the multi-drug resistance gene." Biochem Biophys Res Commun **179**(1): 661-667.

The effect of isoflavones on the growth of the human breast carcinoma cell lines, MDA-468 (estrogen receptor negative), and MCF-7 and MCF-7-D-40 (estrogen receptor positive), has been examined. Genistein is a potent inhibitor of the growth of each cell line (IC50 values from 6.5 to 12.0 micrograms/ml), whereas biochanin A and daidzein are weaker growth inhibitors (IC50 values from 20 to 34 micrograms/ml). The isoflavone beta-glucosides, genistin and daidzin, have little effect on growth (IC50 values greater than 100 micrograms/ml). The presence of the estrogen receptor is not required for the isoflavones to inhibit tumor cell growth (MDA-468 vs MCF-7 cells). In addition, the effects of genistein and biochanin A are not attenuated by overexpression of the multi-drug resistance gene product (MCF-7-D40 vs MCF-7 cells).

Petrovics, G., et al. (2004). "Elevated expression of PCGEM1, a prostate-specific gene with cell growth-promoting function, is associated with high-risk prostate cancer patients." Oncogene **23**(2): 605-611.

PCGEM1 is a novel, highly prostate tissue-specific, androgen-regulated gene. Here, we demonstrate that PCGEM1 expression is significantly higher in prostate cancer (CaP) cells of African-American men than in Caucasian-American men (P=0.0002). Further, increased PCGEM1 expression associates with normal prostate epithelial cells of CaP patients with a family history of CaP (P=0.0400). PCGEM1 overexpression in LNCaP and in NIH3T3 cells promotes cell proliferation and a dramatic increase in colony formation, suggesting a biological role of PCGEM1 in cell growth regulation. Taken together, the cell proliferation/colony formation-promoting functions of PCGEM1 and the association of its increased expression with high-risk CaP patients suggest the potential roles of PCGEM1 in CaP onset/progression, especially in these high-risk groups.

Pio, R., et al. (2004). "Alpha CP-4, encoded by a putative tumor suppressor gene at 3p21, but not its alternative splice variant alpha CP-4a, is underexpressed in lung cancer." Cancer Res **64**(12): 4171-4179.

alpha CP-4 is an RNA-binding protein coded by PCBP4, a gene mapped to 3p21, a common deleted region in lung cancer. In this study we characterized the expression of alpha CP-4 and alpha CP-4a, an alternatively spliced variant of alpha CP-4, in lung cancer cell lines and non-small cell lung cancer

(NSCLC) samples from early stage lung cancer patients. In NSCLC biopsies, an immunocytochemical analysis showed cytoplasmic expression of alpha CP-4 and alpha CP-4a in normal lung bronchiolar epithelium. In contrast, alpha CP-4 immunoreactivity was not found in 47% adenocarcinomas and 83% squamous cell carcinomas, whereas all of the tumors expressed alpha CP-4a. Besides, lack of alpha CP-4 expression was associated with high proliferation of the tumor (determined by Ki67 expression). By fluorescence in situ hybridization, >30% of NSCLC cell lines and tumors showed allelic losses at PCBP4, correlating with the absence of the protein. On the other hand, no mutations in the coding region of the gene were found in any of the 24 cell lines analyzed. By Northern blotting and real-time reverse transcription-PCR, we detected the expression of alpha CP-4 and alpha CP-4a messages in NSCLC and small cell lung cancer cell lines. Our data demonstrate an abnormal expression of alpha CP-4 in lung cancer, possibly associated with an altered processing of the alpha CP-4 mRNA leading to a predominant expression of alpha CP-4a. This may be considered as an example of alternative splicing involved in tumor suppressor gene inactivation. Finally, induction of alpha CP-4 expression reduced cell growth, in agreement with its proposed role as a tumor suppressor, and suggesting an association of this RNA-binding protein with lung carcinogenesis.

Prasad, K. A. and J. G. Church (1991). "EGF-dependent growth inhibition in MDA-468 human breast cancer cells is characterized by late G1 arrest and altered gene expression." Exp Cell Res **195**(1): 20-26.

The MDA-468 human breast cancer cell line displays the unusual phenomenon of growth inhibition in response to pharmacological concentrations of EGF. This study was initiated with the objective of elucidating the cellular mechanisms involved in EGF-induced growth inhibition. Following EGF treatment the percentage of MDA-468 cells in G1 phase increased, together with a concomitant depletion in S and G2/M phase populations, as revealed by flow cytometry of DNA content. The apparent G1 block in the cell cycle was confirmed by treating the cells with vinblastine. DNA synthesis was reduced to about 35% of that measured in control, untreated cells after 48 h of EGF treatment, as measured by the incorporation of [3H]thymidine. DNA synthesis returned to normal following the removal of EGF from the growth-arrested cells. In order to locate the EGF-induced event responsible for the G1 arrest more precisely, we examined the expression of certain cell cycle-dependent genes by Northern blot analysis. EGF treatment did not alter either the induction of the early G1 marker, c-myc, or the expression of the late G1 markers, proliferating cell nuclear antigen, and thymidine kinase. However, EGF-treated cells revealed down regulation of p53 and histone 3.2 expression, which are expressed at the G1/S boundary and in S phase, respectively. These results indicate that EGF-induced growth inhibition in MDA-468 human breast cancer cells is characterized by a reversible cell cycle block at the G1/S boundary.

Qiu, S., et al. (2002). "[Study on the effects of combined IL-12 and GM-CSF gene therapy for murine liver cancer]." Zhonghua Gan Zang Bing Za Zhi **10**(6): 413-416.

OBJECTIVE: To study the anti-tumor effects of combined IL-12 and granalocyte-macrophage-colong scimulating factor (GM-CSF) gene therapy on murine hepatocellular carcinoma. METHODS: Twenty-four mice received subcutaneous inoculation of 1 x 10(6) BNL hepatoma cells were randomly divided into the following four groups with different cytokine encoding plasmids (6 mice for each group): (1)pXX-GM-CSF 12.5 microg and pXX-IL-12 12.5 microg; (2)pXX-IL-12 25 microg; (3)pXX-GM-CSF 25 microg; (4)pXX-Neo 25 microg. The plasmids were given through tail vein using a versatile hydrodynamics-based DNA delivery method on day 3 and day 6 after tumor challenge. The growth of tumor and cellular immune response were observed intensively. The changes in serum concentration of IL-12, GM-CSF, and IFN-gamma after plasmids injection were also observed. RESULTS: Co-delivery of IL-12 and GM-CSF could mount stronger anti-tumor effects, longer term enhanced IL-12 expression and lower level of IFN-gamma than did IL-12 alone. CONCLUSIONS: Combined IL-12 and GM-CSF can render a strong anti-tumor effect as well as a potential to lower the side effects.

Qiu, Z., et al. (2007). "RNA interference-mediated signal transducers and activators of transcription 3 gene silencing inhibits invasion and metastasis of human pancreatic cancer cells." Cancer Sci **98**(7): 1099-1106.

Signal transducers and activators of transcription-3 (STAT3), a central cytoplasmic transcription factor, is frequently overexpressed and constitutively activated by tyrosine during malignant transformation. The overexpression and phosphorylation of STAT3 in pancreatic cancer has been described only recently, but the roles and mechanism still remain unclear. In this study, we elucidate the significance of the STAT3 signaling pathway in metastatic potentials of pancreatic cancer. We stably silence the expression of the STAT3 and p-STAT3 by using RNA interference (RNAi) in the pancreatic cancer cell line SW1990, and

then reduce its invasion capacity in vitro and metastasis capacity in vivo compared to parental cells or cells tansfected with a control vector. Furthermore, silencing SW1990 cells with the STAT3 gene by RNAi also led to a decrease of matrix metalloproteinases-2 (MMP-2) and vascular endothelial growth factor (VEGF) at the mRNA and protein level. Collectively, these studies suggest that activation of the STAT3 signaling pathway plays an important role in the progression of pancreatic cancer, and that silence of the STAT3 gene with RNAi may be a useful anti-invasive therapeutic option in pancreatic cancer.

Ralhan, R., et al. (2000). "Association between polymorphism in p21(Waf1/Cip1) cyclin-dependent kinase inhibitor gene and human oral cancer." Clin Cancer Res **6**(6): 2440-2447.

The cyclin-dependent kinase inhibitor gene p21(Waf1/Cip1) plays a central role in inducing cellular growth arrest, terminal differentiation, and apoptosis. Alterations in this gene may adversely affect regulation of these processes and increase susceptibility for cancer. We have recently reported a novel polymorphism in the p21(Waf1/Cip1) gene in the Indian population and its association with esophageal cancer. An A-->G transition at codon 149 resulted in amino acid substitution from aspartate to glycine in the proliferating cell nuclear antigen binding COOH-terminal domain of p21(Waf1/Cip1) that may affect PCNA-p21(Waf1/Cip1) interactions, thereby affecting regulation of cellular proliferation, and may increase susceptibility for development of cancer. In a parallel study in our laboratory, we searched for putative p21(Waf1/Cip1) mutations in oral premalignant and malignant lesions. No somatic mutation was detected in exon 2 of p21(Waf1/Cip1). Interestingly, a codon 149 polymorphism variant (A-->G) was identified in 11 of 30 (37%) premalignant lesions (7 of 19 hyperplastic lesions and 4 of 11 dysplastic lesions) and 11 of 30 (37%) squamous cell carcinomas (SCCs). This codon 149 variant was also identified in paired lymphocytes of all of the patients with premalignant lesions and SCCs harboring the variant allele, suggesting the occurrence of a polymorphism. Lymphocyte DNA isolated from 50 unrelated age- and gender-matched healthy subjects was screened for this polymorphism. Seven of 50 (14%) normal controls harbored the A-->G codon 149 variant allele. Immunohistochemical analysis of p21(Waf1/Cip1) protein expression showed immunoreactivity in 19 of these 30 (63%) oral premalignant lesions and 16 of 30 (53%) SCCs. The most intriguing features of the study were: (a) the significant increase in frequency of this polymorphism not only in patients with oral SCCs (P = 0.038), but also in patients with premalignant lesions (P = 0.038), compared with normal controls; and (b) the significantly higher frequency of p21(Waf1/Cip1) variants (codon 149) in oral premalignant lesions (10 of 11 cases) and SCCs (11 of 11 cases) with wild-type p53 (P = 0.045) than in lesions with p53 mutations, suggesting that this polymorphism affects the p53 pathway and may play a vital role in oral tumorigenesis. Furthermore, overexpression of p21 protein in oral lesions harboring missense mutations in the p53 gene suggest a p53-independent role for p21 in the pathogenesis of oral cancer.

Ram, T. G., et al. (2000). "Blocking HER-2/HER-3 function with a dominant negative form of HER-3 in cells stimulated by heregulin and in breast cancer cells with HER-2 gene amplification." Cell Growth Differ **11**(3): 173-183.

Amplification and overexpression of the HER-2 (neu/ erbB-2) gene in human breast cancer are clearly important events that lead to the transformation of mammary epithelial cells in approximately one-third of breast cancer patients. Heterodimer interactions between HER-2 and HER-3 (erbB-3) are activated by neu differentiation factor/heregulin (HRG), and HER-2/HER-3 heterodimers are constitutively activated in breast cancer cells with HER-2 gene amplification. This indicates that inhibition of HER-2/HER-3 heterodimer function may be an especially effective and unique strategy for blocking the HER-2-mediated transformation of breast cancer cells. Therefore, we constructed a bicistronic retroviral expression vector (pCMV-dn3) containing a dominant negative form of HER-3 in which most of the cytoplasmic domain was removed for introduction into cells. By using a bicistronic retroviral vector in which the antibiotic resistance gene and the gene of interest are driven by a single promoter, we attained 100% coordinate coexpression of antibiotic resistance with the gene of interest in target cell populations. Breast carcinoma cells with HER-2 gene amplification (21 MT-1 cells) and normal mammary epithelial cells without HER-2 gene amplification from the same patient (H16N-2 cells) were infected with pCMV-dn3 and assessed for HER-2/ HER-3 receptor tyrosine phosphorylation, p85PI 3-kinase and SHC protein activation, growth factor-dependent and -independent proliferation, and transformed growth in culture. Dominant negative HER-3 inhibited the HRG-induced activation of HER-2/HER-3 and signaling in H16N-2 and 21 MT-1 cells as well as the constitutive activation of HER-2/HER-3 and signaling in 21 MT-1 cells. Responses to exogenous HRG were strongly inhibited by dominant negative HER-3. In contrast, the proliferation of cells stimulated by epidermal growth factor was not apparently affected by dominant negative HER-3. The

growth factor-independent proliferation and transformed growth of 21 MT-1 cells were also strongly inhibited by dominant negative HER-3 in anchorage-dependent and independent growth assays in culture. Furthermore, the HRG-induced or growth factor-independent proliferation of 21 MT-1 cells was inhibited by dominant negative HER-3, whereas the epidermal growth factor-induced proliferation of these cells was not: this indicates that dominant negative HER-3 preferentially inhibits proliferation induced by HER-2/HER-3.

Ramondetta, L., et al. (2000). "Adenovirus-mediated expression of p53 or p21 in a papillary serous endometrial carcinoma cell line (SPEC-2) results in both growth inhibition and apoptotic cell death: potential application of gene therapy to endometrial cancer." Clin Cancer Res **6**(1): 278-284.

Papillary serous endometrial carcinoma is an aggressive tumor characterized by late-stage presentation, i.p. spread, and poor prognosis. It is histologically similar to serous papillary carcinoma of the ovary. Preclinical studies have shown that adenovirus-mediated expression of p53 in ovarian cancer cell lines causes growth inhibition and apoptosis in vitro and in vivo. Such studies provide the rationale for Phase I Adp53 gene therapy clinical trials in ovarian cancer. In the present study, we compared the efficacy of adenoviral vectors containing p53 (Adp53) or p21 (Adp21) in a papillary serous endometrial tumor cell line (SPEC-2) that contains mutated p53. Growth assays revealed that both Adp53 and Adp21 were efficacious in decreasing cell proliferation as assessed by anchorage-dependent and anchorage-independent growth assays. However, as compared with Adp53, the effects of Adp21 tended to be more transient and less marked. Strikingly, Adp21, but not Adp53, induced a G1 arrest in SPEC-2 endometrial adenocarcinoma cells. In contrast, as assessed by induction of hypodiploid peaks, free DNA ends detected by a terminal deoxynucleotidyl transferase-based assay, and annexin V positivity, p53 was more effective than p21 in inducing cell death by apoptosis. Compatible with the more efficient induction of apoptosis, Adp53, but not Adp21, induced a marked increase in expression of the preapoptotic molecule BAX without a concomitant change in expression of the antiapoptotic mediator Bcl-2. The differential effects of Adp53 and Adp21 on cell cycle progression and apoptosis may be related to the reversibility of p21-induced cell cycle arrest and the irreversibility of p53-induced apoptosis. Thus, at least in the papillary serous endometrial carcinoma cell line SPEC-2, Adp53 may be more effective than Adp21 as a gene therapeutic. Nevertheless, these preclinical studies suggest that papillary serous endometrial carcinoma is a potential target for p53- or p21-mediated gene therapy.

Ranzani, G. N., et al. (1995). "p53 gene mutations and protein nuclear accumulation are early events in intestinal type gastric cancer but late events in diffuse type." Cancer Epidemiol Biomarkers Prev **4**(3): 223-231.

We screened for p53 alterations in 71 early gastric cancers of differing histological types and growth patterns, 18 advanced cancers of diffuse type, 19 dysplastic lesions, and 12 extensive intestinal metaplasia cases. Tumors were investigated for gene mutations (exons 5-8) with PCR-based denaturing gradient gel electrophoresis and sequencing techniques, and for protein accumulation with immunohistochemical methods. Nontumor samples were studied with immunohistochemistry alone. Of the early cancers, intestinal tumors showed a much higher p53 mutation frequency (41%) than did diffuse cancers (4%). When comparing early and advanced tumors of the same type, we observed a similarity in mutation frequency (41 versus about 50%) for intestinal tumors, and a significant increase for diffuse tumors (from 4 to 33%). Immunopositive case distribution between tumor types and stages paralleled that of mutated cases. Immunohistochemical and genetic analysis gave concordant results for all samples with gene mutations. Eighteen of the 65 (28%) nonmutated tumors displayed significant immunoreactivity. Early tumors that massively penetrated the submucosa, i.e., the early tumors for which prognosis is worst, showed the highest frequency both of p53 gene mutation and of nonmutated protein accumulation. Twelve of 19 dysplastic lesions showed significant immunoreactivity, whereas intestinal metaplasias proved unreactive in all but a few cells. Our results yield two implications: that p53 alterations have a crucial and early role in gastric carcinogenesis of intestinal type, likely acting at the transition step between metaplasia and dysplasia; and that the alterations are mainly associated with tumor progression in cancer of diffuse type.

Rao, G., et al. (2004). "Facilitating role of preprotachykinin-I gene in the integration of breast cancer cells within the stromal compartment of the bone marrow: a model of early cancer progression." Cancer Res **64**(8): 2874-2881.

Despite early detection of breast cancer, patients' survival may be compromised if the breast cancer cells (BCCs) enter the bone marrow (BM). It is highly probable that BCCs enter the BM long before clinical detection. An in vitro coculture model with BM stroma and BCCs (cell lines; primary cells from stage III BC, n = 7, and stage M0, n = 3) mimicked early entry of

BCCs into the BM. In coculture, BCCs exhibit contact inhibition and do not require otherwise needed growth supplements. Stromal growth rate was increased 2-fold in coculture. The inclusion of BCCs in stromal support of long-term culture-initiating cell assay frequencies show no difference (38 +/- 3 versus 36 +/- 6). Nontumorigenic breast cells (patients and cell lines) did not survive in coculture, suggesting that the model could select for malignant population in surgical breast tissues. Cocultures were able to select cells with 73 +/- 7% cloning efficiencies and with the ability to form cocultures with BM stroma. Preprotachykinin-I (PPT-I), a gene that is conserved by evolution, facilitates BCC integration as part of the stromal compartment. This was deduced as follows: (a) nontumorigenic breast cells (n = 4) genetically engineered to express PPT-I and led to anchorage-independent growth, foci formation, and formation of cocultures; and (b) suppression of PPT-I in BCCs (n = 5) with pPMSKH1-PPT-I small interfering RNA reverted the cells to nontumorigenic phenotypes and was undetectable in the BM nude mice. The evidence supports that the PPT-I gene facilitates the integration of BCCs in the stromal compartment during a period before clinical detection, without disrupting hematopoietic activity.

Reddy, M. K., et al. (1995). "Inhibitors of angiotensin-converting enzyme modulate mitosis and gene expression in pancreatic cancer cells." Proc Soc Exp Biol Med **210**(3): 221-226.

The angiotensin-converting enzyme (ACE) inhibitor captopril inhibits mitosis in several cell types that contain ACE and renin activity. In the present study, we evaluated the effect of the ACE inhibitors captopril and CGS 13945 (10(-8) to 10(-2) M) on proliferation and gene expression in hamster pancreatic duct carcinoma cells in culture. These cells lack renin and ACE activity. Both ACE inhibitors produced a dose-dependent reduction in tumor cell proliferation within 24 hr. Captopril at a concentration of 0.36 mM and CGS 13945 at 150 microM decreased cellular growth rate to approximately half that of the control. Neither drug influenced the viability or the cell cycle distribution of the tumor cells. Slot blot analysis of mRNA for four genes, proliferation associated cell nuclear antigen (PCNA), K-ras, protein kinase C-beta (PKC-beta) and carbonic anhydrase II (CA II) was performed. Both ACE inhibitors increased K-ras expression by a factor of 2, and had no effect on CA II mRNA levels. Captopril also lowered PCNA by 40% and CGS 13945 lowered PKC-beta gene expression to 30% of the control level. The data demonstrate that ACE inhibitors exhibit antimitotic activity and differential gene modulation in hamster pancreatic duct carcinoma cells. The absence of renin and ACE activity in these cells suggests that the antimitotic action of captopril and CGS 13945 is independent of renin-angiotensin regulation. The growth inhibition may occur through downregulation of growth-related gene expression.

Reinholz, M. M., et al. (2010). "Expression profiling of formalin-fixed paraffin-embedded primary breast tumors using cancer-specific and whole genome gene panels on the DASL (R) platform." BMC Med Genomics **3**: 60.

BACKGROUND: The cDNA-mediated Annealing, extension, Selection and Ligation (DASL) assay has become a suitable gene expression profiling system for degraded RNA from paraffin-embedded tissue. We examined assay characteristics and the performance of the DASL 502-gene Cancer Panel v1 (1.5K) and 24,526-gene panel (24K) platforms at differentiating nine human epidermal growth factor receptor 2- positive (HER2+) and 11 HER2-negative (HER2-) paraffin-embedded breast tumors. METHODS: Bland-Altman plots and Spearman correlations evaluated intra/inter-panel agreement of normalized expression values. Unequal-variance t-statistics tested for differences in expression levels between HER2 + and HER2 - tumors. Regulatory network analysis was performed using Metacore (GeneGo Inc., St. Joseph, MI). RESULTS: Technical replicate correlations ranged between 0.815-0.956 and 0.986-0.997 for the 1.5K and 24K panels, respectively. Inter-panel correlations of expression values for the common 498 genes across the two panels ranged between 0.485-0.573. Inter-panel correlations of expression values of 17 probes with base-pair sequence matches between the 1.5K and 24K panels ranged between 0.652-0.899. In both panels, erythroblastic leukemia viral oncogene homolog 2 (ERBB2) was the most differentially expressed gene between the HER2 + and HER2 - tumors and seven additional genes had p-values < 0.05 and log2 -fold changes > |0.5| in expression between HER2 + and HER2 - tumors: topoisomerase II alpha (TOP2A), cyclin a2 (CCNA2), v-fos fbj murine osteosarcoma viral oncogene homolog (FOS), wingless-type mmtv integration site family, member 5a (WNT5A), growth factor receptor-bound protein 7 (GRB7), cell division cycle 2 (CDC2), and baculoviral iap repeat-containing protein 5 (BIRC5). The top 52 discriminating probes from the 24K panel are enriched with genes belonging to the regulatory networks centered around v-myc avian myelocytomatosis viral oncogene homolog (MYC), tumor protein p53 (TP53), and estrogen receptor alpha (ESR1). Network analysis with a two-step extension also showed that the eight discriminating genes common to the 1.5K and 24K panels are functionally linked together through MYC,

TP53, and ESR1. CONCLUSIONS: The relative RNA abundance obtained from two highly differing density gene panels are correlated with eight common genes differentiating HER2 + and HER2 - breast tumors. Network analyses demonstrated biological consistency between the 1.5K and 24K gene panels.

Reyes, N., et al. (2004). "Effect of linoleic acid on proliferation and gene expression in the breast cancer cell line T47D." Cancer Lett **209**(1): 25-35.

Human and animal studies have linked n-6 polyunsaturated fatty acids with mammary carcinogenesis. We investigated the cellular and molecular effects of linoleic acid on the human breast cancer cell line T47D. Linoleic acid had a stimulatory effect on the growth of T47D cells, associated with an increase in the proportion of cells in the S phase of the cell cycle. Microarray, functional group and quantitative PCR analyses indicate that linoleic acid may affect T47D cell growth by modulation of the estrogen receptor (ERalpha), the G13alpha G protein, and p38 MAP kinase gene expression as well genes involved in RNA transcription and cell cycle regulation.

Rizk, N. P., et al. (1999). "The evaluation of adenoviral p53-mediated bystander effect in gene therapy of cancer." Cancer Gene Ther **6**(4): 291-301.

Because many tumors have mutated p53, one potential strategy proposed for cancer gene therapy is the introduction of the wild-type p53 gene into tumor cells. One puzzling aspect of this approach is that currently available gene transfer protocols result in a small percentage of tumor cells being transduced in vivo, thus implicating a "bystander effect" to achieve therapeutic efficacy. Because bystander effects in the context of p53-mediated gene therapy have not been well characterized, we evaluated the role of in vitro and in vivo bystander effects of adenovirally delivered p53 (AdWTp53). Using human tumor cell lines that did not express p53 protein but were infectible with adenovirus and showed sensitivity to p53-mediated apoptosis, we were unable to demonstrate an AdWTp53-mediated in vitro bystander effect, despite seeing strong bystander effects when cells were infected with an adenovirus containing the suicide gene herpes simplex virus thymidine kinase and treated with ganciclovir. In contrast, in vivo flank mixing studies using one of these cell lines showed a weak but significant p53-mediated bystander effect (a 40% inhibition of tumor growth). This bystander effect translated into a small survival advantage in an established intraperitoneal tumor model when tumor burden was low at the time of viral instillation. The survival advantage was lost, however, when tumor burden was increased. This study indicates that treatment of human tumors using AdWTp53 may be possible; however, because of the weak bystander effect in vivo, effective treatment will likely require a large percentage of tumor cells to be transduced.

Rocco, J. W., et al. (1998). "p16INK4A adenovirus-mediated gene therapy for human head and neck squamous cell cancer." Clin Cancer Res **4**(7): 1697-1704.

Inactivation of the tumor suppressor gene p16INK4A is the most common genetic alteration in human head and neck squamous cell cancer (HNSCC), making it an ideal target for gene replacement. We constructed a replication-defective, recombinant adenovirus capable of directing a high level of p16INK4A protein expression (Ad5-p16) to investigate its benefit in treating HNSCC. Initial in vitro experiments in four human HNSCC cell lines demonstrated that Ad5-p16 treatment significantly inhibits cell growth with up to 96% efficiency. Flow cytometric analysis showed that Ad5-p16 induced a maximum G1-S cell cycle arrest of 90%. Subsequent studies in a nude mouse model demonstrated that Ad5-p16 treatment significantly reduced (cell line 011) or stabilized (cell line 012) established tumors when compared with control treatments ($P < 0.008$). These results demonstrate for the first time a significant antitumor effect of Ad5-p16 against human HNSCC in vivo and support the potential application of Ad5-p16 to treat locally advanced, unresectable, or metastatic head and neck cancer, as well as microscopic residual disease after surgical resection.

Rohr, U. P., et al. (2003). "Non-small lung cancer cells are prime targets for p53 gene transfer mediated by a recombinant adeno-associated virus type-2 vector." Cancer Gene Ther **10**(12): 898-906.

In this study, we elucidated the potential of recombinant adeno-associated virus type-2 (rAAV-2) vectors for lung cancer gene therapy. Cell lines of the three major histological subtypes of non-small cell lung cancer (NSCLC) were highly susceptible for rAAV-2 showing transduction rates between 63.4 and 98.9%. In contrast, cell lines of small cell carcinomas were resistant to rAAV-2 infection. For restoration of p53 function in p53 deficient NSCLC, a rAAV-2 vector was constructed containing wt p53 cDNA. Following transduction with rAAV-p53, cell growth of all NSCLC cell lines was significantly reduced in a dose-dependent manner between 44 and 71.7% in comparison with rAAV-GFP transduced cells. The reduction of tumor cell growth was associated with increased apoptosis. Adding cisplatin to rAAV-p53-infected cells led to a significant growth inhibition between 81 and 91% indicating a synergistic effect between cisplatin and rAAV-p53. Interestingly, the

tumor cells surviving cisplatin and rAAV-p53 treatment were inhibited in their ability to form colonies as reflected by a reduction of colony growth between 57 and 90.4%. In conclusion, rAAV-2 vectors exhibit a strong tropism for NSCLC. Successful inhibition of tumor cell growth following transduction with a rAAV-p53 vector underlines the potential role of rAAV-2 in cancer gene therapy.

Rosell, R., et al. (1995). "Mutated K-ras gene analysis in a randomized trial of preoperative chemotherapy plus surgery versus surgery in stage IIIA non-small cell lung cancer." Lung Cancer **12 Suppl 1**: S59-70.

The observation that the proteins encoded by ras genes play a central role in the signalling pathways used by cells to respond to growth factors and the fact that mutated ras proteins are constantly promoting cell division have led to a PCR-based hunt for additional clinical information. In the present study, K-ras analysis draws the following conclusions: (1) K-ras point mutation frequency was higher in the surgery group (10 of 24 patients) than in the chemotherapy-surgery group (3 of 20 patients). (2) Mutated K-ras was predominantly observed at codon 12 but five mutations appeared at codon 61. (3) Mutations were identified in the squamous cell carcinoma histological NSCLC subtype except in four cases corresponding to adenocarcinoma. (4) A multifarious pattern of substitutions, especially at codon 12, were noted with aspartic K 12 substitutions more prone to develop bone metastases. (5) Although a genotypic K-ras classification of NSCLC may not yet be formulated, our accumulated data (unpublished) suggest a trend toward it. (6) Patients with mutated K-ras tumors in the surgery group had no different survival than those with normal K-ras. However our pooled data as well as other authors' results assert that mutated K-ras constitute an additional prognostic datum that deserves to be included together with TNM classification. In the design of new preoperative (neoadjuvant) chemotherapy trials, stratification of tumors by K-ras status deserves to be further investigated in order to correlate with response, relapse and survival. Mutated K-ras genotype merits further research. Finally, the paradigm of uneven histological distribution and mutated K-ras spectra among researchers should serve as a stimulus to search for further contributions in this field.

Roth, J. A. (1998). "Gene replacement strategies for lung cancer." Curr Opin Oncol **10**(2): 127-132.

Considerable evidence has accumulated that cancer has a genetic origin based on the development of somatic mutations in families of genes responsible for critical functions of cellular DNA repair, growth control, and division. Restoration of the function of a single pivotal gene product appears sufficient to mediate antitumor effects that are potentially clinically significant. For example, restoration of wild-type p53 function in the cancer cell by gene transfer is sufficient to cause either cell-cycle arrest or apoptosis. This effect is not restricted to p53 but has been observed for oncogenes and other tumor suppressor genes as well. Genes can be delivered with sufficient efficiency by direct intratumoral injection to mediate tumor regression as shown in preclinical studies and phase I clinical trials in non-small cell lung cancer. Although clinical trials of gene replacement are in the earliest stages, this treatment offers a unique mechanism of action with a potentially high therapeutic index.

Sadanandam, A., et al. (2010). "High gene expression of semaphorin 5A in pancreatic cancer is associated with tumor growth, invasion and metastasis." Int J Cancer **127**(6): 1373-1383.

Semaphorin 5A (SEMA5A) is an axonal regulator molecule, which belongs to the Semaphorin family of proteins. Previously, we identified SEMA5A as a putative marker for aggressive pancreatic tumors. However, the expression, localization and functional significance of SEMA5A in pancreatic tumors remain unclear. In our study, we hypothesized that SEMA5A expression modulates pancreatic tumor growth and metastasis. We analyzed the constitutive expression and localization of SEMA5A in patient pancreatic tumors ($n = 33$) and unmatched normal pancreatic ($n = 8$) tissues and human pancreatic cancer cell lines ($n = 16$) with different histopathological characteristics. We observed significantly higher expression of SEMA5A protein expression ($p < 0.05$) in human pancreatic tumor tissue samples compared to normal pancreatic tissues. Similarly, the pancreatic cancer cell lines with higher tumorigenic and metastatic potentials as xenografts in nude mice expressed higher levels of SEMA5A mRNA compared to those with lower tumorigenic and metastatic potentials. Furthermore, we examined the functional role of SEMA5A in pancreatic tumor growth and invasion. Ectopic expression of mouse full-length Sema5A in Panc1 (SEMA5A negative) cells significantly ($p < 0.05$) enhanced tumorigenesis, growth and metastasis in vivo as well as proliferation, invasiveness and homotypic aggregation in vitro. Together, these data demonstrate that the expression of SEMA5A in pancreatic cancer cells regulates tumorigenesis, growth, invasion and metastasis, and it also suggests a novel target for diagnosis and treatment of pancreatic cancer.

Saeki, T., et al. (2002). "Inhibition of human lung cancer growth following adenovirus-mediated mda-7

gene expression in vivo." Oncogene **21**(29): 4558-4566.

Overexpression of the melanoma differentiation associated gene-7 (mda-7) in vitro results in suppression of lung cancer cell proliferation. However, the ability of MDA-7 to suppress lung cancer in vivo has not been previously demonstrated. In this study, we investigated the possibility of inducing overexpression of the mda-7 gene in human non-small cell lung carcinoma cells in vivo and its effects on tumor growth. Adenovirus-mediated overexpression of MDA-7 in p53-wild-type A549 and p53-null H1299 subcutaneous tumors resulted in significant tumor growth inhibition through induction of apoptosis. In addition, decreased CD31/PECAM expression and upregulation of APO2/TRAIL were observed in tumors expressing MDA-7. In vivo studies correlated well with in vitro inhibition of lung tumor cell proliferation and endothelial cell differentiation mediated by Ad-mda7. These data demonstrate that Ad-mda7 functions as a multi-modality anti-cancer agent, possessing both, pro-apoptotic and anti-angiogenic properties. We demonstrate for the first time the potential therapeutic effects of Ad-mda7 in human lung cancer.

Saimura, M., et al. (2002). "Intraperitoneal injection of adenovirus-mediated NK4 gene suppresses peritoneal dissemination of pancreatic cancer cell line AsPC-1 in nude mice." Cancer Gene Ther **9**(10): 799-806.

NK4, composed of the N-terminal hairpin and subsequent four-kringle domains of hepatocyte growth factor (HGF), acts not only as a competitive antagonist for HGF but also as a potent angiogenesis inhibitor. This study was designed to assess a therapeutic potential of adenovirus-mediated NK4 gene transfer for disseminated pancreatic cancer cells in the peritoneal lavage of nude mice. We constructed a recombinant adenovirus NK4 (Ad-NK4), which encodes a secretable form of human NK4. In vitro migration of AsPC-1 (human pancreatic cancer cell line) was stimulated by HGF, and it was completely inhibited by Ad-NK4 transfection. Weekly intraperitoneal injections of Ad-NK4 could suppress the development of tumor nodules in a nude mouse peritoneal dissemination model. NK4 expression was detected in the disseminated nodules, liver, pancreas, spleen, and mesenterium. Immunohistochemical study of the disseminated tumors showed a remarkable decrease in microvessel density and an increase in number of apoptotic tumor cells in the Ad-NK4-treated mice. Survival of the Ad-NK4-treated mice was significantly improved. This study indicates that the intraperitoneal transduction of adenovirus-mediated NK4 gene may be a useful therapeutic modality to prevent the development of peritoneal dissemination of pancreatic cancer.

Saito, Y., et al. (2003). "Adenovirus-mediated transfer of the PTEN gene inhibits human colorectal cancer growth in vitro and in vivo." Gene Ther **10**(23): 1961-1969.

The tumor-suppressor gene PTEN encodes a multifunctional phosphatase that is mutated in a variety of human cancers. PTEN inhibits the phosphatidylinositol 3-kinase pathway and downstream functions, including activation of Akt/protein kinase B (PKB), cell survival, and cell proliferation in tumor cells carrying mutant- or deletion-type PTEN. In such tumor cells, enforced expression of PTEN decreases cell proliferation through cell-cycle arrest at G1 phase accompanied, in some cases, by induction of apoptosis. More recently, the tumor-suppressive effect of PTEN has been reported in ovarian and thyroid tumors that are wild type for PTEN. In the present study, we examined the tumor-suppressive effect of PTEN in human colorectal cancer cells that are wild type for PTEN. Adenoviral-mediated transfer of PTEN (Ad-PTEN) suppressed cell growth and induced apoptosis significantly in colorectal cancer cells (DLD-1, HT29, and SW480) carrying wtPTEN than in normal colon fibroblast cells (CCD-18Co) carrying wtPTEN. This suppression was induced through downregulation of the Akt/PKB pathway, dephosphorylation of focal adhesion kinase (FAK) and mitogen-activated protein kinase (MAPK) and cell-cycle arrest at the G2/M phase, but not the G1 phase. Furthermore, treatment of human colorectal tumor xenografts (HT-29, and SW480) with Ad-PTEN resulted in significant (P=0.01) suppression of tumor growth. These results indicate that Ad-PTEN exerts its tumor-suppressive effect on colorectal cancer cells through inhibition of cell-cycle progression and induction of cell death. Thus Ad-PTEN may be a potential therapeutic for treatment of colorectal cancers.

Sakakura, C., et al. (1995). "Inhibition of colon cancer cell proliferation by antisense oligonucleotides targeting the messenger RNA of the Ki-ras gene." Anticancer Drugs **6**(4): 553-561.

Point mutations that activate the Ki-ras proto-oncogene are present in approximately 50% of human colorectal tumors and the activated Ki-ras gene is considered to play an important role in colorectal cancer cell proliferation. Five different colon cancer cell lines and two kinds of control cell lines were treated with antisense oligonucleotides complementary to the messenger RNA of Ki-ras. Treatment with antisense oligonucleotides at concentrations between 10 and 40 microM significantly and dose-dependently

inhibited cell growth, colony formation and Ki-ras protein production of the colon cancer cells with activated Ki-ras, but did not affect the normal cells and colon cancer cells without Ki-ras mutation. These results show that use of synthetic oligonucleotides is an effective way of producing antisense-mediated changes in the behavior of human colon cancer cells with an activated Ki-ras gene.

Sakurada, A., et al. (1999). "Adenovirus-mediated delivery of the PTEN gene inhibits cell growth by induction of apoptosis in endometrial cancer." Int J Oncol **15**(6): 1069-1074.

PTEN, a gene encoding a dual specificity phosphatase, is frequently altered in endometrial carcinoma. Moreover, these alterations are observed even in atypical hyperplasia of the endometrium. This evidence suggests that mutation of PTEN is an early genetic alteration involved in endometrial carcinogenesis. Adenovirus-mediated gene transfer was carried out using Ishikawa 3 H 12 and RL95-2, the endometrial cancer cell lines with completely inactivated PTEN, together with endometrial cancer cell lines HEC1-A and KLE expressing wild-type PTEN as the control. The PTEN transgene significantly suppressed cell growth in vitro through induction of apoptosis in cells lacking wild-type PTEN. Furthermore, the ex vivo tumor formation by Ishikawa 3 H 12 cells was completely inhibited by the introduction of wild-type PTEN. However, neither regression nor progression was observed in inoculated tumors of either cell line by in vivo introduction of the PTEN gene. These results suggest that PTEN may be a good candidate for gene therapy in patients with endometrial carcinoma.

Sato, M., et al. (1997). "Induction of cyclin-dependent kinase inhibitor, p21WAF1, by treatment with 3,4-dihydro-6-[4-(3,4)-dimethoxybenzoyl)-1-piperazinyl]-2(1H)-quinoline (vesnarinone) in a human salivary cancer cell line with mutant p53 gene." Cancer Lett **112**(2): 181-189.

It has been found by PCR-SSCP analysis and direct DNA sequencing that a human salivary adenosquamous carcinoma-forming cell line, TYS, has a mutant p53 gene at codon 281Asp-->His. When TYS cells were treated with a differentiation-inducing agent, vesnarinone, cellular proliferation was significantly inhibited on the basis of MTT assay. In addition, it has been found by Northern blotting and/or immunoblotting that expression of p21WAF1 and transforming growth factor-beta (TGF-beta) is up-regulated by treating TYS cells with vesnarinone. TGF-beta 1 alone also induced p21WAF1 expression in TYS cells. Moreover, it has been shown by ELISA that the treatment of TYS cells with vesnarinone results in the enhanced generation of latent TGF-beta 1. The expression of TGF-beta receptor (T beta R), including T beta R-I, T beta R-II and T beta R-III, on TYS cells was detected by affinity cross-linking using 125I-TGF-beta 1 and addition of active TGF-beta 1 into serum-free culture medium inhibited the growth of TYS cells in a concentration-dependent manner. These findings suggest that vesnarinone might directly induce expression of p21WAF1 gene in TYS cells, the product of which may be associated with the inhibition of cell growth and induce differentiation.

Sauer, M. K. and I. L. Andrulis (2005). "Identification and characterization of missense alterations in the BRCA1 associated RING domain (BARD1) gene in breast and ovarian cancer." J Med Genet **42**(8): 633-638.

BACKGROUND: BRCA1 associated RING domain protein (BARD1) was originally identified due to its interaction with the RING domain of BRCA1. BARD1 is required for S phase progression, contact inhibition and normal nuclear division, as well as for BRCA1 independent, p53 dependent apoptosis. METHODS: To investigate whether alterations in BARD1 are involved in human breast and ovarian cancer, we used single strand conformation polymorphism analysis and sequencing on 35 breast tumours and cancer cell lines and on 21 ovarian tumours. RESULTS: Along with the G2355C (S761N) missense mutation previously identified in a uterine cancer, we found two other variants in breast cancers, T2006C (C645R) and A2286G (I738V). The T2006C (C645R) mutation was also found in one ovarian tumour. A variant of uncertain consequence, G1743C (C557S), was found to be homozygous or hemizygous in an ovarian tumour. Eleven variants of BARD1 were characterised with respect to known functions of BARD1. None of the variants appears to affect localisation or interaction with BRCA1; however, putative disease associated alleles appear to affect the stability of p53. These same mutations also appear to abrogate the growth suppressive and apoptotic activities of BARD1. CONCLUSIONS: These activities allowed us to identify one of the rare variants (A2286G; I738V) as a neutral polymorphism rather than a detrimental mutation, and suggested that G1743C (C557S) is not a polymorphism but may contribute to the cancer phenotype.

Sazawa, A., et al. (2002). "Adenovirus mediated gelsolin gene therapy for orthotopic human bladder cancer in nude mice." J Urol **168**(3): 1182-1187.

PURPOSE: Gelsolin is an actin regulatory protein that is undetectable or reduced in human bladder tumors compared with normal epithelial cells. Whether the over expression of gelsolin could inhibit

tumor growth was investigated in an orthotopic bladder cancer nude mouse model using recombinant adenovirus encoding wild-type gelsolin (Ad-GSN). MATERIALS AND METHODS: The 2 human bladder cancer cell lines KU-7 and UMUC-2 were transduced with Ad-GSN in vitro. Flow cytometric analysis was done to examine the cell cycle after transducing the adenovirus. Cell growth was compared with control groups of these cells transduced with adenovirus containing the Escherichia coli beta-galactosidase gene Ad-betagal. In vivo KU-7 cells were introduced into the bladder of nude mice (day 0), followed by 3 injections into the urethra (days 2 to 4) with Ad-GSN or Ad-betagal (1 x 10 pfu). At 8 days after initial adenovirus exposure (day 10) each bladder was sectioned and stained, and the mass of the tumor was digitally determined. RESULTS: Bladder cancer cell growth (KU-7 and UMUC-2) was inhibited after these cells were transduced with Ad-GSN in vitro. Based on flow cytometric analysis over expression of gelsolin may cause these cells to arrest or delay at the G2/M phase of the cell cycle. In the orthotopic bladder cancer model the mass of the tumor was approximately 90% less in Ad-GSN treated animals than in controls. CONCLUSIONS: Ad-GSN provides a significant tumor suppressive effect on human bladder cancer cells in this orthotopic nude mouse model. Adenovirus mediated over expression of gelsolin may be useful therapy for human bladder cancer.

Schmidt, L. J., et al. (2009). "Effects of the 5 alpha-reductase inhibitor dutasteride on gene expression in prostate cancer xenografts." Prostate **69**(16): 1730-1743.

BACKGROUND: In the prostate, androgens play a crucial role in normal and cancerous growth; hence the androgenic pathway has become a target of therapeutic intervention. Dutasteride is a 5 alpha-reductase (5AR) inhibitor currently being evaluated both for chemoprevention and treatment of prostate cancer. Dutasteride inhibits both 5AR I and II enzymes, effectively blocking conversion of testosterone to dihydrotestosterone (DHT) in the prostate. This greatly reduces the amount of the active ligand DHT available for binding to the androgen receptor (AR) and stimulating proliferation, making this a good candidate for chemoprevention of prostate cancer. In this study, we sought to determine how dutasteride is functioning at the molecular level, using a prostate cancer xenograft model. METHODS: Androgen-responsive LuCaP 35 xenograft tumors were grown in Balb/c mice. Subcutaneously implanted time-release pellets were used for drug delivery. Microarray analysis was performed using the Affymetrix HG-U133Av2 platform to examine changes in gene expression in tumors following dutasteride treatment. RESULTS: Dutasteride significantly reduced tumor growth in LuCaP 35 xenografts by affecting genes involved in apoptotic, cytoskeletal remodeling, and cell cycle pathways among others. Notably, genes in the Rho GTPase signaling pathway, shown to be important in androgen-deprivation conditions, were significantly up-regulated. CONCLUSION: We have identified multiple pathways outside of the androgenic pathway in prostate cancer xenografts affected by treatment with dutasteride. These findings provide insights into the function of dutasteride within the tumor microenvironment, potentially allowing for development of agents that can be used in combination with this drug to further enhance its effectiveness.

Seki, T., et al. (2001). "Mechanism of growth-inhibitory effect of cisplatin on human pancreatic cancer cells and status of p53 gene." Anticancer Res **21**(3B): 1919-1924.

Pancreatic cancer is a devastating malignant tumor in humans and the development of new modalities of treatment is needed. We studied the mechanism of the growth-inhibitory effect of cisplatin (CDDP) on human pancreatic cancer cells in connection with the status of the p53 gene and expression of the bcl-2 family. COLO-357 cells with wild-type p53 gene and T3M4, Panc-1 and AsPC-1 cells with mutant-p53 gene were used. Growth of these cells was inhibited by CDDP in a dose-dependent manner in both serum-deprived and serum-supplemented conditions. CDDP induced apoptosis of COLO-357 and T3M4 cells in the serum-supplemented condition, whereas necrosis of these cells was induced by CDDP at high concentrations in the serum-deprived condition. Although expression of bax mRNA and its protein product were enhanced, while bcl-2 protein was decreased by CDDP in COLO-357 cells, expression of mRNA of the bcl-2 family and protein product were not influenced by CDDP in T3M4 cells. Increased expression of bax and reduced expression of bcl-2 are involved in the growth-inhibitory effect of CDDP on pancreatic cancer cells with wild-type p53 gene.

Sekine, S., et al. (2002). "Target disruption of the mutant beta-catenin gene in colon cancer cell line HCT116: preservation of its malignant phenotype." Oncogene **21**(38): 5906-5911.

Most colorectal carcinomas harbor genetic alterations that result in stabilization of beta-catenin. A colorectal carcinoma cell line, HCT116, which has both mutated and wild-type beta-catenin genes, was engineered by homologous recombination to investigate the significance of beta-catenin gene mutation. As expected, the mutant allele-targeted

clones showed decreased beta-catenin expression and downregulation of T-cell factor (TCF)/lymphoid enhancer factor (LEF)-dependent transcription. Morphologically, targeted clones were only minimally altered under usual culture conditions, but under low serum conditions, mutant allele-targeted clones still grew in plane, in contrast to parental cell line and wild allele-targeted clones, which formed spheroids. The mutant allele-targeted clones showed no significant changes in growth rate and anchorage-independent growth in vitro, and displayed rather increased growth in vivo. Although beta-catenin stabilization affects some biological characteristics including adhesive properties, it may not have growth-promoting effects at least in some colorectal carcinomas.

Sheikh, M. S., et al. (1993). "Estrogen receptor-negative breast cancer cells transfected with the estrogen receptor exhibit increased RAR alpha gene expression and sensitivity to growth inhibition by retinoic acid." J Cell Biochem **53**(4): 394-404.

We and others have shown previously that retinoic acid (RA) selectively inhibits the growth of estrogen receptor (ER)-positive human breast carcinoma (HBC) cells and ER-negative cells are refractory to RA inhibition of growth. The ER-negative cells inherently express lower levels of RAR alpha and retinoic acid response element (RARE)-mediated RA-induced CAT activity. In this study we report that when ER-negative MDA-MB-231 cells were transfected with the ER gene they not only expressed higher levels of RAR alpha and RARE-mediated RA-induced CAT gene expression, but their growth was not inhibited by RA. Estrogen enhanced RAR alpha gene expression not only in established ER-positive cell lines but also in ER-transfected MDA-MB-231 cells. The estrogen effect appears to be direct and at the gene transcription level since it did not alter the stability of RAR alpha mRNA and cycloheximide failed to block estrogen-mediated enhancement of RAR alpha gene expression. Our data strongly suggest that ER-mediated enhancement of RAR alpha levels plays an important role in RA inhibition of HBC growth. In addition, we also report here that HBC cells appear to express a unique isoform (s) of RAR alpha which was detected only when the full-length RAR alpha cDNA was used as a probe; the RAR alpha 1 and RAR alpha 2 specific probes failed to hybridize with the HBC specific RAR alpha message.

Shi, Y. E., et al. (1997). "Antitumor activity of the novel human breast cancer growth inhibitor, mammary-derived growth inhibitor-related gene, MRG." Cancer Res **57**(15): 3084-3091.

A novel human tumor growth inhibitor was identified by differential cDNA sequencing. The predicted amino acid sequence of this tumor-suppressing factor has a significant sequence homology to mouse mammary-derived growth inhibitor and thus was named mammary-derived growth inhibitor-related gene (MRG). MRG was found to be expressed in normal and benign human breast tissues but not in breast carcinomas. In situ hybridization analysis demonstrated a stage-specific MRG expression as follows. MRG was barely detectable in breast carcinomas, showed partial and weak expression in benign hyperplasia, but was expressed at a high level in normal breast epithelial cells. To determine if MRG can modulate in vivo growth of human breast cancers, we transfected a full-length MRG cDNA into MDA-MB-231 human breast cancer cells and studied the orthotopic growth of MRG transfectants versus control transfectants in the mammary fat pad of athymic nude mice. Overexpression of MRG in human breast cancer cells significantly suppressed cell proliferation in vitro and tumor growth in an orthotopic nude mouse model. These results suggest that MRG has tumor-suppressing activity, and the loss of MRG expression may be involved in the development and progression of breast cancer.

Shiau, A. L., et al. (2001). "Retrovirus-mediated transfer of prothymosin gene inhibits tumor growth and prolongs survival in murine bladder cancer." Gene Ther **8**(21): 1609-1617.

To explore the potential use of prothymosin alpha (ProT), a putative thymic hormone, in gene therapy for bladder cancer, we generated a replication-defective recombinant retroviral vector encoding ProT and tested its antitumor effect on the MBT-2 murine bladder cancer. C3H/HeN mice injected with MBT-2 cells in conjunction with retroviruses encoding ProT exhibited smaller tumor mass, lower tumor incidence and higher survival rate, as well as higher antitumor cytotoxic activities compared with those injected with control viruses. However, such effects were not observed in severe combined immunodeficiency mice, suggesting that ProT exerts antitumor effects through its immunomodulatory activities. Cell growth in monolayer culture and colony formation in soft agar were enhanced in ProT gene-modified MBT-2 clones, and such growth-promoting activities of ProT could be reversed if its nuclear localization signal (NLS) was deleted. To circumvent the proliferation-promoting effect of ProT on tumor cells, a retroviral vector encoding ProT lacking NLS was constructed. Our results showed that retroviruses encoding NLS-deleted ProT was more efficacious than those encoding wild-type ProT in prolonging survival of tumor-bearing

mice. This is the first report indicating that ProT, in particular NLS-deleted ProT, delivered by retroviral vectors may be further explored for the treatment of bladder cancer.

Shimizu, M., et al. (1998). "Effect on colon cancer cells of human interferon-beta gene entrapped in cationic multilamellar liposomes." Biochem Mol Biol Int **44**(6): 1235-1243.

When cultured cells of human colon cancer cell line SW480 were transfected with human interferon-beta (hIFN-beta) gene by means of cationic multilamellar liposomes, the endogenously produced hIFN-beta exhibited a remarkable anti-proliferative effect on the cells, which was more effective than that of exogenously added hIFN-beta. This effect lasted for several days, and was blocked completely by the addition of sufficient amounts of anti-hIFN-beta antibody. From experiments using a transwell plate and an infusion pump, we found that endogenously produced hIFN-beta acted effectively on the cells around the transfectants and that the growth-inhibitory effect was totally retained upon continuous dilution of the medium. These data indicate that hIFN-beta expressed endogenously by transfer of its gene acted on these cancer cells mainly in a paracrine manner. Although the transfection with hIFN-gamma gene also revealed a definite growth-inhibitory effect on the same tumor cells, the extent was less than that of hIFN-beta gene.

Shinderman-Maman, E., et al. (2016). "The thyroid hormone-alphavbeta3 integrin axis in ovarian cancer: regulation of gene transcription and MAPK-dependent proliferation." Oncogene **35**(15): 1977-1987.

Ovarian carcinoma is the fifth common cause of cancer death in women, despite advanced therapeutic approaches. alphavbeta3 integrin, a plasma membrane receptor, binds thyroid hormones (L-thyroxine, T4; 3,5,3'-triiodo-L-thyronine, T3) and is overexpressed in ovarian cancer. We have demonstrated selective binding of fluorescently labeled hormones to alphavbeta3-positive ovarian cancer cells but not to integrin-negative cells. Physiologically relevant T3 (1 nM) and T4 (100 nM) concentrations in OVCAR-3 (high alphavbeta3) and A2780 (low alphavbeta3) cells promoted alphav and beta3 transcription in association with basal integrin levels. This transcription was effectively blocked by RGD (Arg-Gly-Asp) peptide and neutralizing alphavbeta3 antibodies, excluding T3-induced beta3 messenger RNA, suggesting subspecialization of T3 and T4 binding to the integrin receptor pocket. We have provided support for extracellular regulated kinase (ERK)-mediated transcriptional regulation of the alphav monomer by T3 and of beta3 monomer by both hormones and documented a rapid (30-120 min) and dose-dependent (0.1-1000 nM) ERK activation. OVCAR-3 cells and alphavbeta3-deficient HEK293 cells treated with alphavbeta3 blockers confirmed the requirement for an intact thyroid hormone-integrin interaction in ERK activation. In addition, novel data indicated that T4, but not T3, controls integrin's outside-in signaling by phosphorylating tyrosine 759 in the beta3 subunit. Both hormones induced cell proliferation (cell counts), survival (Annexin-PI), viability (WST-1) and significantly reduced the expression of genes that inhibit cell cycle (p21, p16), promote mitochondrial apoptosis (Nix, PUMA) and tumor suppression (GDF-15, IGFBP-6), particularly in cells with high integrin expression. At last, we have confirmed that hypothyroid environment attenuated ovarian cancer growth using a novel experimental platform that exploited paired euthyroid and severe hypothyroid serum samples from human subjects. To conclude, our data define a critical role for thyroid hormones as potent alphavbeta3-ligands, driving ovarian cancer cell proliferation and suggest that disruption of this axis may present a novel treatment strategy in this aggressive disease.

Sica, G., et al. (1999). "Effect of leuprorelin acetate on cell growth and prostate-specific antigen gene expression in human prostatic cancer cells." Eur Urol **35 Suppl 1**: 2-8.

OBJECTIVES: We investigated modulation of cell growth and prostate-specific antigen (PSA) gene expression in prostatic cancer cells by the luteinizing hormone-releasing hormone analog (LH-RHa), leuprorelin acetate, alone or combined with other agents. METHODS: The effect of the analog on proliferation of both androgen-sensitive and -insensitive prostate cancer cells, maintained in different culture conditions, was evaluated by cell counts at various intervals of time. Basal expression of PSA gene and its variations were determined by a reverse transcriptase-polymerase chain reaction assay. RESULTS: LH-RHa is ineffective in regulating cell growth, when used alone in both hormone-sensitive and -insensitive cell lines. Nevertheless, it counteracts the stimulatory action of androgens on proliferation of LNCaP cells, which respond to low concentrations of dihydrotestosterone. Moreover, LH-RHa has an inhibitory effect on the mitogenic action of epidermal growth factor (EGF) in androgen-unresponsive PC-3 cells. The analog reduces PSA gene expression in both hormone-sensitive and -insensitive cells. Interestingly, it counteracts the gene expression induced by androgens in LNCaP cells and by EGF in PC-3 cells. CONCLUSIONS: These data show that LH-RHa may behave like a negative growth factor, which directly

regulates cell growth and PSA gene expression. Moreover, our findings support the idea that growth factors may interfere with the androgen signalling pathway.

Siemens, D. R., et al. (2000). "Viral vector delivery in solid-state vehicles: gene expression in a murine prostate cancer model." J Natl Cancer Inst **92**(5): 403-412.

BACKGROUND: Although there are increasingly more clinical trials involving gene therapy, efficient gene transfer remains a major hurdle to success. To enhance the efficiency of delivery of viral vectors in gene therapy protocols, we evaluated the effect of various matrices to act as a vehicle for recombinant virus during intratumoral injection. METHODS: The ability of several vehicles (catgut spacer, polyglycolic acid, chromic catgut, and gelatin sponge matrix) to deliver the canarypox virus ALVAC to the cells of the murine prostate cancer cell line RM-1 was studied in vitro and in vivo. ALVAC recombinants encoding the murine cytokines interleukin 2 (IL-2), interleukin 12 (IL-12), and tumor necrosis factor-alpha (TNF-alpha) were used to assess enhancement of antitumor activity after intratumoral inoculation. Confirmatory experiments were conducted by use of another mouse prostate cancer cell line, RM-11, and a mouse bladder cancer cell line, MB-49. All statistical tests were two-sided. RESULTS: The gelatin sponge matrix proved to be the most effective solid-state vehicle for delivering viral vectors to cells in culture. In addition, this matrix statistically significantly enhanced expression of ALVAC-delivered reporter genes in tumor models when compared with fluid-phase delivery of virus ($P = .037$ for the RM-1 model and $P = .03$ for the MB-49 model). Statistically significant growth inhibition of established tumors was observed when a combination of the three recombinant ALVAC viruses expressing IL-2, IL-12, and TNF-alpha was delivered with the matrix in comparison with 1) fluid-phase intratumoral injection of the ALVAC recombinants, 2) no treatment, or 3) treatment with parental ALVAC (all $P < .05$). CONCLUSIONS: Viral vector delivery in a solid-state vehicle resulted in improved recombinant gene expression in vivo and translated to greater inhibition of tumor growth in an immunotherapy protocol for heterotopic tumor nodules. The efficient delivery of reporter genes described herein may prove useful in many solid tumor gene therapy protocols.

Singh, P., et al. (1996). "Gastrin gene expression is required for the proliferation and tumorigenicity of human colon cancer cells." Cancer Res **56**(18): 4111-4115.

The majority of human colon cancers express the gastrin gene, and a significant percentage bind gastrin-like peptides. However, it is not known if gastrin gene products are physiologically relevant to the growth and proliferation of human colon cancers. To investigate the functional role of gastrin gene expression, we examined the effect of gastrin antisense (AS) RNA expression on the growth and tumorigenicity of colon cancer cells. The full-length human gastrin cDNA was cloned in the AS direction in a retroviral vector under the transcriptional control of human cytomegalovirus promoter. Three representative human colon cancer cell lines that expressed negligible (Colo-205A) to significant (Colo-320 and HCT-116) levels of gastrin mRNA were transfected with either AS or control vectors and subjected to various growth studies in vitro and in vivo. The proliferative and tumorigenic potential of the AS clones from the gastrin-expressing cell lines was significantly suppressed compared to that of the control clones, whereas the growth of Colo-205A-AS cells (the negative control) was similar to that of the Colo-205A-C-cells, indicating the relative specificity of the antitumorigenic effects of AS gastrin RNA expression. We believe that this is the first evidence that supports a possible critical role of gastrin gene expression in the tumorigenicity of human colon cancers that express the gastrin gene. Because > 60-80% of human colon cancers express the gastrin gene, it can be expected that the growth of a significant percentage of these cancers may be critically dependent on the expression of gastrin gene products. Therapeutic measures, such as the AS strategy used in the present study, may therefore prove to be useful in treating human colon cancers in the future.

Soler, M. N., et al. (2000). "Gene therapy of rat medullary thyroid cancer by naked nitric oxide synthase II DNA injection." J Gene Med **2**(5): 344-352.

BACKGROUND: Nitric oxide (NO), produced by NO synthase II (NOS II), is the main mediator of the tumoricidal action of activated macrophages. In the present study we examined the potential of the NOS II gene as a suicide gene for medullary thyroid cancer (MTC) therapy. METHODS: We orthotopically transplanted rMTC 6-23 cells into the inbred strain of Wag/Rij rats and constructed a plasmid carrying the NOS II gene under the control of the cytomegalovirus (CMV) promoter. RESULTS: Successive injections of tumor cells (Day 0) and naked DNA (Day 2) caused strong inhibition of tumor growth (50%, $p < 0.05$). Plasmid injection into established tumors (14-day tumors) resulted in the development of large cavities due to tumor cell destruction, with a significant reduction in tumor tissue volume (35%, $p < 0.05$). Adjacent quiescent tissues were unaffected. Cell death occurred by apoptosis as demonstrated by specific

labeling. Macrophages and CD4+ lymphocytes were recruited in the treated tumors. However, tumor-specific T lymphocytes were undetectable in the spleen of treated rats. In control experiments using Lac Z as a reporter gene, expression of beta-galactosidase was detected in only 1% of the tumor cells. CONCLUSIONS: Despite a low gene transfer efficiency, NOS II plasmid produced a strong anti-tumor action resulting from its marked 'bystander' effect mainly due to NO production and diffusion. Therefore the NOS II gene appears to be a promising suicide gene therapy of human cancer.

Sorrells, D. L., et al. (1999). "Competitive PCR to detect eIF4E gene amplification in head and neck cancer." Head Neck **21**(1): 60-65.

BACKGROUND: The protein eukaryotic initiation factor 4E (elF4E) binds to messenger ribonucleic acid (mRNA) as the initial step in protein synthesis. Overexpression of elF4E results in upregulation of specific proteins essential to cell growth and division. Overexpression of elF4E has been found in head and neck squamous cell carcinoma (HNSCC) and breast carcinoma. This study's purpose is to determine whether elF4E overexpression is present and associated with elF4E gene amplification in HNSCC. METHODS: Competitive polymerase chain reaction (PCR) was performed on eight HNSCC and seven intraoral benign lesions to determine the copy number of elF4E relative to a reference gene, gastrin. Western blots were performed to quantify elF4E protein expression. RESULTS: All eight HNSCC specimens demonstrated significant (p <.005) overexpression of elF4E protein (14.1+/-10.4) and elF4E gene amplification (4.5+/-1.2). Benign tissue did not exhibit elF4E protein overexpression or gene amplification. CONCLUSIONS: Overexpression and associated gene amplification of elF4E were present in HNSCC but not in benign tissue. Gene amplification of elF4E may be an important mechanism for elF4E overexpression.

Spitz, F. R., et al. (1996). "In vivo adenovirus-mediated p53 tumor suppressor gene therapy for colorectal cancer." Anticancer Res **16**(6B): 3415-3422.

BACKGROUND: The p53 tumor suppressor gene is altered in up to 70% of colorectal cancers. MATERIALS AND METHODS: We infected the colorectal cancer cell lines SW620 and KM12L4, in which p53 is mutated, with the replication-defective adenovirus Ad5/CMV/p53 to evaluate the effects of adenovirus-mediated wild-type p53 gene transfer. Gene transduction was measured by cytochemical staining of cells infected with the Ad5/CMV/beta-gal virus and expression of the wildtype p53 protein in these cells was demonstrated by immunoblotting. RESULTS: Significant suppression of in vitro cell proliferation and induction of apoptosis (as measured by TUNEL assay labeling) were observed following Ad5/CMV/p53 infection. More importantly, similar effects were observed in vivo in an established nude mouse subcutaneous tumor model; significant suppression of tumor growth (60%-70%) and induction of apoptosis were observed following intratumoral injections of Ad5/CMV/p53. CONCLUSION: This form of therapy may provide a novel approach to colorectal cancer.

Srivastava, M., et al. (2001). "ANX7, a candidate tumor suppressor gene for prostate cancer." Proc Natl Acad Sci U S A **98**(8): 4575-4580.

The ANX7 gene is located on human chromosome 10q21, a site long hypothesized to harbor a tumor suppressor gene (s) (TSG) associated with prostate and other cancers. To test whether ANX7 might be a candidate TSG, we examined the ANX7-dependent suppression of human tumor cell growth, stage-specific ANX7 expression in 301 prostate specimens on a prostate tissue microarray, and loss of heterozygosity (LOH) of microsatellite markers at or near the ANX7 locus. Here we report that human tumor cell proliferation and colony formation are markedly reduced when the wild-type ANX7 gene is transfected into two prostate tumor cell lines, LNCaP and DU145. Consistently, analysis of ANX7 protein expression in human prostate tumor microarrays reveals a significantly higher rate of loss of ANX7 expression in metastatic and local recurrences of hormone refractory prostate cancer as compared with primary tumors (P = 0.0001). Using four microsatellite markers at or near the ANX7 locus, and laser capture microdissected tumor cells, 35% of the 20 primary prostate tumors show LOH. The microsatellite marker closest to the ANX7 locus showed the highest rate of LOH, including one homozygous deletion. We conclude that the ANX7 gene exhibits many biological and genetic properties expected of a TSG and may play a role in prostate cancer progression.

Stoll, V., et al. (2005). "Dominant negative inhibitors of signalling through the phosphoinositol 3-kinase pathway for gene therapy of pancreatic cancer." Gut **54**(1): 109-116.

BACKGROUND: Ras signalling is frequently aberrant in pancreatic cancer so that there is constitutive activation of the phosphatidylinositol 3-kinase (PI3K) and AKT/protein kinase B pathway, as well as the RAF/MEK/ERK pathway. AIMS: In the present study we investigated the role of the PI3K/AKT pathway in malignant transformation of pancreatic cancer cells. METHODS: A genetic approach was used to interfere with signal transduction

in vitro and in vivo. RASN17, a dominant negative mutant of RAS, was applied to inhibit the PI3K/AKT pathway upstream of PI3K. The regulatory p85beta subunit of PI3K and the negative regulator PTEN were utilised to inhibit the pathway at the level of PI3K, and AAA-AKT, a dominant negative mutant of AKT was employed to interfere with PI3K/AKT signalling at the level of AKT. RESULTS: Antiproliferative, proapoptotic, and anticancer effects were documented, showing that inhibition of the PI3K pathway in these cell lines suppresses tumour cell growth in vitro and reduces growth in nude mice. CONCLUSIONS: The PI3K/AKT pathway represents a potential therapeutic target for pancreatic cancer, and gene therapy may be one approach to produce selective inhibition.

Sumitomo, K., et al. (2000). "Expression of a TGF-beta1 inducible gene, TSC-36, causes growth inhibition in human lung cancer cell lines." Cancer Lett **155**(1): 37-46.

TSC-36 (TGF-beta1-stimulated clone 36) is a TGF-beta1 inducible gene whose product is an extracellular glycoprotein that contains a single follistatin module. TSC-36 is highly expressed in the lung, but its physiological function is unknown. In an attempt to elucidate it, we investigated the effect of TSC-36 on proliferation of human lung cancer cell lines. We found a correlation between expression of TSC-36 and cell growth: TSC-36 mRNA was not detected in cells derived from small cell lung cancer (SCLC) cells, a highly aggressive neoplasm, but was detected in some non-small cell lung cancer (NSCLC) cells, a moderately aggressive neoplasm. This suggested an antiproliferative function for TSC-36. To address this question, NSCLC PC-14 cells, which express very low level of TSC-36 protein, were transfected with TSC-36 cDNA and the proliferative capacity of stable transfectants was determined by measuring the doubling time, colony forming activity in soft agar and the level of incorporation of (3)H-thymidine into DNA. Under normal culture conditions, the transfected cells showed a longer doubling time, lower plating efficiency and lower rate of DNA synthesis than the parental cells and the control neo transfectant cells. These findings suggested that expression of TSC-36 caused growth inhibition in human lung cancer cells.

Sumitomo, M., et al. (1999). "Overexpression of IL-1ra gene up-regulates interleukin-1beta converting enzyme (ICE) gene expression: possible mechanism underlying IL-1beta-resistance of cancer cells." Br J Cancer **81**(2): 277-286.

We investigated the interaction of endogenous interleukin (IL)-1beta, IL-1ra, and interleukin-1beta converting enzyme (ICE) in four human urological cancer cell lines, KU-19-19, KU-1, KU-2 and KU-19-20. Northern blot analysis showed that IL-1beta gene was expressed in all cell lines. On the other hand, in KU-19-19 and KU-19-20, the gene expressions of both IL-1ra and ICE were suppressed. MTT assay revealed that IL-1beta (10 ng ml (-1)) promoted cell growth in KU-19-19 and KU-19-20, while it inhibited in KU-1 and KU-2. An ICE inhibitor, Acetyl-Tyr-Val-Ala-Asp-CHO (YVAD-CHO) blocked IL-1beta-induced growth inhibition in KU-1 and KU-2. Overexpression of the secretory type IL-1ra with adenovirus vector (AxlL-1ra) enhanced ICE gene expression, while exogenous IL-1ra (100 ng ml (-1)) did not enhance it. Furthermore, AxIL-1ra treatment promoted endogenous IL-1beta secretion and induced significant growth inhibition and apoptotic cell death on KU-19-19 and KU-19-20. Treatment with either IL-1ra (100 ng ml (-1)), IL-1beta antibody (100 microg ml (-1)), or YVAD-CHO blocked AxlL-1ra-induced cell death in KU-19-19 and KU-19-20. These results suggest that IL-1beta-sensitivity depends on the level of ICE gene expression, which is regulated by the level of endogenous slL-1ra expression. This is a first report on the intracellular function of slL-1ra and these findings may provide key insights into the mechanism underlying the viability of cancer cells.

Suzuki, S., et al. (2001). "Coexpression of the partial androgen receptor enhances the efficacy of prostate-specific antigen promoter-driven suicide gene therapy for prostate cancer cells at low testosterone concentrations." Cancer Res **61**(4): 1276-1279.

The prostate specific antigen (PSA) promoter/enhancer has been clearly demonstrated to be tissue specific, and has been applied to prostate-specific gene therapy. However, the transcription of the PSA gene is strictly androgen dependent, and its promoter activity is very weak at low concentrations of testosterone, which are generally observed in prostatic cancer patients treated with androgen deprivation. In this study, we used a partial androgen receptor (ARf) containing amino acids 232-429 and 481-657 to transactivate the PSA gene without androgens. We made two expression vectors, ARfPPLUC and ARfPPTK. They contained ARf cDNA driven by cytomegalovirus promoter and cDNAs of either firefly luciferase (LUC) or herpes simplex virus thymidine kinase (TK) driven by PSA promoter/enhancer (PP). The expressed ARf enhanced the PP activity by about 110-fold in the PSA-producing prostate cancer cell line, LNCaP, under low testosterone concentrations. Moreover, in a PSA-nonproducing prostate cancer cell line, DU145, ARf also enhanced the PP activity by about 60-fold in an androgen-independent manner. In a growth inhibition assay, ARfPPTK treated with ganciclovir was found to

inhibit the cell growth of LNCaP cells much more effectively than PPTK. Furthermore, in contrast to PPTK, ARfPPTK also had an inhibitory effect on DU145 cells. This system is thus considered to provide a useful therapeutic option in patients with prostate cancer who are receiving hormonal therapy.

Takaoka, A., et al. (1998). "Suppression of invasive properties of colon cancer cells by a metastasis suppressor KAI1 gene." Oncogene **16**(11): 1443-1453.

KAI1 is a potential metastatic suppressor gene for prostate cancer. We found by Northern blot analysis that six of ten (60%) gastric and colon cancer cell lines exhibited undetectable or very low expression level of KAI1 mRNA. The effects of KAI1 on the adhesion, motility and invasiveness of colon cancer cells was therefore investigated by using two kinds of stable transfectants, i.e., antisense transfectants of BM314 cells whose KAI1 mRNA expression was suppressed by transfer of antisense KAI1 cDNA and sense transfectants of DLD-1 cells with the enhanced KAI1 mRNA by sense cDNA transfer. The following results were obtained: (1) KAI1 gene expression had no significant effect on in vitro cell growth rate of colon cancer BM314 and DLD-1 cells; (2) Cell aggregation assay showed that KAI1 enhanced the Ca++-independent aggregatability of those colon cancer cells; (3) It was revealed by cell motility and invasion assays that KAI1 suppressed both the motility and in vitro invasiveness of those cells and (4) Furthermore, both the binding to fibronectin and the migration on fibronectin-coated plates of those cells were inhibited by KAI1 expression. These suggest that reduced KAI1 gene expression may contribute to the invasiveness and metastatic ability of colon cancer cells.

Takei, Y., et al. (1998). "Isolation of a novel TP53 target gene from a colon cancer cell line carrying a highly regulated wild-type TP53 expression system." Genes Chromosomes Cancer **23**(1): 1-9.

We established a colon cancer cell line SW480-LOWTP53-1 carrying a wild-type TP53 transgene that is inducible under control of the lactose operon. Induction of this transgene by isopropyl-beta-D-thiogalactoside (IPTG) arrests growth of the transfected cells. To investigate cellular responses related to the TP53 signaling pathway to induce growth arrest, we applied a differential display method to screen mRNAs isolated from this cell line and looked for genes whose expression was activated or suppressed after induction of wild-type TP53. Subsequent Northern blot analysis confirmed that expression of one novel gene was regulated by wild-type TP53. The cDNA, termed TP53TG1 (TP53 target gene 1), contained an open reading frame of 270 nucleotides encoding 90 amino acids. Under conditions of cellular stress (ultraviolet irradiation or exposure to bleomycin or cisplatin), expression of TP53TG1 was induced in a wild-type TP53-dependent manner, indicating that this gene is likely to play an important role in the signaling pathway of TP53 and may function in response to cellular damage.

Takeyama, Y., et al. (2010). "Knockdown of ZEB1, a master epithelial-to-mesenchymal transition (EMT) gene, suppresses anchorage-independent cell growth of lung cancer cells." Cancer Lett **296**(2): 216-224.

We found that among four master epithelial-to-mesenchymal transition (EMT)-inducing genes (ZEB1, SIP1, Snail, and Slug) ZEB1expression was most significantly correlated with the mesenchymal phenotype (high Vimentin and low E-cadherin expression) in non-small cell lung cancer (NSCLC) cell lines and tumors. Furthermore, ZEB1 knockdown with RNA interference in three NSCLC cell lines with high ZEB1 expression suppressed to varying degrees mass culture growth and liquid colony formation but in all cases dramatically suppressed soft agar colony formation. In addition, ZEB1 knockdown induced apoptosis in one of the three lines, indicating that the growth inhibitory effects of ZEB1 knockdown occurs in part through the activation of the apoptosis pathway. These results suggest that inhibiting ZEB1 function may be an attractive target for NSCLC therapeutic development.

Tanaka, M. and H. B. Grossman (2001). "Connexin 26 gene therapy of human bladder cancer: induction of growth suppression, apoptosis, and synergy with Cisplatin." Hum Gene Ther **12**(18): 2225-2236.

The connexin 26 (Cx26) gene encodes a protein involved in gap junctional intercellular communication and is a putative tumor suppressor. We constructed a Cx26 adenovirus vector (Ad-Cx26) and used it to infect human bladder cancer cell lines UM-UC-3, UM-UC-6, UM-UC-14, and T24. Infection with Ad-Cx26 suppressed the growth of these cell lines in vitro and prevented tumor formation in vivo. Cell cycle accumulation or arrest at the G (1) phase was noted in UM-UC-3 cells and at the G (2)/M phase in UM-UC-6, UM-UC-14, and T24 cells. Apoptosis was noted in UM-UC-3, UM-UC-6, and UM-UC-14 cells both in vitro and in vivo. These effects were not seen with control adenovirus (Ad-CTR) or mock infection. Ad-Cx26 did not significantly alter the growth of the immortalized normal human bladder cell line SV-HUC. Direct injection of Ad-Cx26 into established UM-UC-3 and UM-UC-14 tumors in nude mice resulted in

Cx26 expression, apoptosis, and significantly decreased growth compared with Ad-CTR treated tumors. Delayed resumption of tumor growth was associated with loss of Cx26 expression. Combination therapy with Ad-Cx26 and cisplatin resulted in decreased growth in vitro compared with either agent alone. We explored combination therapy with Ad-Cx26 and cisplatin to improve the in vivo efficacy of Cx26 gene therapy. In vivo therapy with Ad-Cx26 and cisplatin resulted in long-term suppression of tumor growth. These data demonstrate that combining gene and chemotherapy can result in dramatic synergy in vivo.

Tayeb, M. T., et al. (2003). "CYP3A4 and VDR gene polymorphisms and the risk of prostate cancer in men with benign prostate hyperplasia." Br J Cancer **88**(6): 928-932.

Prostate cancer (PRCa) is one of the most common causes of cancer death in men and determinants of PRCa risk remain largely unidentified. Benign prostatic hyperplasia (BPH) is found in the majority of ageing men and has been associated with PRCa. Many candidate genes have been suggested to be involved in PRCa, such as those that are central to cellular growth and differentiation in the prostate gland. The vitamin D receptor (VDR) and CYP3A4 have been shown to be involved in the regulation of cell proliferation and differentiation in prostate cells. Genetic variations of these genes have been associated with PRCa in case-control studies and may be useful to detect BPH patients that have a higher risk of developing PRCa. The association between CYP3A4 and VDR TaqI SNPs and the risk of developing PRCa have been investigated in this study by determining the variant genotype frequencies of both SNPs in 400 patients with BPH who have been followed clinically for a median of 11 years. The results of this study showed that the incidence rate of PRCa was higher in BPH patients having CYP3A4 variant genotype compared to those with wild type (relative risk (RR)=2.7; 95% CI=0.77-7.66). No association between variant genotype and risk of developing PRCa was observed with the VDR TaqI variant genotype. In addition, the results of combined genotype analysis of these two SNPs showed a borderline significant association between CYP3A4 and VDR TaqI combined variant genotypes and PRCa risk (RR=3.43; 95% CI=0.99-11.77). While independent confirmation is required in further studies, these results provide a potential tool to assist prediction strategies for this important disease.

Tekmal, R. R. and V. R. Durgam (1997). "A novel in vitro and in vivo breast cancer model for testing inhibitors of estrogen biosynthesis and its action using mammary tumor cells with an activated int-5/aromatase gene." Cancer Lett **118**(1): 21-28.

We recently showed that the cellular gene int-5/aromatase in BALB/c mammary alveolar hyperplastic nodule (D2 HAN/D2 tumor cells) is activated as a result of mouse mammary tumor virus integration within the 3' untranslated region of the aromatase gene. In the present study, we evaluated the effect of various aromatase inhibitors on androstenedione-mediated tumor cell growth. Also, we compared the effect of the non-steroidal aromatase inhibitor (CGS 16949A) on the inhibition of tumor growth. Our results show that D2 tumor cells respond well to various aromatase inhibitors and antiestrogens. We examined the usefulness of this model by using D2 tumor cells to simulate postmenopausal breast cancer employing both in vitro cell culture and in vivo ovariectomized (OVX) nude mouse. Unlike DMBA-induced tumors or other models, D2 tumor cells form very rapid tumors within a few days in intact mice or OVX nude mice with androstenedione supplementation and respond well to an aromatase inhibitor. This model with its known mechanism of aromatase activation should be useful for studying the role of intra-tumoral estrogen in mammary cancer, for evaluating the effects of aromatase inhibitors and antiestrogens, and for comparing breast cancer treatments.

Thompson, A. M., et al. (1990). "Gene expression in oestrogen-dependent human breast cancer xenograft tumours." Br J Cancer **62**(1): 78-84.

Xenograft tumours from an oestrogen-dependent human breast cancer cell line MCF-7 have been established and characterised in thymectomised, irradiated female CBA strain mice. There was evidence for selection in xenografts of a subpopulation of MCF-7 cells with an altered pattern of gene expression as measured by mRNA levels compared with the original cells in vitro. Tumorigenicity increased significantly on repeated animal passage but oestrogen dependence was retained. Following injection of the mice with oestrogen, mitosis was induced in the tumour cells with associated increases in thymidine uptake and percentage of cells in S-phase. In accord with these changes, c-myc and p53 expression were increased and TGF-beta was suppressed. Thereafter the expression of the c-myc and p53 genes fell whilst that of the TGF-beta gene was induced as the oestrogenic-stimulus declined. The oestrogen-regulated mRNA pS2 showed a biphasic response to oestrogen and levels declined as the serum oestrogen fell to undetectable levels. This xenograft system demonstrates that changes in transcription of oncogenes, growth factor and oestrogen-regulated genes can be detected in vivo in response to oestrogen.

It thus provides an in vivo model for studies of the biochemical and molecular basis for therapeutic manipulation of hormone-sensitive human breast cancer.

Tomida, S., et al. (2004). "Gene expression-based, individualized outcome prediction for surgically treated lung cancer patients." Oncogene **23**(31): 5360-5370.

Individualized outcome prediction classifiers were successfully constructed through expression profiling of a total of 8644 genes in 50 non-small-cell lung cancer (NSCLC) cases, which had been consecutively operated on within a defined short period of time and followed up for more than 5 years. The resultant classifier of NSCLCs yielded 82% accuracy for forecasting survival or death 5 years after surgery of a given patient. In addition, since two major histologic classes may differ in terms of outcome-related expression signatures, histologic-type-specific outcome classifiers were also constructed. The resultant highly predictive classifiers, designed specifically for nonsquamous cell carcinomas, showed a prediction accuracy of more than 90% independent of disease stage. In addition to the presence of heterogeneities in adenocarcinomas, our unsupervised hierarchical clustering analysis revealed for the first time the existence of clinicopathologically relevant subclasses of squamous cell carcinomas with marked differences in their invasive growth and prognosis. This finding clearly suggests that NSCLCs comprise distinct subclasses with considerable heterogeneities even within one histologic type. Overall, these findings should advance not only our understanding of the biology of lung cancer but also our ability to individualize postoperative therapies based on the predicted outcome.

Tomizawa, Y., et al. (2001). "Inhibition of lung cancer cell growth and induction of apoptosis after reexpression of 3p21.3 candidate tumor suppressor gene SEMA3B." Proc Natl Acad Sci U S A **98**(24): 13954-13959.

Semaphorins SEMA3B and its homologue SEMA3F are 3p21.3 candidate tumor suppressor genes (TSGs), the expression of which is frequently lost in lung cancers. To test the TSG candidacy of SEMA3B and SEMA3F, we transfected them into lung cancer NCI-H1299 cells, which do not express either gene. Colony formation of H1299 cells was reduced 90% after transfection with wild-type SEMA3B compared with the control vector. By contrast, only 30-40% reduction in colony formation was seen after the transfection of SEMA3F or SEMA3B variants carrying lung cancer-associated single amino acid missense mutations. H1299 cells transfected with wild-type but not mutant SEMA3B underwent apoptosis. We found that lung cancers (n = 34) always express the neuropilin-1 receptor for secreted semaphorins, whereas 82% expressed the neuropilin-2 receptor. Because SEMA3B and SEMA3F are secreted proteins, we tested conditioned medium from COS-7 cells transfected with SEMA3B and SEMA3F and found that medium from wild-type SEMA3B transfectants reduced the growth of several lung cancer lines 30-90%, whereas SEMA3B mutants or SEMA3F had little effect in the same assay. Sequencing of sodium bisulfite-treated DNA showed dense methylation of CpG sites in the SEMA3B 5' region of lung cancers not expressing SEMA3B but no methylation in SEMA3B-expressing tumors. These results are consistent with SEMA3B functioning as a TSG, the expression of which is inactivated frequently in lung cancers by allele loss and promoter region methylation.

Tomoda, K., et al. (1998). "Cationic multilamellar liposome-mediated human interferon-beta gene transfer into cervical cancer cell." Anticancer Res **18**(3A): 1367-1371.

Interferons (IFNs) have antineoplastic activity, but it has been reported that treatment with IFN alone is not effective in many cancers. To enhance the effect of growth inhibition on tumor cells by raising the concentration, we attempted the transfection of cervical cancer cells, HeLa cells, with human interferon-beta (HuIFN-beta) cDNA contained in the expression vector pRSV delivered by cationic multilamellar liposomes, which resulted in the secretion of HuIFN-beta into the medium. The concentration of HuIFN-beta in the medium was 22 IU/ml by 72 hours after transfection of 10 ng DNA, and provoked around 45-fold cell growth inhibitory effect compared with that of exogenously added HuIFN-beta (1000 IU/ml). This strong growth inhibition was considered to be due to the action of HuIFN-beta in a paracrine manner, and a notable fraction of the cell death was apoptotic.

Tong, Q., et al. (2003). "Growth inhibiting effects of antisense eukaryotic expression vector of proliferating cell nuclear antigen gene on human bladder cancer cells." Chin Med J (Engl) **116**(8): 1203-1206.

OBJECTIVE: To explore the growth inhibiting effects on human bladder cancer by antisense RNA targeting the proliferating cell nuclear antigen (PCNA) gene. METHODS: The eukaryotic expression vector for antisense PCNA cDNA was constructed and transferred into a bladder cancer EJ cell line. The PCNA expression in the cancer cells was detected by RT-PCR and Western blotting assays. The in vitro

proliferation activities of the transferred cells were observed by growth curve, tetrazolium bromide (MTT) colorimetry, tritiated thymidine ((3)H-TdR) incorporation, flow cytometry and clone formation testing, while its in vivo anti-tumor effects were detected on nude mice allograft models. RESULTS: After the antisense vector, pLAPSN, was transferred, cellular PCNA expression was inhibited at both protein and mRNA levels. The growth rates of EJ cells were reduced from 27.91% to 62.07% ($P < 0.01$), with an inhibition of DNA synthesis rate by 52.31% ($P < 0.01$). Transferred cells were blocked at G (0)/G (1) phases in cell-cycle assay, with the clone formation ability decreased by 50.81% ($P < 0.01$). The in vivo carcinogenic abilities of the transferred cancer cells were decreased by 54.23% ($P < 0.05$). CONCLUSIONS: Antisense PCNA gene transfer could inhibit the growth of bladder cancer cells in vitro and in vivo, which provided an ideal strategy for gene therapy of human cancers.

Trapasso, F., et al. (2006). "Genetic ablation of Ptprj, a mouse cancer susceptibility gene, results in normal growth and development and does not predispose to spontaneous tumorigenesis." DNA Cell Biol **25**(6): 376-382.

Ptprj is a ubiquitously expressed murine gene encoding a receptor-type protein tyrosine phosphatase, which has recently been proposed as a candidate gene on the locus Scc1 for colon cancer susceptibility. It has been demonstrated that PTPRJ, the human homologue of Ptprj, is involved in the control of cell growth and adhesion, being furthermore altered in several types of cancer including mammary, thyroid, lung, colon, and pancreatic cancers. To investigate the biological functions of Ptprj, we have generated mice deficient in this receptor protein tyrosine phosphatase. Ptprj-deficient mice are viable, fertile, and show no gross anatomical alterations. Furthermore, neither changes in life span nor spontaneous tumor appearance were observed in Ptprj-null mice. Our results indicate that Ptprj is dispensable for normal growth and development in mice.

Trougakos, I. P., et al. (2004). "Silencing expression of the clusterin/apolipoprotein j gene in human cancer cells using small interfering RNA induces spontaneous apoptosis, reduced growth ability, and cell sensitization to genotoxic and oxidative stress." Cancer Res **64**(5): 1834-1842.

Clusterin/Apolipoprotein J (CLU) is a heterodimeric ubiquitously expressed secreted glycoprotein that is implicated in several physiological processes and is differentially expressed in many severe physiological disturbances, including tumor formation and in vivo cancer progression. Despite extensive efforts, clarification of CLU's biological role has been exceptionally difficult and its precise function remains elusive. Short RNA duplexes, referred to as small interfering RNAs (siRNAs), provide a new approach for the elucidation of gene function in human cells. Here, we describe siRNA-mediated CLU gene silencing in osteosarcoma and prostate human cancer cells and illustrate that CLU mRNA is amenable to siRNA-mediated degradation. Our data demonstrate that CLU knockdown in human cancer cells induces significant reduction of cellular growth and higher rates of spontaneous endogenous apoptosis. Moreover, CLU knockdown cancer cells were significantly sensitized to both genotoxic and oxidative stress induced by chemotherapeutic drugs and H (2)O (2), respectively. These effects were more pronounced in cell lines that express high endogenous steady-state levels of the CLU protein and occur through hyperactivation of the cellular apoptotic machinery. Overall, our results reveal that, in the distinct cellular contexts of the osteosarcoma and prostate cancer cells assayed, CLU is a central molecule in cell homeostasis that exerts a cytoprotective function. The described CLU-specific siRNA oligonucleotides that can potently silence CLU gene expression may thus prove valuable agents during antitumor therapy or at other pathological conditions where CLU has been implicated.

Tsai, L. C., et al. (1997). "Effects of tamoxifen and retinoic acid on cell growth and c-myc gene expression in human breast and cervical cancer cells." Anticancer Res **17**(6D): 4557-4562.

The effects of estradiol, tamoxifen, and retinoic acid on the proliferation of breast and cervical cancer cells were investigated. Estrogen stimulated only MCF-7 cell growth, whereas tamoxifen and retinoic acid inhibited the proliferation of all cells studied. Northern blot analysis indicated that estradiol up-regulates c-myc mRNA level in all cell lines studied regardless of the estrogen receptor status in the cells. On the contrary, tamoxifen inhibits c-myc gene expression in all cell lines studied except in MCF-7 cells where the c-myc transcript was not affected. The inhibitory effect of tamoxifen on c-myc gene expression and cell proliferation in estrogen receptor-negative cells suggest an estrogen receptor-independent mechanism. The results also suggest that different mechanisms are involved in the regulation of cell growth and c-myc gene expression in different cancer cells by estrogen and tamoxifen.

Tsao, Y. P., et al. (1999). "Adenovirus-mediated p21((WAF1/SDII/CIP1)) gene transfer induces apoptosis of human cervical cancer cell lines." J Virol **73**(6): 4983-4990.

p21((WAF1/SDII/CIP1)) (p21) arrests cell growth by inhibiting cyclin-depend kinases. To explore the potential of using p21 for the gene therapy of cervical cancer, we infected human papillomavirus (HPV)-positive cervical cancer cells (HeLa, SiHa, and Z172) and HPV-negative cervical cancer cells (C33A) with recombinant adenovirus encoding p21 cDNA. The results revealed that effective inhibition of cell growth could be achieved by sense p21 adenovirus but not antisense p21 adenovirus infection and occurred through apoptosis as measured by DNA fragmentation and chromatin condensation. Apoptosis was also observed in xenografts of human cervical cancer cells infected with sense p21 adenovirus, as confirmed by in situ terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL). The apoptosis was not prevented by overexpression of the bcl-2 transgene. To sum up, the apoptotic effect suggests that p21 should be a tumoricidal agent instead of a tumoristatic agent in preventing cervical cancers. In addition, our report substantiates the combination of the high efficiency of adenovirus vector-mediated gene delivery and the apoptotic effect of p21.

Tsunemitsu, Y., et al. (2004). "Molecular therapy for peritoneal dissemination of xenotransplanted human MKN-45 gastric cancer cells with adenovirus mediated Bax gene transfer." Gut **53**(4): 554-560.

BACKGROUND: Gene therapy is an innovative therapeutic approach for cancer. An adenoviral vector expressing the tumour suppressor p53 gene (Ad/p53) is currently under clinical evaluation for various cancers. We recently developed a binary adenoviral vector system that can express the strong proapoptotic gene Bax (Ad/PGK-GV16+Ad/GT-Bax: Ad/Bax). AIMS: To evaluate the potential of Bax gene therapy for gastric cancer, we assessed its antitumour effect in comparison with that of p53. METHODS: The human gastric cancer cell lines MKN-1, MKN-7, MKN-28, and MKN-45 were treated with Ad/Bax or Ad/p53, and cell viability, transgene expression, and caspase activation were assessed in vitro. To compare the antitumour effects of Ad/Bax and Ad/p53 treatment in vivo, subcutaneous tumours and peritoneal dissemination of MKN-45 cells were generated in nude mice. Each mouse underwent intratumoral or intraperitoneal administration of viruses and the growth of implanted tumours was observed after treatment. RESULTS: Treatment with Ad/Bax and Ad/p53 resulted in marked Bax and p53 protein expression and effective apoptosis induction in MKN-1, MKN-7, and MKN-28 cells in vitro. In contrast, MKN-45 cells showed resistance to Ad/p53 and only treatment with Ad/Bax resulted in activation of caspase 3 expression and massive apoptosis. Ad/Bax treatment was more effective in suppressing both subcutaneous and peritoneally disseminated MKN-45 tumours compared with Ad/p53 treatment. CONCLUSION: Ad/Bax treatment significantly inhibited the growth of even p53 resistant gastric cancer in vitro and in vivo. Therefore, adenovirus mediated Bax gene transfer may be useful in gene therapy for gastric cancers.

Tsunoo, H., et al. (2002). "Effect of transfection with human interferon-beta gene entrapped in cationic multilamellar liposomes in combination with 5-fluorouracil on the growth of human esophageal cancer cells in vitro." Anticancer Res **22**(3): 1537-1543.

When human esophageal cancer cells were transfected with the human interferon-beta (hIFN-beta) gene entrapped in cationic multilamellar liposomes, the growth of all cancer cells tested was suppressed in a dose-dependent manner. The 50% inhibitory concentration (IC50) of the hIFN-beta gene entrapped in the liposomes ranged from 16 to 176 ng plasmid DNA/ml culture medium. Among the 10 cell lines examined, NUEC3, NUEC4, TE-3 and WSSC cell lines were highly susceptible to transfection with this gene entrapped in the liposomes. The IC50 values of the hIFN-beta gene entrapped in the liposomes with respect to cell growth were positively-correlated with those of exogenous cytokine hIFN-beta, suggesting that the antiproliferative effect of hIFN-beta gene entrapped in the liposomes can be mainly ascribed to the function of hIFN-beta produced by cells transfected with the gene. Two days after transfection with the liposome-entrapped gene, the concentration of hIFN-beta secreted into the medium was determined. Even though the level of hIFN-beta observed in the medium was lower than that of the IC50 of exogenously added hIFN-beta, the inhibitory potency of the hIFN-beta gene entrapped in the liposomes on the cell growth was remarkable. When the esophageal cancer cells were treated with 5-fluorouracil (5-FU) in the presence of a low concentration of liposome-entrapped-gene, the rate of growth inhibition of these cells increased over that caused by either 5-FU or hIFN-beta gene entrapped in the liposomes alone. All these data suggest that combination therapy with the hIFN-beta gene entrapped in cationic multilamellar liposomes and the anticancer drug 5-FU would be beneficial for preoperative treatment of carcinoma of the esophagus.

Tu, S. P., et al. (2005). "Gene therapy for colon cancer by adeno-associated viral vector-mediated transfer of survivin Cys84Ala mutant." Gastroenterology **128**(2): 361-375.

BACKGROUND AND AIMS: Reactivation of survivin expression is involved in carcinogenesis and

angiogenesis in colon cancer. Previous in vitro studies showed that mutation of the cysteine residue at position 84 (Cys84Ala) of survivin generates a dominant-negative mutant that triggers mitotic catastrophe and apoptosis. We investigated the therapeutic effect of the adeno-associated virus (AAV)-mediated survivin mutant (Cys84Ala) on colon cancer. METHODS: Survivin mutant (Cys84Ala) (Sur-Mut (Cys84Ala)) was cloned into the AAV expression vector pAM/CAG-WPRE.poly (A) to generate recombinant AAV-Sur-Mut (Cys84Ala) virus. Cell proliferation, apoptosis, mitotic catastrophe, and tumor growth were measured in vitro and in vivo. RESULTS: Transduction of colon cancer cells with rAAV-Sur-Mut (Cys84Ala) inhibited cell proliferation and induced apoptosis and mitotic catastrophe in vitro. rAAV-Sur-Mut (Cys84Ala) sensitized colon cancer cells to chemotherapeutic drugs. Furthermore, expression of survivin mutant mediated by AAV inhibited tumorigenesis in colon cancer cells. Intratumoral injection of rAAV-Sur-Mut (Cys84Ala) significantly induced apoptosis and mitotic catastrophe and inhibited angiogenesis and tumor growth in a colon cancer xenograft model in vivo. No obvious cytotoxicity to other tissues was observed. More importantly, rAAV-Sur-Mut (Cys84Ala) expression strongly enhanced the antitumor activity of 5-Fluorouracil (5-FU), resulting in regression of established tumors. CONCLUSIONS: Our results showed that rAAV-Sur-Mut (Cys84Ala) induced apoptosis and mitotic catastrophe and inhibited tumor angiogenesis and tumor growth. Thus, use of AAV-mediated survivin mutant (Cys84Ala) is a promising strategy in colon cancer gene therapy.

Ueki, T., et al. (2001). "Silencing of the caspase-1 gene occurs in murine and human renal cancer cells and causes solid tumor growth in vivo." Int J Cancer **91**(5): 673-679.

Renal cell cancer is a unique solid tumor that occasionally shows spontaneous regression even at an advanced stage, of which the underlying mechanism is not well understood. To investigate a potential role of the pro-apoptotic molecule caspase-1 in the growth regulation of renal cell cancer, we created transfectants expressing exogenous caspase-1 from a murine renal cancer cell line, Renca. Overexpression of caspase-1 did not affect the growth of Renca cells in vitro at the exponential phase but induced apoptotic cell death at 50% to 75% confluence, whereas control cells underwent apoptosis only after reaching 100% confluence. When implanted into the flank of a syngeneic BALB/c mouse, caspase-1-overexpressing Renca cells did not effectively establish growth as a solid tumor, forming a measurable tumor in only 7 of 11 (64%) animals, whereas control cells formed a tumor in 6 of 6 (100%) animals. The growth of tumors from caspase-1-overexpressing cells slowed down markedly after the tumors reached 5 to 10 mm in diameter, and histological examination of such tumors revealed numerous apoptotic cells positively stained by TUNEL assay. Interestingly, endogenous caspase-1 was not detected in the tumors from control cells, which re-expressed caspase-1 when they were re-cultured and exposed to a demethylation reagent, 5-aza-2'-deoxycytidine. Furthermore, treatment of a human renal cancer cell line, ACHN, with 5-aza-2'-deoxycytidine also caused recovery of caspase-1 expression, which was not detected before treatment. These data suggest that silencing of caspase-1 through DNA methylation may be involved in the oncogenesis of some renal cell cancers growing as a solid tumor.

Ueno, M., et al. (2001). "Tumor-specific chemo-radio-gene therapy for colorectal cancer cells using adenovirus vector expressing the cytosine deaminase gene." Anticancer Res **21**(4A): 2601-2608.

We studied the effect of suicide gene therapy using an adenovirus vector expressing the cytosine deaminase (CD) gene combined with irradiation therapy (chemo-radio-gene therapy) for human colorectal cancer cells. Since serum CEA levels are elevated in patients with some malignant tumors including colorectal cancer, we applied the CEA promoter to chemo-radio-gene therapy, expecting tumor-specific expression of the CD gene. In in vitro study, we succeeded in selective expression of the target CD gene and growth inhibition in only CEA-producing tumor cells; Further the inhibitory effect was enhanced by combination with radiation therapy in an irradiation dose-dependent manner. In addition, in in vivo study, a significant growth inhibition was observed in chemo-radio-gene therapy in comparison with radiation therapy alone or suicide gene therapy alone. Thus, we suggest that tumor-specific chemo-radio-gene therapy may be a useful strategy for human colorectal cancer.

Unni, E., et al. (2004). "Osteopontin is a potential target gene in mouse mammary cancer chemoprevention by Se-methylselenocysteine." Breast Cancer Res **6**(5): R586-592.

BACKGROUND: Se-methylselenocysteine (MSC) is a naturally occurring organoselenium compound that inhibits mammary tumorigenesis in laboratory animals and in cell culture models. Previously we have documented that MSC inhibits DNA synthesis, total protein kinase C and cyclin-dependent kinase 2 kinase activities, leading to prolonged S-phase arrest and elevation of growth-arrested DNA damage genes, followed by caspase activation and apoptosis in a synchronized TM6 mouse

mammary tumor model. The aim of the present study was to examine the efficacy of MSC against TM6 mouse mammary hyperplastic outgrowth (TM6-HOG) and to determine in vivo targets of MSC in this model system. METHODS: Twenty mammary fat pads each from female Balb/c mice transplanted with TM6-HOG and fed with 0.1 ppm selenium and with 3 ppm selenium respectively, were evaluated at 4 and 12 weeks after transplantation for growth spread, proliferative index and caspase-3 activity. Thirteen mice transplanted with TM6-HOG in each selenium group were observed for tumor formation over 23 weeks. Tumors from mice in both groups were compared by cDNA array analysis and data were confirmed by reverse transcription-polymerase chain reaction. To determine the effect of MSC on the expression of the novel target gene and on cell migration, experiments were performed in triplicate. RESULTS: A dietary dose of 3 ppm selenium significantly reduced the growth spread and induced caspase-3 activity in mammary fat pads in comparison with mice fed with the basal diet (0.1 ppm selenium). The extended administration (23 weeks) of 3 ppm selenium in the diet resulted in a tumor incidence of 77% in comparison with 100% tumor incidence in 0.1 ppm selenium-fed animals. The size of TM6 tumors in the supplemented group was smaller (mean 0.69 cm2) than in the mice fed with the basal diet (mean 0.93 cm2). cDNA array analysis showed a reduced expression of osteopontin (OPN) in mammary tumors of mice fed with the 3 ppm selenium diet in comparison with OPN expression in tumors arising in 0.1 ppm selenium-fed mice. A 24-hour treatment of TM6 cells with MSC significantly inhibited their migration and also reduced their OPN expression in comparison with untreated cells. CONCLUSIONS: OPN is a potential target gene in the inhibition of mammary tumorigenesis by selenium.

Vernejoul, F., et al. (2002). "Antitumor effect of in vivo somatostatin receptor subtype 2 gene transfer in primary and metastatic pancreatic cancer models." Cancer Res **62**(21): 6124-6131.

Our previous studies conducted in pancreatic cancer models established in nude mice and hamsters revealed that cloned somatostatin receptor subtype 2 (sst2) gene expression induced both antioncogenic and local antitumor bystander effects in vivo. In the present study, in vivo gene transfer of sst2 was investigated in two transplantable models of primary and metastatic pancreatic carcinoma developed in hamsters. LacZ reporter or mouse sst2 genes were expressed by means of two different delivery agents: an adenoviral vector and a synthetic polycationic carrier [linear polyethylenimine (PEI)]. sst2 was injected into either exponentially growing pancreatic primary tumors or hepatic metastases, and then transgene expression and tumor progression were investigated 5-6 days after gene transfer. Molecular mechanisms involved in the inhibition of tumor growth were also analyzed. Both adenovirus- and PEI-mediated in vivo gene transfer in primary pancreatic tumors induced an increase of beta-galactosidase activity and expression of sst2 transgene nRNA (100% and 86% of tumors for adenovirus and PEI vector, respectively). Adenoviral vector-based sst2 gene transfer resulted in significant reduction of pancreatic tumor growth ($P < 0.05$). Using PEI vector, both pancreatic primary tumor growth and metastatic tumor growth were also significantly slackened as compared with both LacZ-treated and untreated control groups ($P < 0.02$). Moreover, the proliferative index decreased significantly ($P < 0.005$), whereas apoptosis increased ($P < 0.005$) in tumors transferred with sst2 gene. The increase of apoptosis correlated with an activation of the caspase-3 and poly (ADP-ribose) polymerase pathways. We concluded that in both primary and metastatic pancreatic cancer models, the synthetic gene delivery system can achieve in vivo sst2 gene transfer and results in a significant antitumor effect characterized by an increase of apoptosis and an inhibition of cell proliferation. This new strategy of gene therapy allows the restoration of expression of an antioncogenic molecule and could be promising for the treatment of advanced pancreatic cancer.

Vikhanskaya, F., et al. (1993). "Effects of DNA damaging agents on gene expression in two human cancer cell lines." Cell Mol Biol (Noisy-le-grand) **39**(8): 855-862.

In two human cancer cell lines, the breast mcf-7 and the T-cell leukemia MOLT4, we investigated the cytotoxicity of four antineoplastic agents having different mechanisms of action. We selected doxorubicin as a DNA-topoisomerase II inhibitor, FCE24517 (a Distamycin A derivative) as a DNA minor groove binder with specificity for AT bases, melphalan as an alkylating agent and cis-platinum as an alkylating agent able to form DNA-intrastrand crosslinks. From the cytotoxicity experiments a moderately toxic (less than 10% of growth inhibition) and a highly toxic (about 75% growth inhibition) dose were selected to evaluate the expression of genes involved in cell proliferation and in cell response to extracellular insults. The expression was evaluated at early times (60 min.) and 24 hrs. after treatment. At the concentrations utilized in both cell lines we could not find any alteration in the expression of p53, gas-1 and heat shock 70. After melphalan treatment down regulation of c-myc and of the H2A histone was seen at high doses, while no significant alteration of their expression was seen with the other drugs.

Viney, J. L. (1995). "Transgenic and gene knockout mice in cancer research." Cancer Metastasis Rev **14**(2): 77-90.

Transgenic animal technology, and the use of germline manipulation for the creation of targeted gene mutations, has resulted in a plethora of murine models for cancer research. Our understanding of some of the important issues regarding the mechanisms controlling cell division, differentiation and death has dramatically advanced in recent years through exploitation of these techniques to generate transgenic mice. In particular, the generation of mice with targeted mutations in genes encoding proteins of oncological interest has proved to be a useful way of elucidating the function of these gene products in vivo. Transgenic mouse models have provided some insight into the complex oncogenic events contributing to cellular dysregulation and the loss of growth control that can lead to tumorigenesis. These animal studies have highlighted the fact that there are many different stages at which the loss of cell cycle control can occur, as a result of mutations affecting proteins anywhere from the cell surface to the nucleus. Although mutations affecting growth factors, growth factor receptors, signal transduction molecules, cytoplasmic proteins or nuclear proteins might appear to be very distinct, the end result of these changes may be accelerated and unchecked cell growth ultimately leading to cancer. It is beyond the scope of this review to mention every animal model that has been developed for cancer research, especially since many of the early studies have been covered extensively in previous reviews. This article will instead focus on a small selection of transgenic and knockout animal models which exemplify how proteins from distinct localisations along multiple pathways can contribute to loss of cell cycle control and the pathogenesis of cancer.

von Gruenigen, V. E., et al. (1998). "In vivo studies of adenovirus-based p53 gene therapy for ovarian cancer." Gynecol Oncol **69**(3): 197-204.

OBJECTIVES: To test the safety, efficacy, and toxicity of gene therapy using wild-type p53-expressing adenovirus (Ad-CMV-p53) in a nude mouse model with intraperitoneal (i.p.) 2774 human ovarian cancer cell line that contains a p53 mutation. STUDY DESIGN: An initial study of adenovirus tolerance was determined in nude mice by a single i.p. injection of increasing doses of Ad-CMV-p53. Nude mice were implanted with an LD100 dose of 1 x 10(7) cells. To study the efficacy and specificity of Ad-CMV-p53 treatment, the mice received treatment with different adenovirus constructs. One group received Ad-CMV-p53 and another group received a control adenovirus construct, Ad-CMV-beta gal. To study the treatment response to Ad-CMV-p53, the mice were divided into groups and received various treatment schedules of 1 x 10(8) pfu of Ad-CMV-p53. RESULTS: The mice tolerated Ad-CMV-p53 without adverse effects at doses of 1 x 10(8) pfu. The response to Ad-CMV-p53 showed significant survival duration in each dose regimen, with a survival time greater than that of untreated animals (P = 0.0173). However, no statistically significant survival advantage was observed between Ad-CMV-p53- and Ad-CMV-beta gal-treated mice. CONCLUSIONS: These studies show that at the adenovirus dose and administration regimen used, there is effective but not specific 2774 tumor growth inhibition in vivo. Efficient introduction of biologically active genes into tumor cells would greatly facilitate cancer therapy. Thus, although promising, these results caution that much effort will be required to realize the potential for clinical application of adenovirus-based ovarian cancer gene therapy.

von Knebel Doeberitz, M., et al. (1990). "Growth-regulating functions of human papillomavirus early gene products in cervical cancer cells acting dominant over enhanced epidermal growth factor receptor expression." Cancer Res **50**(12): 3730-3736.

Squamous cell carcinomas of the human anogenital tract are usually associated with infection of specific types of human papillomaviruses (HPV 16, 18, 31, 33, 35). The intracellular concentration of human papillomavirus early gene products E6 and E7 has been directly linked to the proliferative capacity of cervical cancer cells. Since the expression rate of epidermal growth factor receptor correlates to growth properties in squamous carcinoma cell lines, it has been presumed that human papillomavirus early genes influence cell growth via enhanced epidermal growth factor receptor expression. This hypothesis implies that growth regulation by epidermal growth factor receptor overexpression dominates over a growth-regulatory influence of human papillomavirus early gene products in squamous carcinoma cells. To test this hypothesis epidermal growth factor receptor expression was analyzed in various clones of the C4-1 cervical cancer cell line which, upon dexamethasone treatment, express either increased or decreased levels of human papillomavirus 18 early gene products. In C4-1 clones expressing reduced levels of viral E6/E7 gene products upon glucocorticoid treatment expression of epidermal growth factor receptor was the same as in those clones displaying increased levels of papillomavirus proteins under identical culture conditions. The growth rate of the cells correlated with the level of viral gene products rather than with the

expression of epidermal growth factor receptor. These findings suggest that unregulated overexpression of epidermal growth factor receptor is not the dominant mechanism of growth control in papillomavirus-positive carcinoma cells. Other, yet unknown pathways associated with papillomavirus early genes are essentially involved in growth control mechanisms of human cervical cancer cells.

Wada, Y., et al. (2001). "Gene therapy for bladder cancer using adenoviral vector." Mol Urol **5**(2): 47-52.

BACKGROUND AND PURPOSE: Bladder cancer is common. Current treatment for patients with superficial bladder cancer involves transurethral resection followed by adjuvant bacillus Calmette-Guerin (BCG) administration. Adjuvant BCG has been reported to be effective in 38% to 68% of patients; however, more than 30% of patients do not respond. Because p53 mutations are common among superficial bladder cancers, we tested the feasibility of using p53 as a gene therapy agent for targeting superficial tumors, which are easily accessible using an intravesical approach. MATERIALS AND METHODS: Wild-type p53 was transduced into various human and murine bladder cancer cell lines (HTB9, KU-1, and MBT-2) using a recombinant adenoviral vector (Ad5CMV-p53) in vitro. Also, subcutaneous tumors were established and then treated with intratumoral injection of Ad5CMV-p53 or control viruses. RESULTS: In vitro assays revealed significant growth suppression of target cells by Ad5CMV-p53 in comparison with those receiving the control Ad5-CMV-PA vector or untreated control cells. In vivo studies using subcutaneous bladder tumor models established in syngeneic mice demonstrated that the rate of tumor growth and volume was reduced to a greater extent by 14 days of intratumoral injection of Ad5CMV-p53 rather than Ad5CMV-PA. Furthermore, the survival of host animals bearing tumors that were infected with Ad5CMV-p53 was significantly longer than that of the control group treated with Ad5CMV-PA ($P < 0.01$). CONCLUSION: Our data suggest that Ad5CMV-p53 is effective in suppressing bladder cancer growth and improving host survival.

Wallqvist, A., et al. (2003). "Linking the growth inhibition response from the National Cancer Institute's anticancer screen to gene expression levels and other molecular target data." Bioinformatics **19**(17): 2212-2224.

MOTIVATION: Data mining tools are proposed to establish mechanistic connections between chemotypes and specific cellular functions. Drawing on a previous study that classified the cellular response patterns of growth inhibition measurements log (GI (50)) from the National Cancer Institute's (NCI's) anticancer screen, we have examined additional data for mRNA expression, sets of known molecular targets and mutational status against these same tumor cell lines to relate chemosensitivity more precisely to biochemical pathways. RESULTS: Our analysis finds that gene expression levels do not, in general, correlate with log (GI (50)) measurements, instead they reflect a generic toxic condition. Within the remaining set of non-generic conditions, examples were found where a correlation suggesting a biochemical basis for cellular cytotoxicity could be supported. These included reconfirmation of previously observed associations between mutant and wild-type status of p53, and chemosensitivity to alkylating agents, while extending these results to reveal associations with gamma-induced expressions of MDM2, WAF1 and GADD45, signals that were not apparent in measurements of basal mRNA expression levels for any of these genes. Additional examinations revealed that mRNA expression levels directly correlated with paclitaxel chemosensitivity to mitosis, while also identifying additional chemotypes as P-glycoprotein substrates. Our analysis revealed well-known direct associations between p16 mutant status and chemotypes implicated in cell cycle control, and extended these results to include expression levels for three additional tyrosine kinase proteins (TEK, transgelin and hCdc4). Links were also found that suggested associations between chemosensitivity and the endocrine, paracrine ligand-receptor loops, via expression of the adrenergic receptor, calcium second messenger pathways via expression levels of carbonic anhydrase and cellular communication pathways via fibrillin.

Watts, C. K., et al. (1994). "Antiestrogen regulation of cell cycle progression and cyclin D1 gene expression in MCF-7 human breast cancer cells." Breast Cancer Res Treat **31**(1): 95-105.

The molecular mechanisms by which antiestrogens inhibit breast cancer cell proliferation are not well understood. Using cultured breast cancer cell lines, we studied the effects of antiestrogens on proliferation and cell cycle progression and used this information to select candidate cell cycle regulatory genes that are potential targets for antiestrogens. Under estrogen- and serum-free conditions antiestrogens inhibited proliferation of MCF-7 cells stimulated with insulin. Cells were blocked at a point in G1 phase. These effects are comparable with those in serum- and estrogen-containing medium and were also seen to a lesser degree in nude mice bearing MCF-7 tumors. Similar observations with other peptide mitogens suggest that the process inhibited by antiestrogens is common to estrogen and growth factor activated pathways. Other studies have identified G1

cyclins as potential targets for growth factor and steroid hormone/steroid antagonist regulation of breast epithelial cell proliferation. In MCF-7 cells growing in the presence of fetal calf serum, cyclin D1 mRNA was rapidly down-regulated by steroidal and nonsteroidal antiestrogens by an apparently estrogen receptor mediated mechanism. Cyclin D1 gene expression was maximally inhibited before effects on entry into S phase and inhibition was therefore not merely a consequence of changes in cell cycle progression. Together with data on the effects of antiestrogens in serum-free conditions [1], these results suggest down-regulation of cyclin D1 by antiestrogens may be a general phenomenon in estrogen receptor-positive breast cancer cells, independent of culture conditions and class of antiestrogen. These observations are compatible with the hypothesis that reductions in cyclin D1 levels may mediate in part the action of antiestrogens in blocking entry of cells into S phase.

Werner, H. and R. Sarfstein (2014). "Transcriptional and epigenetic control of IGF1R gene expression: implications in metabolism and cancer." Growth Horm IGF Res **24**(4): 112-118.

IGF1R plays an important role in protection from apoptosis, regulation of cell growth, differentiation and oncogenic transformation. IGF1R aberrations lead to intrauterine and postnatal growth failure, microcephaly, mental retardation and deafness. High levels of IGF1R are detected in a diversity of human tumors. IGF1R gene transcription is controlled by complex interactions involving DNA-binding and non DNA-binding transcription factors. This review highlights selected examples of a series of tumor suppressors, including the breast cancer gene-1 (BRCA1), p53, the Wilm's tumor protein-1 (WT1) and the von Hippel-Lindau gene (VHL), whose mechanisms of action involve regulation of IGF1R gene expression. IGF1R gene transcription is also dependent on the presence of stimulatory nuclear proteins, including zinc-finger protein Sp1, EWS-WT1, E2F1, Kruppel-like factor-6 (KLF6), high-mobility group A1 (HMGA1), and others. Loss-of-function of tumor suppressor genes, usually caused by mutations, may result in non-functional proteins unable to control IGF1R promoter activity. Impaired regulation of the IGF1R gene is linked to defective cell division, chromosomal instability and increased incidence of cancer.

Wiechen, K., et al. (1999). "Suppression of the c-erbB-2 gene product decreases transformation abilities but not the proliferation and secretion of proteases of SK-OV-3 ovarian cancer cells." Br J Cancer **81**(5): 790-795.

The overexpression of the c-erbB-2 oncogene product has been reported in approximately 20-30% of human ovarian cancers and has been correlated with a poor prognosis in ovarian cancer patients. To investigate the function of p185(c-erbB-2) in human ovarian cancer cells, a c-erbB-2-specific single-chain antibody (scFv-5R) was expressed in the c-erbB-2-overexpressing SK-OV-3 cell line using a retroviral expression vector. Eight individual clones expressing the single-chain antibody were isolated. These clones have a prominent retention of the cell surface p185(c-erbB-2). In this study we compared the proliferation rate, the anchorage-independent growth, the secretion of matrix metalloproteases and of the urokinase-type plasminogen activator. The clones expressing the c-erbB-2 single-chain antibody, the control cells harbouring the empty vector and the parental SK-OV-3 cells they all had similar proliferation rates in the presence of 10% serum and secreted similar amounts of matrix metalloproteases and of the urokinase-type plasminogen activator. However, the expression of the c-erbB-2 oncogene product offers a strong growth advantage under serum-reduced conditions with 1% serum. In contrast to the parental SK-OV-3 and empty vector control cells, the scFv-5R-expressing clones were not able to grow anchorage-independently. These findings suggest that c-erbB-2 enhances transformation abilities of SK-OV-3 ovarian cancer cells without affecting the secretion of proteases and the proliferation of SK-OV-3 ovarian cancer cells in the presence of high concentrations of serum.

Wierer, M., et al. (2013). "PLK1 signaling in breast cancer cells cooperates with estrogen receptor-dependent gene transcription." Cell Rep **3**(6): 2021-2032.

Polo-like kinase 1 (PLK1) is a key regulator of cell division and is overexpressed in many types of human cancers. Compared to its well-characterized role in mitosis, little is known about PLK1 functions in interphase. Here, we report that PLK1 mediates estrogen receptor (ER)-regulated gene transcription in human breast cancer cells. PLK1 interacts with ER and is recruited to ER cis-elements on chromatin. PLK1-coactivated genes included classical ER target genes such as Ps2, Wisp2, and Serpina3 and were enriched in developmental and tumor-suppressive functions. Performing large-scale phosphoproteomics of estradiol-treated MCF7 cells in the presence or absence of the specific PLK1 inhibitor BI2536, we identified several PLK1 end targets involved in transcription, including the histone H3K4 trimethylase MLL2, the function of which on ER target genes was impaired by PLK1 inhibition. Our results propose a mechanism for the tumor-suppressive role of PLK1 in mammals as an interphase transcriptional regulator.

Wilczynska, U., et al. (2001). "Combined delivery of an antiangiogenic protein (angiostatin) and an immunomodulatory gene (interleukin-12) in the treatment of murine cancer." Acta Biochim Pol **48**(4): 1077-1084.

We investigated the feasibility of a novel therapeutic approach to treat neoplastic diseases in mice. This novel strategy consists in delivering a protein (angiostatin) with strong antiangiogenic properties, followed by administration of the interleukin 12 gene that is strongly immunomodulatory and has also some antiangiogenic effects. When angiostatin-mediated antiangiogenic therapy was used in combination with intratumor delivery of the IL-12 gene (a strategy much safer than IL-12 protein administration), this produced a synergistic therapeutic effect.

Wilson, G. D., et al. (2014). "Gene expression changes during repopulation in a head and neck cancer xenograft." Radiother Oncol **113**(1): 139-145.

BACKGROUND/PURPOSE: To investigate temporal changes in global gene expression and pathways involved in the response to irradiation during phases of growth inhibition, recovery and repopulation in a human head and neck squamous cell cancer (HNSCC) xenograft. METHODS AND MATERIALS: Low passage head and neck squamous cancer cells (UT-14-SCC) were injected into the flanks of female nu/nu mice to generate xenografts. After tumors reached a size of 500 mm3, they were treated with either sham RT or 15Gy in one fraction. At different time points, days 0, 3, and 10 for controls and days 4, 7, 12, and 21 after irradiation, the tumors were harvested for global gene expression analysis and pathway analysis. RESULTS: The tumors showed growth inhibition through days 4-7 and began the transition to regrowth around the day 12 time point. When comparing the pooled controls to each day of treatment, there were 22, 119, 125, and 25 differentially expressed genes on days 4, 7, 12, and 21 respectively using a p0.01 and a 2-fold cut-off. Gene Ontology (GO), gene set enrichment analysis (GSEA) and sub-network enrichment analysis (SNEA) identified different biological processes, cell process pathways and expression targets to be active on each time point after irradiation. An important observation was that the molecular events on day 12 which represented the transition from growth inhibition to regrowth identified interferon and cytokine related genes and signaling pathways as the most prominent. CONCLUSION: The findings in this study compliment research which has identified components of interferon-related signaling pathways to be involved in radioresistance. Further work will be required to understand the significance of these genes in both radioresistance and treatment response leading to new therapeutic strategies and prognostic tools.

Wilson, L. C., et al. (2003). "Nonsteroidal anti-inflammatory drug-activated gene (NAG-1) is induced by genistein through the expression of p53 in colorectal cancer cells." Int J Cancer **105**(6): 747-753.

Genistein is an isoflavenoid found in soy that has anti-tumorigenic activities. Treatment of colorectal carcinoma HCT-116 cells with 50 microM genistein results in a 50% reduction in cell proliferation and a 6-fold increase in apoptosis. Genistein induces nonsteroidal anti-inflammatory drug-activated gene 1 (NAG-1), a protein with antitumorigenic activities, in a time- and concentration-dependent manner in HCT-116 cells. In addition, p53 and p21 are induced in HCT-116 cells. The induction of p53 (3 hr) precedes the induction of NAG-1 (12 hr), suggesting that genistein-induced NAG-1 expression is mediated by p53. In contrast, NAG-1 is not induced by genistein in the p53-negative colorectal carcinoma cell line HCT-15. Luciferase reporter constructs of the NAG-1 promoter containing 2 p53 sites showed that the p53 sites within the NAG-1 promoter are critical to genistein-induced NAG-1 expression in p53-positive U2OS cells. The expression of p53 was critical for NAG-1 promoter activity since no promoter activity was observed with genistein treatment in HCT-15 cells. However, genistein-induced promoter activity was restored in HCT-15 cells by transfection with wild-type p53. Together our data suggest a relationship between genistein, p53 and NAG-1 forming a novel pathway responsible for the antitumorigenic activity of genistein.

Wolf, J. K., et al. (1999). "Growth suppression of human ovarian cancer cell lines by the introduction of a p16 gene via a recombinant adenovirus." Gynecol Oncol **73**(1): 27-34.

OBJECTIVE: The cell cycle regulatory protein p16 (CDKN2/cyclin dependent kinase 4 inhibitor/multiple tumor suppressor-1) causes cell cycle arrest at the G1 checkpoint by inhibiting activity of cyclin D-CDK4 complexes. The purpose of this study is to assess the effect of introduction of the p16 gene into two ovarian cancer cell lines via a recombinant adenoviral vector (Ad5CMV-p16). METHODS: Cells lines used were SKOV3, which has a p16 deletion, and OVCA420, which has normal p16. Transduction efficiency was established by infecting cells with an adenovirus containing the Escherichia coli beta-galactosidase gene (Ad5CMV-beta-gal) at multiplicity of infection from 0 to 1000 and staining for X-gal. Cells were infected with Ad5CMV-p16 and cell growth was assessed by counting cells every other

day for up to 7 days. Western blotting was done to assess for p16 expression after infection. Fluorescence-activated cell sorting after staining with propidium iodide was done to assess the effect of p16 on the cell cycle. RESULTS: The SKOV3 cell line was transduced with the adenovirus at a slightly lower MOI than the OVCA420 cell line. Growth of the Ad5CMV-p16-infected cells was suppressed 75-80% by cell count in both cell lines and caused morphologic changes of the cells consistent with apoptosis. The p16 protein expression was seen to increase within 24 h after introduction of the p16 gene. G1 arrest of cells occurred beginning 24 h after introduction of the p16 gene. CONCLUSIONS: These results suggest that Ad5CMV-p16 may be further studied as a potential therapeutic agent for ovarian cancer as introduction of the p16 gene into ovarian cancer cell lines causes a G1 arrest and attenuation of growth, regardless of the endogenous p16 status of the cells.

Wosikowski, K., et al. (1997). "Altered gene expression in drug-resistant human breast cancer cells." Clin Cancer Res **3**(12 Pt 1): 2405-2414.

It is increasingly recognized that drug-resistant cells undergo transitions not directly linked to "classical" drug resistance. We examined the expression of growth factors, growth factor receptors, and the estrogen receptor in 17 drug-resistant and 2 revertant human breast cancer sublines to provide an understanding of the phenotypic changes that occur and how these changes could affect the biology of the cell. These sublines were derived from five parental human breast cancer cell lines (MCF-7, ZR75B, T47D, MDA-MB-231, and MDA-MB-453). The expression of estrogen receptor was absent or decreased in 6 of the 15 resistant MCF-7, ZR75B, and T47D sublines. Increases of as much as 49-fold compared to parental levels were observed in transforming growth factor alpha, epidermal growth factor receptor, c-erbB2, and/or c-erbB3 mRNA expression in 14 of the 17 resistant sublines. Altered amphiregulin and insulin-like growth factor-I receptor expression was observed in nine and four drug-resistant sublines, respectively. No major alterations were observed in epidermal growth factor and c-erbB4 expression. Few alterations were observed in two sublines derived from estrogen receptor-negative cells. Higher levels of phosphotyrosine residues were detected in a subset of the resistant sublines, indicating an increased tyrosine kinase activity in these cells. Interestingly, decreased growth rates were observed in all of the sublines, despite up-regulated growth factor-related gene expression. Taken together, these data suggest that loss of estrogen receptor, increased expression of growth factor pathway genes, and decreased growth rate regularly occur in drug-resistant breast cancer cells. Although we do not know whether the altered expression of growth factor pathway genes is linked as a cause or a consequence of the reduced growth rate, it is well established that decreased growth rate confers drug resistance. These phenotypic changes in drug-resistant human breast cancer cells could serve to initiate, support, or extend the drug resistance.

Yamato, H., et al. (1995). "In vivo evidence for progressive activation of parathyroid hormone-related peptide gene transcription with tumor growth and stimulation of osteoblastic bone formation at an early stage of humoral hypercalcemia of cancer." J Bone Miner Res **10**(1): 36-44.

The present study was undertaken to clarify in vivo the temporal profile of parathyroid hormone-related peptide (PTHRP) gene expression as well as bone histomorphometric features as a function of tumor growth, using an athymic rat model associated with humoral hypercalcemia of malignancy (HHM). Tumor-bearing animals exhibited hypercalcemia, hypophosphatemia, and increased circulating levels of PTHRP, and died within 3 weeks. Steady-state PTHRP mRNA levels and the transcription rate of PTHRP gene in the tumors were markedly increased with tumor growth. RNAse mapping analysis revealed that both upstream and downstream promoters of the human PTHRP gene were utilized in the tumors and became progressively activated with time. Bone histomorphometric analysis showed that osteoclastic bone resorption was progressively increased throughout the course, whereas osteoblastic bone formation was stimulated more than 2-fold at a very early stage (day 6 after tumor implantation) and then markedly suppressed thereafter on day 12 and day 18 compared with age-matched control animals. These results provide in vivo evidence that PTHRP gene transcription is progressively activated with tumor growth and that activation of osteoblasts does occur at a very early phase of HHM syndrome in contrast to the marked suppression of bone formation at later stages.

Yan, R. L., et al. (2002). "[Experimental study of anti-VEGF hairpin ribozyme gene inhibiting expression of VEGF and proliferation of ovarian cancer cells]." Ai Zheng **21**(1): 39-44.

BACKGROUND & OBJECTIVE: Growth of solid tumor metastases are critically dependent on angiogenesis. Vascular endothelial growth factor (VEGF), also known as vascular permeability factor, has been identified as one of the most potent inducers of tumor associated angiogenesis, studies have shown that VEGF plays an important role in angiogenesis which is associated with epithelial ovarian cancer. Until now, many strategies for gene therapy have been developed. Among them is Ribozyme-based

therapeutics for cancer which might be devised to inhibit tumor growth or prevent metastases. Angiogenesis is required for sustained tumor growth, making the VEGF pathway another promising target for either small molecule or nucleic acid-based therapeutics. Little is known about the role of VEGF in ovarian tumorigenecity. We propose to block the autocrine and/or paracrine pathway of VEGF in ovarian cancer using anti-VEGF hairpin ribozyme gene to see whether the growth of tumor cells could be inhibited and to further exploit its mechanisms. METHODS: Anti-VEGF hairpin ribozyme gene eukaryotic expression vector was introduced into ovarian cancer SKOV3 cells by lipofectin mediation and positive clones were screened by G418; Ribozyme expression was confirmed by RNA dot blot; The VEGF expression of SKOV3 cells before or after transfection were detected by immunohistochemical and immunofluorescence and flow cytometer immunofluorescence methods, MTT, colony forming, soft agar colony forming, and FCM were used to observe the effect of proliferation to ovarian cancer cells. RESULTS: VEGF expression decreased distinctly in SKOV3-RZ cells. The growth of transfected SKOV3-RZ cells were slower, The average colony forming efficiency and soft agar colony forming efficiency of SKOV3-RZ cells (12.7 +/- 1.4 and 9.4 +/- 2.0, respectively) decreased distinctly ($P < 0.001$). The SKOV3-RZ cells of G1 stage increased ($P < 0.01$), the SKOV3-RZ cells of S stage were reduced ($P < 0.01$). CONCLUSIONS: Anti-VEGF hairpin ribozyme gene can inhibit the proliferation of ovarian cancer SKOV3 cells. This provides a experimental basis for cure human ovarian cancer with antiangiogenesis method.

Yanagie, H., et al. (2009). "Tumor growth suppression by adenovirus-mediated introduction of a cell-growth-suppressing gene tob in a pancreatic cancer model." Biomed Pharmacother **63**(4): 275-286.

TOB (transducer of ErbB-2) is a tumor suppressor that interacts with protein-tyrosine kinase receptors, including ErbB-2. Introduction of the tob gene into NIH3T3 cells results in cell growth suppression. In this study, we evaluated the effect of tob expression in pancreatic cell lines (AsPC-1, BxPC-3, SOJ) and discuss the tumor-suppressing effects of adenoviral vector expressing tob cDNA. We first measured the levels of endogenous tob mRNA being expressed in all pancreatic cancer cell lines. Then, we examined the effect of adenoviral vector containing tob cDNA (Ad-tob vector) on cancer cell lines. The viral vector was expanded with transfection in 293 cells. The titer of the vector was 350x10(6) pfu/ml. These cancer cells were able to be transfected with MOI 20 without adenoviral toxicity. The transfection of Ad-tob vector results in growth suppression of SOJ and AsPC-1 cell lines. The magnitude of the expression of the Ad-tob gene in cancer is correlated to tumor suppressive activity. We prepared pancreatic cancer peritonitis models using a peritoneal injection of AsPC-1 cells. In this model, bloody ascites and multiple tumor nodules were seen at the mesentery after 16 days. AdCAtob (50x10(6) pfu/day) was administered from day 5 to day 9 after 4 days of peritoneal injection of 2x10(6) AsPC-1 cells. Tumor growth suppression occurred 10 days after peritoneal injection of AdCAtob compared with the control group. There were no tumor nodules in the abdomen and no bloody ascites. These results suggest that the peritoneal injection of AdCAtob has potential to suppress the formation of pancreatic cancer peritonitis, and can be applied for chemotherapy-resistant cancer peritonitis.

Yang, G., et al. (2003). "Silencing of H-ras gene expression by retrovirus-mediated siRNA decreases transformation efficiency and tumorgrowth in a model of human ovarian cancer." Oncogene **22**(36): 5694-5701.

To examine the role of H-ras in the development of human ovarian cancer, we used small inhibitory RNA (siRNA) to silence its expression in human ovarian cancer cell lines and assessed the effects of its silencing on proliferation, apoptosis, and tumorgrowth. First, we developed a retrovirus-based delivery system that allowed long-term stable expression of the desired siRNA. Retrovirus-mediated expression of siRNA against green fluorescence protein (GFP) reduced its expression more than 90% in four cancer cell lines. We then constructed three retroviruses that expressed siRNAs targeting the H-rasV (12) mutation (H1/siRNA) or either of two wild-type sequences of the H-ras gene (H2/siRNA and H3/siRNA) and used these retroviruses to infect T80H and SKOV-3 cells. In T80H cells (a genetically transformed human ovarian surface epithelial cell line whose tumorigenicity depends on H-rasV (12) expression), infection with the H1/siRNA and H2/siRNA, but not with H3/siRNA, decreased T80H proliferation, increased G (0)/G (1) arrest and apoptosis, blocked transformation in vitro, and suppressed tumor growth in nude mice. In SKOV-3 cells (a human ovarian cancer cell line that contains high levels of wild-type H-ras protein but no H-rasV (12) mutation), introduction of the H2/siRNA construct, but not H1/siRNA or H3/siRNA, produced similar effects, demonstrating that the suppression of tumorgrowth by siRNA was sequence-specific. We conclude that H-ras is involved in maintenance of tumorgrowth of human ovarian cancer, and that retrovirus-mediated siRNA expression against H-ras expression is a powerful tool to dissect ras-signaling

pathways and may be used therapeutically against ovarian cancer.

Yang, Q., et al. (2002). "[Gene transfer of murine Flt3 ligand mediated by adenoviral vector efficiently induces growth inhibition of murine liver cancer]." Zhonghua Yi Xue Za Zhi **82**(11): 775-779.

OBJECTIVE: To observe the in vivo therapeutic effects of murine Flt3 ligand (mFL) mediated by recombinant adenoviral vector on murine liver cancer. METHODS: Murine liver cancer cell line Hepal-6 was infected with adenovirus in vitro. The infection efficacy was measured by green fluorescence protein (GFP) expression and the amount of mFL in supernatant was measured by ELISA 48 hrs following infection of Hepal-6. AdmFL, Ad-null, and PBS were added into the culture of Hepal-6 cells, the number of cells was counted every other day for 14 days. A murine liver cancer model was established by subcutaneous inoculation of Hepal-6 cells. A single dosage of 1 x 10(9) expression forming unit (efu) of Ad-mFL, Ad-null or PBS was injected intratumorally. The tumor volume and survival rate were measured twice a week. Twenty days after treatment of adenoviral vectors, three treated mice with their tumor disappearing were killed and their spleen was taken. The splenocytes from these tumor free mice were adoptively transferred to naive mice to whom Hepal-6 cells were inoculated 3 days thereaftter and then the tumor volume was measured once a week.for 4 weeks. 38 days after administration of adenoviral vectors, tumor free animals were rechallenged by parental Hepal-6 cells or syngenic EL4 lymphoma cells at the opposite sites of the original inoculation sites, and the tumor volume was also measured once a week for 4 weeks. RESULTS: Adenoviral vector efficiently infected Hepal-6 cells in intro, and lead to the secretion of high levels of mFL protein (80.5 +/- 7.3 ng/10(6)/24 h) in the supernatant. The growth of Hepal-6 tumor was significantly inhibited by one single intratumoral administration of Ad-mFL in 90% of the treated mice, and the tumor gradually grew in the two other groups. Two of the 7 mice (30%) in PBS group died in 17 days, 14% still lived in 37 days, and all died within 45 days. The mice in the Ad-null group began to die since the 21(st) day, only 11% of them were still alive in 60 days. All mice in the Ad-mFL group were alive at the 60(th) day after tumor implantation. Adoptive transfer of splenocytes to the animals receiving Ad-mFL treatment protected effectively them against a subsequent challenge with the identical tumor cells. Rechallenge of the Ad-mFL cured mice with the parental Hepal-6 cells resulted in complete inhibition of tumor growth. The growth of inoculated EL4 lymphoma cells gradually grew equally in the controls and the experimental mice. CONCLUSION: FL gene transfer mediated by recombinant adenoviral vector has potent therapeutic effects on Hepal-6 liver cancer, and develops long-lasting specific antitumor immunity, which may become a potent cancer gene therapy candidate for further clinic application.

Yang, W., et al. (2001). "sFlt-1 gene-transfected fibroblasts: a wound-specific gene therapy inhibits local cancer recurrence." Cancer Res **61**(21): 7840-7845.

Local recurrence occurs frequently at the site of injury after surgical resection. On the other hand, fibroblasts have been shown to accumulate in the injured area to heal and remodel the damaged tissues. Therefore, fibroblasts are likely to be useful as wound-specific vectors for delivery of genes to sites of surgically injury. The present study was performed to investigate wound-specific migration of exogenously administered fibroblasts and efficacy of gene therapy using genetically engineered fibroblasts in an i.p. wound recurrence model in rats. We demonstrated that fibroblasts transfected with the GFP gene accumulated specifically around the site of injury immediately after i.p. injection. Then, fibroblasts transfected with an adenovirus designated as AdFex that encoded the soluble form of Flt-1 (sFlt-1), a vascular endothelial growth factor receptor, were administered i.p. to the rats to examine inhibition of tumor growth. At day 16 after implantation, a significantly smaller tumor volume and less microvessel density in wound sites were observed in the AdFex/fibroblast-treated rats than in controls. Furthermore, this treatment also resulted in an improved survival rate. In conclusion, autologous fibroblasts show promise as a wound-specific vector for gene therapy, and administration of sFlt-1 gene-engineered fibroblasts contributed to local control of the tumor around the injured tissue.

Yang, W. M., et al. (2006). "LRIG1, a candidate tumour-suppressor gene in human bladder cancer cell line BIU87." BJU Int **98**(4): 898-902.

OBJECTIVES: To determine the effects of LRIG1 on the growth, migration and invasion of bladder cancer cells and the mechanisms underlying such effects. MATERIALS AND METHODS: The plasmid pLRIG1-green fluorescence protein (GFP) was transfected into BIU87 bladder cancer cells by Lipofectamine2000 (Invitrogen, Groningen, the Netherlands), and the cells that expressed LRIG1 stably were screened out by G418. The changes in LRIG1 and epidermal growth factor receptor (EGFR) protein levels were measured by Western blot; growth curves were estimated by the tetrazolium (MTT) assay; then cell-cell adhesion, cell-matrix adhesion and cell invasion assays were used to measure proliferation,

adhesion and invasion in LGIR1-transfected and control cells. RESULTS: The LRIG1 protein level in pLRIG1-GFP transfected cells was significantly higher than that in control cells, while the EGFR protein level was significantly lower. pLRIG1-GFP transfected cells had less proliferation than control cells. Contrasting with non-LRIG1-transfected cells, the invasion and cell-matrix adhesion ability of pLRIG1-GFP transfected cells decreased markedly, and conversely the homotypic cell-cell adhesion ability was significantly higher. CONCLUSIONS: LRIG1 might act as a tumour-suppressor gene, participating in negative feedback control of EGFR expression, which inhibits bladder cancer cells from growth, migration and invasion.

Yang, Y. J., et al. (2003). "[Expression of osm gene driven by hTERT gene promoter inhibits cancer cells proliferation in vitro]." Ai Zheng **22**(6): 575-578.

BACKGROUND & OBJECTIVE: Specific gene expression plays an important role in cancer gene therapy. Construction of specific expression vector is the basis of cancer gene therapy. This study was conducted to explore the expression specificity of osm (oncostatin M) gene driven by human telomerase reverse transcriptase (hTERT) gene promoter in tumor cells with telomerase activity and to investigate the growth inhibitory capability of expression of osm gene on telomerase-positive tumor cells. METHODS: The authors constructed the expression vector of osm gene afforded by the hTERT promoter and investigated its effect on tumors in vitro using reverse transcription polymerase chain reaction (RT-PCR), transient transfection, and MTT method. RESULTS: Expression of extrinsic osm gene driven by hTERT gene promoter was detected in HepG2 cell with telomerase activity, and not detected in human embryonic lung fibroblast (HEL) cell without telomerase activity. After transfection of phTERT-osm, the proliferation of HepG2, HeLa, Glc, and A549 cells showed significant inhibitory effect, and the inhibitory rate was 12.4-46%. No inhibitory effect appeared in HEL cell. CONCLUSION: The expression of osm gene under the control of hTERT gene promoter can restrict toxic effect to telomerase-positive tumor cells, and alleviate the toxic effect on normal cells without telomerase activity.

Ye, D., et al. (2001). "Growth inhibition of interleukin-2 receptor gene-transduced peripheral blood lymphocytes on human ovarian cancer cells." Chin Med J (Engl) **114**(3): 303-307.

OBJECTIVE: To investigate the growth inhibition of interleukin-2 receptor (IL-2R) gene-transduced peripheral blood lymphocytes (PBLs) on human ovarian cancer cells. METHODS: Interleukin-2 (IL-2) and IL-2R genes were transfected into human ovarian cancer cell line 3AO and PBLs, respectively, using the same Fugene vector. Twenty-four hours later transfected and nontransfected PBLs were cocultured with transfected and nontransfected 3AO for 48 hours. cytotoxicity of PBLs on 3AO was detected by the MTT assay. RESULTS: The morphology of IL-2-transduced 3AO and IL-2R-transduced PBLs remained unchanged. 3AO cells could be transfected with the IL-2 gene and expressed IL-2 mRNA, and PBLs could be transfected with the IL-2R gene and expressed IL-2R mRNA. IL-2 transduced 3AO cells enhanced their response to the cytotoxicity of PBLs. Furthermore, growth inhibition of PBLs to 3AO cells increased significantly when the IL-2R was transfected into PBLs and when the IL-2 gene was transfected into 3AO cells and the two were combined. CONCLUSIONS: IL-2R gene transduced PBLs are able to enhance their cytotoxicity on IL-2 gene transduced ovarian cancer cells. This method may be a new way to investigate IL-2 gene therapy for ovarian cancer.

Ye, X. and M. Wu (1992). "Retrovirus mediated transfer of antisense human c-myc gene into human esophageal cancer cells suppressed cell proliferation and malignancy." Sci China B **35**(1): 76-83.

A retroviral vector, called pDAM3, containing the neomycin resistant gene and the antisense human c-myc gene fragment (the third exon and 3' flanking sequence) was constructed. pDAM3 was introduced into amphotropic packaging cells PA317 by the calcium phosphate precipitation method. Several G418-resistant PA317 clones were isolated. The virus titer of these cell lines was determined by infectivity of their culture fluid to NIH/3T3 cells. The highest titer obtained was 8 x 10(5) G418-resistant colony forming units/ml. Clonal and pooled G418-resistant PA317 colonies with high titers were expanded and analyzed by Southern blot for the presence of intact viral sequences. All cell lines were found to harbor the internal sequences of the pDAM3 vector without any rearrangement. Recombinant virus DAM3 infected human esophageal cancer cell line EC8712 efficiently. The DAM3-infected EC8712 (called EC-DAM3) was found to contain the full DAM3 sequence (4.8 kb) by Southern blot analysis. Antisense myc RNA expressed in the EC-DAM3 cell was detected by RNA hybridization. Further studies indicated that [3H]-thymidine incorporation in EC-DAM3 cells was reduced by 45% in average compared to that in untreated EC8712 cells. Growth rate of EC-DAM3 cells also decreased about 50%. DAM3-infected EC8712 cells lost their ability of forming tumor in nude mice. It thus appeared that the antisense myc gene introduced into EC8712 cells via retrovirus

vector was capable of inhibiting cell proliferation and malignancy.

Yeap, B. B., et al. (1999). "Differential posttranscriptional regulation of androgen receptor gene expression by androgen in prostate and breast cancer cells." Endocrinology **140**(7): 3282-3291.

Androgens, via the androgen receptor (AR), modulate the growth and proliferation of prostate and breast cancer cells. However, the molecular mechanisms underlying the regulation of AR gene expression by androgen in these cells remain to be fully elucidated. To explore differences in AR gene expression between these hormone-responsive tumor cell types, we studied androgen-responsive LNCaP prostate cancer and AR positive MDA453 breast cancer cells. Dihydrotestosterone (DHT) 10 nM increased LNCaP cell proliferation and the proportion of LNCaP cells in S-phase of the cell cycle but inhibited MDA453 cell proliferation and reduced the proportion of MDA453 cells in S-phase of cell cycle. In both these cell lines, DHT decreased total AR messenger RNA (mRNA) but increased AR protein. In LNCaP cells, DHT down-regulated AR mRNA transcription but stabilized AR mRNA. In contrast, in MDA453 cells, DHT had no effect on AR mRNA transcription but destabilized AR mRNA. In summary, transcriptional down-regulation induced by androgens in LNCaP cells results in down-regulation of steady-state AR mRNA despite an androgen-induced increase in AR mRNA stability. However, in MDA453 cells, posttranscriptional destabilization of AR mRNA appears to be the predominant mechanism resulting in down-regulation of AR mRNA by androgen. These results demonstrate cell-specific and divergent regulation of AR mRNA turnover by androgen and identify a novel pathway of androgen-induced posttranscriptional destabilization and down-regulation of AR mRNA in human breast cancer cells. Furthermore, these data establish an important role for posttranscriptional pathways in the regulation of AR gene expression by androgen in human prostate and breast cancer cells.

Yoshioka, N., et al. (2002). "Suppression of anchorage-independent growth of human cancer cell lines by the TRIF52/periostin/OSF-2 gene." Exp Cell Res **279**(1): 91-99.

In searching for genes that suppress the viral transformation of primary cells, we have isolated a number of TRIF (transcript reduced in F2408) genes that are expressed well in primary rat embryo fibroblasts (REFs) but poorly in spontaneously immortalized rat fibroblast cell lines derived from REFs. One of these genes, TRIF52, is a rat homologue of the mouse protein periostin, which is suspected of being involved in oncogenesis. We found here that periostin mRNA expression is markedly downregulated in a variety of human cancer cell lines and human lung cancer tissues. Human cancer cell lines with reduced endogenous periostin gene expression that were infected with a recombinant retrovirus containing the periostin gene had reduced anchorage-independent growth. Mutational analysis revealed that the C-terminal region of periostin is sufficient to convey the anchorage-independent growth-suppressive activity of the protein. These observations together suggest that periostin may serve to inhibit the development of human cancers by acting as a tumor suppressor.

Yu, J. X., et al. (2007). "Pathway analysis of gene signatures predicting metastasis of node-negative primary breast cancer." BMC Cancer **7**: 182.

BACKGROUND: Published prognostic gene signatures in breast cancer have few genes in common. Here we provide a rationale for this observation by studying the prognostic power and the underlying biological pathways of different gene signatures. METHODS: Gene signatures to predict the development of metastases in estrogen receptor-positive and estrogen receptor-negative tumors were identified using 500 re-sampled training sets and mapping to Gene Ontology Biological Process to identify over-represented pathways. The Global Test program confirmed that gene expression profilings in the common pathways were associated with the metastasis of the patients. RESULTS: The apoptotic pathway and cell division, or cell growth regulation and G-protein coupled receptor signal transduction, were most significantly associated with the metastatic capability of estrogen receptor-positive or estrogen-negative tumors, respectively. A gene signature derived of the common pathways predicted metastasis in an independent cohort. Mapping of the pathways represented by different published prognostic signatures showed that they share 53% of the identified pathways. CONCLUSION: We show that divergent gene sets classifying patients for the same clinical endpoint represent similar biological processes and that pathway-derived signatures can be used to predict prognosis. Furthermore, our study reveals that the underlying biology related to aggressiveness of estrogen receptor subgroups of breast cancer is quite different.

Yuan, B. Z., et al. (2003). "DLC-1 gene inhibits human breast cancer cell growth and in vivo tumorigenicity." Oncogene **22**(3): 445-450.

The human DLC-1 (deleted in liver cancer 1) gene was cloned from a primary human hepatocellular carcinoma (HCC) and mapped to the chromosome

8p21-22 region frequently deleted in common human cancers and suspected to harbor tumor suppressor genes. DLC-1 was found to be deleted or downregulated in a significant number of HCCs. We expanded our investigations to other cancers with recurrent deletions of 8p22, and in this study examined alterations of DLC-1 in primary human breast tumors, human breast, colon, and prostate tumor cell lines. Genomic deletion of DLC-1 was observed in 40% of primary breast tumors, whereas reduced or undetectable levels of DLC-1 mRNA were seen in 70% of breast, 70% of colon, and 50% of prostate tumor cell lines To see whether DLC-1 expression affects cell growth and tumorigenicity, two breast carcinoma cell lines lacking the expression of endogenous gene were transfected with the DLC-1 cDNA. In both cell lines, DLC-1 transfection caused significant growth inhibition and reduction of colony formation. Furthermore, introduction of the DLC-1 cDNA abolished the in vivo tumorigenicity in nude mice, suggesting that the DLC-1 gene plays a role in breast cancer by acting as a bona fide tumor suppressor gene.

Yuan, F., et al. (1999). "Altered growth and viral gene expression in human papillomavirus type 16-containing cancer cell lines treated with progesterone." Cancer Invest **17**(1): 19-29.

This study explores interactions between high-risk human papillomavirus type 16 (HPV-16) and the female sex hormone progesterone in the growth of tumor cells and viral oncogene expression. For both the cervical cancer cell line CaSki containing integrated HPV-16 DNA and the laryngeal carcinoma cell line HEp-2 transfected with HPV-16 DNA, prolonged progesterone treatment enhances their colony formation efficiency both on plastic surface and in soft agar. In contrast, progesterone has no effect on the HPV-negative cervical cancer cell line C-33A or the untransfected HEp-2 parental cells. Progesterone increases HPV-16 E6/E7 oncogene transcription in both HPV-16-containing cell lines. A detectable increase requires at least 3 days of treatment, and this delayed response may be due, at least in part, to increased stability of viral transcripts as determined by actinomycin D treatment. The progesterone antagonist RU 486 and nuclease-resistant oligomers containing HPV-16 progesterone response element are able to abrogate the enhancement by progesterone on cell growth and E6/E7 gene transcription. Taken together, these results support the notion that progesterone can be a cofactor in HPV-related malignancies.

Zerbini, L. F., et al. (2006). "A novel pathway involving melanoma differentiation associated gene-7/interleukin-24 mediates nonsteroidal anti-inflammatory drug-induced apoptosis and growth arrest of cancer cells." Cancer Res **66**(24): 11922-11931.

Numerous studies show that nonsteroidal anti-inflammatory drugs (NSAIDs) are effective in chemoprevention or treatment of cancer. Nevertheless, the mechanisms underlying these antineoplastic effects remain poorly understood. Here, we report that induction of the cancer-specific proapoptotic cytokine melanoma differentiation associated gene-7/interleukin-24 (MDA-7/IL-24) by several NSAIDs is an essential step for induction of apoptosis and G (2)-M growth arrest in cancer cells in vitro and inhibition of tumor growth in vivo. We also show that MDA-7/IL-24-dependent up-regulation of growth arrest and DNA damage inducible 45 alpha (GADD45alpha) and GADD45gamma gene expression is sufficient for cancer cell apoptosis via c-Jun NH (2)-terminal kinase (JNK) activation and growth arrest induction through inhibition of Cdc2-cyclin B checkpoint kinase. Knockdown of GADD45alpha and GADD45gamma transcription by small interfering RNA abrogates apoptosis and growth arrest induction by the NSAID treatment, blocks JNK activation, and restores Cdc2-cyclin B kinase activity. Our results establish MDA-7/IL-24 and GADD45alpha and GADD45gamma as critical mediators of apoptosis and growth arrest in response to NSAIDs in cancer cells.

Zha, Y., et al. (2000). "[The use of gene gun in cancer gene therapy]." Zhonghua Yi Xue Za Zhi **80**(7): 522-525.

OBJECTIVE: With Helios gene gun, the report genes EGFP and Lac-Z were transfected to several cultured mammalian tumor cell lines in vitro and mice skin in vivo, respectively. A stable long time exogene's expression were got. Then, the pWRG3142, which carried mGM-CSF gene was delivered to the abdominal skin of C57BL/6 mice using gene gun. Pathological sections showed the local transproteins' expression companying with a profound inflammation reaction characterized by neutrophilic infiltration. ELISA assay of transfected mouse's serum sample indicated a high improvement of transgenic proteins level, which demonstrated that transgenic GM-CSF secreted from treated skin into the bloodstream effectively. In B16 melanoma tumor model, mice immunized with mGM-CSF expression plasmids could be partially protected from 1 x 10(5) B16 cells challenge and exhibited a drastically reduced tumor growth rate. Finally, we concluded that exogenes could be transfected into cultured cell lines and mice skin effectively by gene gun technology, and gene gun mediated in vivo delivery of GM-CSF cDNA should be further developed for potential clinical testing as an approach for human cancer gene therapy.

Zhang, F., et al. (2002). "Tumor-infiltrating macrophages are involved in suppressing growth and metastasis of human prostate cancer cells by INF-beta gene therapy in nude mice." Clin Cancer Res **8**(9): 2942-2951.

PURPOSE: This study was to determine the role of tumor-infiltrating macrophages in IFN-beta-induced host defense against prostate cancer. EXPERIMENTAL DESIGN: Efficacy of adenovirus-mediated IFN-beta gene therapy against orthotopic xenografts of human prostate cancer was tested in macrophage-compromised nude mice. Immunohistochemistry and Northern blotting were used to elucidate mechanisms responsible for the IFN-beta gene therapy. RESULTS: PC-3MM2 human prostate cancer cells were inoculated into the prostates of nude mice. Intralesional injection of an adenoviral vector-encoding murine IFN-beta (AdmIFN-beta) but not control vector AdE/1 suppressed growth of PC-3MM2 tumors in a dose-dependent manner, with a maximal reduction of tumor weight by approximately 85% at 2 x 10(9) plaque-forming units. The therapy prevented metastasis, eradicated established metastases in some mice, and prolonged the survival of tumor-bearing mice. The efficacy of AdmIFN-beta therapy was reduced significantly in mice treated with macrophage-selective anti-Mac-1 and anti-Mac-2 antibodies. Moreover, the i.p. injection of the antibodies restored the tumorigenicity of PC-3MM2 cells stably engineered with murine IFN-beta gene. Tumor-infiltrating macrophages, significantly increased in AdmIFN-beta-injected lesions, were depleted by the antibodies. The therapy stimulated expression of the inducible nitric oxide synthase, down-regulated transforming growth factor-beta1 and interleukin-8, reduced microvessel density, and resulted in apoptosis of endothelial cells in the lesions. These effects of AdmIFN-beta were partially diminished in mice treated with the antibodies. CONCLUSIONS: These data suggest that macrophages play an important role in IFN-beta gene therapy and that intralesional delivery of the IFN-beta gene could be an effective therapy for clinically localized human prostate cancer.

Zhang, H., et al. (2016). "Integrated analysis of the miRNA, gene and pathway regulatory network in gastric cancer." Oncol Rep **35**(2): 1135-1146.

Gastric cancer is one of the most common malignant tumors worldwide; however, the efficacy of clinical treatment is limited. MicroRNAs (miRNAs) are a class of small non-coding RNAs that have been reported to play a key role in the development of cancer. They also provide novel candidates for targeted therapy. To date, in-depth studies on the molecular mechanisms of gastric cancer involving miRNAs are still absent. We previously reported that 5 miRNAs were identified as being significantly increased in gastric cancer, and the role of these miRNAs was investigated in the present study. By using bioinformatics tools, we found that more than 4,000 unique genes are potential downstream targets of gastric cancer miRNAs, and these targets belong to the protein class of nucleic acid binding, transcription factor, enzyme modulator, transferase and receptor. Pathway mapping showed that the targets of gastric cancer miRNAs are involved in the MAPK signaling pathway, pathways in cancer, the PI3K-Akt signaling pathway, the HTLV-1 signaling pathway and Ras signaling pathway, thus regulating cell growth, differentiation, apoptosis and metastasis. Analysis of the pathways related to miRNAs may provides potential drug targets for future therapy of gastric cancer.

Zhang, J., et al. (2004). "Cystatin m: a novel candidate tumor suppressor gene for breast cancer." Cancer Res **64**(19): 6957-6964.

The contribution of pericellular proteolysis to tumor progression is well documented. To better understand protease biology and facilitate clinical translation, specific proteolytic systems need to be better defined. In particular, the precise role of endogenous protease inhibitors still needs to be deciphered. We reported previously that cystatin M, a potent endogenous inhibitor of lysosomal cysteine proteases, significantly suppressed in vitro cell proliferation, migration, and Matrigel invasion. Here, we show that scid mice orthotopically implanted with breast cancer cells expressing cystatin M show significantly delayed primary tumor growth and lower metastatic burden in the lungs and liver when compared with mice implanted with mock controls. The incidence of metastasis, however, appeared to be unaltered between the cystatin M group and the control group. Experimental metastasis assays suggest that cystatin M suppressed tumor cell proliferation at the secondary site. By using laser capture microdissection and quantitative reverse transcription-polymerase chain reaction, we found consistent expression of cystatin M in normal human breast epithelial cells, whereas expression was decreased by 86% in invasive ductal carcinoma (IDC) cells of stage I to IV patients. Complete loss of expression of cystatin M was observed in two of three IDCs from stage IV patients. Immunohistochemical studies confirmed that expression of cystatin M in IDCs was partially or completely lost. We propose cystatin M as a novel candidate tumor suppressor gene for breast cancer.

Zhang, J., et al. (2000). "[The growth inhibitory effects by transfection of p16 gene on human pancreatic cancer cell line]." Zhonghua Wai Ke Za Zhi **38**(6): 457-459.

OBJECTIVE: To elevate the growth inhibitory effects by transfection of p16 gene on human pancreatic cancer cell line JF305. METHODS: Recombinant eukaryotic expression vector pDOR-p16 containing exogenous human wt-p16 cDNA and vector containing neomycin resistance gene only were introduced by Liposomes-mediated gene transfection into JF305 cell line which did not express endogenous p16. By using PCR amplification, in situ hybridization, and immunocytochemistry, the clones obtained were detected for efficiency of transfection and effect of vector expression and observed for the changes of their biologic characteristics. RESULTS: Exogenous wt-p16 was successfully transferred into JF305 cells and obtained permanent expression. The growth rate of these transfected JF305 cells in regular medium and soft agar was inhibited. The percentage of phage G (1) cells increased and that of phage S cells decreased by analysing cell cycle. The ultrastructural changes of the cells observed under electron-microscope revealed growth retardation. CONCLUSION: p16 is a candidate for cancer gene replacement therapy of human pancreatic cancer.

Zhang, J. F., et al. (1996). "Treatment of a human breast cancer xenograft with an adenovirus vector containing an interferon gene results in rapid regression due to viral oncolysis and gene therapy." Proc Natl Acad Sci U S A **93**(9): 4513-4518.

Treatment of a human breast cancer cell line (MDA-MB-435) in nude mice with a recombinant adenovirus containing the human interferon (IFN) consensus gene, IFN-con1 (ad5/IFN), resulted in tumor regression in 100% of the animals. Tumor regression occurred when virus was injected either within 24 hr of tumor cell implantation or with established tumors. However, regression of the tumor was also observed in controls in which either the wild-type virus or a recombinant virus containing the luciferase gene was used, although tumor growth was not completely suppressed. Tumor regression was accompanied by a decrease in p53 expression. Two other tumors, the human myelogenous leukemic cell line K562 and the hamster melanoma tumor RPMI 1846, also responded to treatment but only with ad5/IFN. In the case of K562 tumors, there was complete regression of the tumor, and tumors derived from RPMI 1846 showed partial regression. We propose that the complete regression of the breast cancer with the recombinant virus ad5/IFN was the result of two events: viral oncolysis in which tumor cells are being selectively lysed by the replication-competent virus and the enhanced effect of expression of the IFN-con1 gene. K562 and RPMI 1846 tumors regressed only as a result of IFN gene therapy. This was confirmed by in vitro analysis. Our results indicate that a combination of viral oncolysis with a virus of low pathogenicity, itself resistant to the effects of IFN and IFN gene therapy, might be a fruitful approach to the treatment of a variety of different tumors, in particular breast cancers.

Zhang, L., et al. (2010). "The impact of C-MYC gene expression on gastric cancer cell." Mol Cell Biochem **344**(1-2): 125-135.

The upregulation or mutation of C-MYC has been observed in gastric, colon, breast, and lung tumors and in Burkitt's lymphoma. However, little is known about the role C-MYC plays in gastric adenocarcinoma. In the present study, we intended to investigate the influence of C-MYC on the growth, proliferation, apoptosis, invasion, and cell cycle of the gastric cancer cell line SGC7901 and the gastric cell line HFE145. C-MYC cDNA was subcloned into a constitutive vector PCDNA3.1 followed by transfection in normal gastric cell line HFE145 by using liposome. Then stable transfectants were selected and appraised. Specific inhibition of C-MYC was achieved using a vector-based siRNA system which was transfected in gastric cancer cell line SGC7901. The apoptosis and cell cycles of these clones were analyzed by using flow cytometric assay. The growth and proliferation were analyzed by cell growth curves and colony-forming assay, respectively. The invasion of these clones was analyzed by using cell migration assay. The C-MYC stable expression clones (HFE-Myc) and C-MYC RNAi cells (SGC-MR) were detected and compared with their control groups, respectively. HFE-Myc grew faster than HFE145 and HFE-PC (HFE145 transfected with PCDNA3.1 vector). SGC-MR1, 2 grew slower than SGC7901 and SGC-MS1, 2 (SGC7901 transfected with scrambled control duplexes). The cell counts of HFE-Myc in the third, fourth, fifth, sixth, and seventh days were significantly more than those of control groups ($P < 0.05$). Those of SGC-MR1, 2 in the fourth, fifth, sixth, and seventh days were significantly fewer than those of control groups ($P < 0.05$). Cell cycle analysis showed that proportions of HFE-Myc and SGC-MR cells in G0-G1 and G2-M were different significantly with their control groups, respectively ($P < 0.05$). The apoptosis rate of HFE-Myc was significantly higher than those of control groups ($P < 0.05$). Results of colony-forming assay showed that the colony formation rate of HFE-Myc was higher than those of control groups; otherwise, the rate of SGC-MR was lower than those of their control groups ($P < 0.05$). The results of cell migration assay showed that there were no significant

differences between experimental groups and control groups (P > 0.05). In conclusion, C-MYC can promote the growth and proliferation of normal gastric cells, and knockdown of C-MYC can restrain the growth and proliferation of gastric cancer cells. It can induce cell apoptosis and help tumor cell maintain malignant phenotype. But it can have not a detectable influence on the ability of invasion of gastric cancer cells.

Zhang, T., et al. (2010). "PIM-1 gene RNA interference induces growth inhibition and apoptosis of prostate cancer cells and suppresses tumor progression in vivo." J Surg Oncol **101**(6): 513-519.

BACKGROUND: The goal of this study was to investigate the roles of PIM-1 in prostate cancer (CaP) cell proliferation and apoptosis, and to assess the potential of PIM-1 as a target for CaP therapy. METHODS: Using RNAi technology, we knocked down the expression of PIM-1 in PC-3 cell. After siRNA transfection, cell morphology, cell proliferation, cell cycle, and apoptosis rate were analyzed. PIM-1 siRNA with Lipofectamine were injected into xenograft models to evaluate its therapeutic effect. RESULTS: PIM-1 siRNA significantly inhibited PIM-1 expression. In vitro, silencing of the PIM-1 gene resulted in irregular cell morphology, decreased cell proliferation, inhibition of cell-cycle progression, and induction of apoptosis. Compared with control groups, intratumoral injection of PIM-1 siRNA with Lipofectamine in nude mice dramatically suppressed PC-3 tumor progression. CONCLUSIONS: PIM-1 could play important roles in the progression of CaP and may be an interesting target for CaP therapy.

Zhang, X., et al. (2007). "Effects of exogenous p16(ink4a) gene on biological behaviors of human lung cancer cells." J Huazhong Univ Sci Technolog Med Sci **27**(1): 37-40.

The effects of exogenous p16(ink4a) gene on biological behaviors of human lung cancer cell line with homozygous deletion of p16(ink4a) gene were investigated. Exogenous p16(ink4a) gene was transfected by lipofectin into human lung cell line A549, in which p16(ink4a) gene was homozygously deleted. The expression of p16(ink4a) mRNA and protein was detected by RT-PCR and immunocyto-chemistry, respectively. The changes in the behaviors of the transfected cell lines in vitro and in vivo were observed. In the transfected cell line A549, the exogenous p16(ink4a) gene could be stably expressed. The growth of A549 cells transfected with p16(ink4a) gene was obviously slowed down. Flow cytometry revealed that transfection of the exogenous p16(ink4a) gene resulted in A549 cell lines arrest in G1 phase of cell cycle. The tumorigenicity of these transfected cells in nude mice could be inhibited, and the tumor growth of nude mice was significantly suppressed. It was concluded that exogenous p16(ink4a) gene may be stably expressed in human lung cancer cell line A549. The expression of the introduced p16(ink4a) could block lung cancer cells to entry into S phase of cell cycle and inhibit tumor malignant growth both in vitro and in vivo.

Zhang, X., et al. (2001). "Apoptosis induction in prostate cancer cells by a novel gene product, pHyde, involves caspase-3." Oncogene **20**(42): 5982-5990.

A novel gene, pHyde, was recently cloned from Dunning rat prostate cancer cells. A recombinant adenovirus containing pHyde cDNA gene (AdpHyde) was generated to investigate the biological function of pHyde protein. AdpHyde inhibited the growth of human prostate cancer cells. Apoptosis was induced in AdpHyde transduced cells as demonstrated by DAPI (4', 6-diamino-2-phenylindole), TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick and labeling) staining, and flow cytometry assays. Apoptosis was also induced in human xenograft prostate cancer tumors growing in nude mice following treatment with AdpHyde. AdpHyde transduction resulted in a dose-dependent stimulation of caspase-3 activity in DU145 cells which was blocked by DEVD (succinyl-Asp-Glu-Val-Asp-aldehyde) and VAD (benzyloxycarbonyl - Val - Ala - Asp -fluoromethylketone), inhibitors specifically against caspase-3. Moreover, cancer cells that lacked expression of endogenous caspase-3 were not or barely inhibited by pHyde. These results taken together suggest that pHyde inhibits cancer growth by inducing apoptosis through a caspase-3 dependent pathway.

Zhang, Y., et al. (2005). "The Human mineral dust-induced gene, mdig, is a cell growth regulating gene associated with lung cancer." Oncogene **24**(31): 4873-4882.

Environmental or occupational exposure to mineral dusts, mainly silica and asbestos, is associated with an increased incidence of lung inflammation, fibrosis, and/or cancer. To better understand the molecular events associated with these pulmonary diseases, we attempted to identify genes that are regulated by mineral dusts. Using a differential display reverse transcription polymerase chain reaction technique and mRNAs of alveolar macrophages from both normal individuals and coal miners, we identified a novel mineral dust-induced gene named mdig, which had not been fully characterized. The expression of mdig mRNA was detected in alveolar macrophages from coal miners but not from normal subjects. The inducible expression of mdig could be observed in A549 cells exposed to silica particles in a time-dependent manner. The full-length mdig mRNA was

expressed in human lung cancer tissues but was barely detectable in the adjacent normal tissues. In addition, a number of lung cancer cell lines constitutively express mdig. Alternative spliced transcripts of mdig were detected in some lung cancer cell lines. Silencing mdig mRNA expression in A549 lung cancer cells by siRNA-mediated RNA interference inhibits cell proliferation and sensitizes the cells to silica-induced cytotoxicity. These results suggest that the mdig gene may be involved in the regulation of cell growth and possibly the development of cancer.

Zhang, Y. M., et al. (2003). "Effect of ZNRD1 gene antisense RNA on drug resistant gastric cancer cells." World J Gastroenterol **9**(5): 894-898.

AIM: To investigate the expression level of ZNRD1 gene in gastric cancer cells SGC7901 and gastric cancer MDR (multidrug resistant) cells SGC7901/VCR, and to observe the drug sensitizing and proliferation effect of ZNRD1 antisense nucleic acid transduction on SGC7901/VCR cells. METHODS: Amplification of sequences encoding ZNRD1 from SGC7901/VCR cDNA by PCR. The levels of ZNRD1 mRNA expression were demonstrated using semiquantitative reverse transcription polymerase chain reaction (RT-PCR). Eukaryotic expression vector pcDNA3.1-anti ZNRD1 was constructed and transfected into SGC7901/VCR cells by lipofectamine. Immunochemical method was used to detect the expression of protein in SGC7901/VCR cells and transfectants. The cell cycle alteration and the intracellular adriamycin (ADM) accumulation were observed by FACS. Growth curve and drug sensitization of cells for vincristine (VCR) were analyzed with MTT assay. RESULTS: We cloned the open reading frame of full-length ZNRD1. The expression of ZNRD1 showed higher in SGC7901/VCR than in SGC7901 cells. The antisense ZNRD1 drug-resistant clones were selected after gene transfection. Immunochemical results showed that the expression level of ZNRD1 protein was lower in anti ZNRD1-SGC7901/VCR cells than that in non-transfectants. Comparing to SGC7901/VCR and pcDNA3.1-SGC7901/VCR, anti ZNRD1-SGC7901/VCR showed gradually accumulated in G (1) phase, with a concomitant decrease of cell population in S phase. FACS also suggested intracellular ADM accumulation increased 2fold in SGC7901/VCR cells after transfected with antisense ZNRD1. MTT assay showed that transfectants cells proliferation was lagged and more sensitive to VCR than non-transfectants. CONCLUSION: ZNRD1 gene displayed highly expression in VCR resistant gastric cancer cells. Expression of ZNRD1 protein was effectively blocked in anti ZNRD1-SGC7901/VCR cells by gene transfection. ZNRD1 antisense nucleic acid transfection sensitized drug resistant gastric cancer cells to VCR, increased ADM accumulation and inhibited the cells proliferation. ZNRD1 antisense RNA transduction could reverse the MDR of human drug-resistant gastric cancer cell SGC7901/VCR to a degree.

Zhang, Z., et al. (2003). "Antisense therapy targeting MDM2 oncogene in prostate cancer: Effects on proliferation, apoptosis, multiple gene expression, and chemotherapy." Proc Natl Acad Sci U S A **100**(20): 11636-11641.

This study was undertaken to investigate the role of mouse double minute 2 (MDM2) oncogene in prostate cancer growth and the potential of MDM2 as a target for prostate cancer therapy. An antisense anti-human-MDM2 mixed-backbone oligonucleotide was tested in human prostate cancer models with various p53 statuses, LNCaP (p53wt/wt), DU145 (p53mt/mt), and PC3 (p53null). In a dose- and time-dependent manner, it specifically inhibited MDM2 expression and modified expression of several genes, at both mRNA and protein levels. In LNCaP cells, p53, p21, Bax, and hypophosphorylated retinoblastoma tumor suppressor protein (pRb) levels increased, whereas Bcl2, pRb protein, and E2F transcription factor 1 (E2F1) levels decreased. In DU145 cells, p21 levels were elevated and E2F1 levels decreased, although mutant p53, Rb, and Bax levels remained unchanged. In PC3 cells, MDM2 inhibition resulted in elevated p21, Bax, and pRb levels and decreased ppRb and E2F1 levels. In all three cell lines, MDM2 inhibition reduced cell proliferation, induced apoptosis, and potentiated the effects of the chemotherapeutic agents 10-hydroxycamptothecin and paclitaxel. The anti-MDM2 oligonucleotide showed antitumor activity and increased therapeutic effectiveness of paclitaxel in both LNCaP and PC3 xenografts, causing changes in gene expression similar to those seen in vitro. In summary, this study demonstrates that MDM2 has a role in prostate cancer growth via p53-dependent and p53-independent mechanisms and that multiple genes are involved in the process. MDM2 inhibitors such as second-generation antisense oligonucleotides have a broad spectrum of antitumor activities in human cancers regardless of p53 status, providing novel approaches to therapy of human prostate cancer.

Zhao, A. G., et al. (2002). "Effects of Chinese Jianpi herbs on cell apoptosis and related gene expression in human gastric cancer grafted onto nude mice." World J Gastroenterol **8**(5): 792-796.

AIM: To explore the mechanism of the Sijunzi decoction and another Chinese herbal recipe (SRRS) based mainly on the Sijunzi decoction in treatment of gastric cancer. METHODS: A human gastric

adenocarcinoma cell line SGC-7901 grafted onto nude mouse was used as the animal model. The mice were divided into 3 groups, one control and the two representative experimental conditions. Animals in the two experimental groups received either Sijunzi decoction or SRRS over a 40-day period starting at 1st day after grafting. Control animals received saline on an identical schedule. Animals were killed 41 days after being grafted. The effect of therapy was assessed by two ways: (1) tumor size was periodically measured during the life of the animals; (2) tumor weight was determined by a electron balance immediately after the animals killed. For detection of apoptotic cells, apoptotic indices (AI) were examined by the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate fluorescence nick end labeling (TUNEL) method. Morphological alterations were observed with electron microscopy. S-P immunohistochemical method was used to detect the expression of Ki-67 in xenografts. Expression of bcl-2 and p53 was semiquantitatively detected using a reverse transcriptase-polymerase chain reaction (RT-PCR) technique. RESULTS: When compared with controls, tumor growth (size and weight) was significantly inhibited by treatment with the Sijunzi decoction (P<0.05) or SRRS (P<0.01). The tumor inhibitory rate in the Sijunzi decoction group was 34.33 % and SRRS group 46.53 %. AI of human gastric cancer xenografts in nude mice was significantly increased to 16.24+/-3.21 % using TUNEL method and 11.38+/-6.46 % by FACScan in the Sijunzi decoction group compared with the controls (TUNEL: 2.63+/-1.03 %, P<0.01; FACScan: 7.15+/- 1.32 %, P<0.05). SRRS group was also found a significantly increased AI by using TUNEL method and flow cytometry analysis compared with the controls (TUNEL: 13.18+/-3.05 %, P<0.05; FACScan: 11.58+/-5.71 % (P<0.05). Under electron microscope, cell shrinkage, nuclear chromatin condensation, formation of membrane blebs and apoptotic bodies were frequently observed in Sijunzi decoction group and SRRS group. The average labeling index (LI) for Ki-67 in SRRS group was significantly decreased to 8.43+/-2.22 % compared with the control group (10.37+/-4.91 %) (P<0.05). The average labeling index for Ki-67 in sijunzi decoction group was 7.95+/- 2.54 % which was lower than that of the control group, but showed no significance (P=0.07). The expression level of p53 mRNA was lower in both Sijunzi decoction group and SRRS group than that in control group (P<0.05; P<0.01). The expression of bcl-2 mRNA was also decreased in SRRS group compared with the control (P<0.01). CONCLUSION: The inhibition of gastric cancer cell growth in vivo by Chinese Jianpi herbs and SRRS is related to induction of the cell apoptosis which may be involved in aberrant expression of p53 and bcl-2 genes.

Zhao, R., et al. (2017). "Screening of potential therapy targets for prostate cancer using integrated analysis of two gene expression profiles." Oncol Lett **14**(5): 5361-5369.

The aim of the present study was to analyze potential therapy targets for prostate cancer using integrated analysis of two gene expression profiles. First, gene expression profiles GSE38241 and GSE3933 were downloaded from the Gene Expression Omnibus database. Differentially expressed genes (DEGs) between prostate cancer and normal control samples were identified using the Linear Models for Microarray Data package. Pathway enrichment analysis of DEGs was performed using Gene Ontology and the Kyoto Encyclopedia of Genes and Genomes. Furthermore, protein-protein interaction (PPI) networks of DEGs were constructed, on the basis of the Search Tool for the Retrieval of Interacting Genes/Proteins database. The Molecular Complex Detection was utilized to perform module analysis of the PPI networks. In addition, transcriptional regulatory networks were constructed on the basis of the associations between transcription factors (TFs) and target genes. A total of 529 DEGs were identified, including 129 upregulated genes that were primarily associated with to the cell cycle. Additionally, 400 downregulated genes were identified, which were principally enriched in the pathways associated with vascular smooth muscle contraction and focal adhesion. Cell Division Cycle Associated 8, Cell Division Cycle 45, Ubiquitin Conjugating Enzyme E2 C and Thymidine Kinase 1 were identified as hub genes in the upregulated sub-network. Furthermore, the upregulated TF E2F, and the downregulated TF Early Growth Response 1, were identified to be critical in the transcriptional regulatory networks. The identified DEGs and TFs may have critical roles in the progression of prostate cancer, and may be used as target molecules for treating prostate cancer.

Zheng, A. Q., et al. (2005). "Liposome transfected to plasmid-encoding endostatin gene combined with radiotherapy inhibits liver cancer growth in nude mice." World J Gastroenterol **11**(28): 4439-4442.

AIM: To evaluate whether intratumoral injection of liposome-endostatin complexes could enhance the antitumor efficacy of radiation therapy in human liver carcinoma (BEL7402) model. METHODS: Recombinant plasmid pcDNA3.End was transfected into human liver carcinoma cell line (BEL7402) with lipofectamine to produce conditioned medium. Then BEL7402 cells and human umbilical vein endothelial

cells (HUVECs) were treated with the conditioned medium. Cell cycle and apoptosis were analyzed by flow cytometer and endothelial cell proliferation rates were determined by MTT assay. The antitumor efficacy of endostatin gene combined with ionizing radiation in mouse xenograft liver tumor was observed. RESULTS: Endostatin significantly suppressed the S phase fraction and increased the apoptotic index in HUVECs. In contrast, endostatin treatment had no effect on BEL7402 cell apoptosis (2.1+/-0.3% vs 8.9+/-1.3%, t = 8.83, P = 0.009<0.01) or cell cycle distribution (17.2+/-2.3% vs 9.8+/-1.2%, t = 4.94, P = 0.016<0.05). The MTT assay showed that endostatin significantly inhibited the proliferation of HUVECs by 46.4%. The combination of local endostatin gene therapy with radiation therapy significantly inhibited the growth of human liver carcinoma BEL7402 xenografts, the inhibition rate of tumor size was 69.8% on d 28 compared to the untreated group. The tumor volume in the pcDNA3.End combined with radiation therapy group (249+/-83 mm3) was significantly different from that in the untreated group (823+/-148 mm3, t = 5.86, P = 0.009<0.01) or in the pcDNA3 group (717+/-94 mm3, t = 6.46, P = 0.003<0.01). Endostatin or the radiation alone also inhibited the growth of liver tumor in vivo, but their inhibition effects were weaker than those of endostatin combined with radiation, the inhibition rates on d 28 were 44.7% and 40.1%, respectively. CONCLUSION: Endostatin not only significantly suppresses tumor growth but also enhances the antitumor efficacy of radiation therapy in human carcinoma xenograft.

The above contents are the collected information from Internet and public resources to offer to the people for the convenient reading and information disseminating and sharing.

References

1. Abasolo, I., et al. (2003). "Overexpression of adrenomedullin gene markedly inhibits proliferation of PC3 prostate cancer cells in vitro and in vivo." Mol Cell Endocrinol 199(1-2): 179-187.
2. Abdul-Wahab, K., et al. (1999). "Overexpression of insulin-like growth factor II (IGFII) in ZR-75-1 human breast cancer cells: higher threshold levels of receptor (IGFIR) are required for a proliferative response than for effects on specific gene expression." Cell Prolif 32(5): 271-287.
3. Abeysinghe, H. R., et al. (2004). "The role of the THY1 gene in human ovarian cancer suppression based on transfection studies." Cancer Genet Cytogenet 149(1): 1-10.
4. Afonja, O., et al. (2002). "RAR agonists stimulate SOX9 gene expression in breast cancer cell lines: evidence for a role in retinoid-mediated growth inhibition." Oncogene 21(51): 7850-7860.
5. Ahn, W. S., et al. (2003). "A major constituent of green tea, EGCG, inhibits the growth of a human cervical cancer cell line, CaSki cells, through apoptosis, G (1) arrest, and regulation of gene expression." DNA Cell Biol 22(3): 217-224.
6. Ahn, W. S., et al. (2004). "Recombinant adenovirus-p53 gene transfer and cell-specific growth suppression of human cervical cancer cells in vitro and in vivo." Gynecol Oncol 92(2): 611-621.
7. Alkhalaf, M. and A. M. El-Mowafy (2003). "Overexpression of wild-type p53 gene renders MCF-7 breast cancer cells more sensitive to the antiproliferative effect of progesterone." J Endocrinol 179(1): 55-62.
8. Allay, J. A., et al. (2000). "Adenovirus p16 gene therapy for prostate cancer." World J Urol 18(2): 111-120.
9. Anwer, K., et al. (2000). "Cationic lipid-based delivery system for systemic cancer gene therapy." Cancer Gene Ther 7(8): 1156-1164.
10. Bai, J., et al. (1998). "Overexpression of CuZnSOD gene suppresses the growth of hepatocellular cancer cell line HepG2." Chin Med J (Engl) 111(9): 789-792.
11. Bai, M., et al. (2001). "[Influence of suppressor gene p16 on retinoic acid inducing lung cancer cell A549 differentiation]." Zhonghua Jie He He Hu Xi Za Zhi 24(9): 534-536.
12. Baidu. http://www.baidu.com. 2019.
13. Banerjee, A., et al. (1992). "Changes in growth and tumorigenicity following reconstitution of retinoblastoma gene function in various human cancer cell types by microcell transfer of chromosome 13." Cancer Res 52(22): 6297-6304.
14. Bao, J. J., et al. (2002). "Reexpression of the tumor suppressor gene ARHI induces apoptosis in ovarian and breast cancer cells through a caspase-independent calpain-dependent pathway." Cancer Res 62(24): 7264-7272.
15. Bardon, S. and L. Razanamahefa (1998). "Retinoic acid suppresses insulin-induced cell growth and cyclin D1 gene expression in human breast cancer cells." Int J Oncol 12(2): 355-359.
16. Bardon, S., et al. (1998). "Monoterpenes inhibit cell growth, cell cycle progression, and cyclin D1 gene expression in human breast cancer cell lines." Nutr Cancer 32(1): 1-7.
17. Bargou, R. C., et al. (1996). "Overexpression of the death-promoting gene bax-alpha which is downregulated in breast cancer restores sensitivity to different apoptotic stimuli and reduces tumor growth in SCID mice." J Clin Invest 97(11): 2651-2659.
18. Barron-Gonzalez, A. and I. Castro Romero (2004). "Re-expression of estrogen receptor alpha using a tetracycline-regulated gene expression system induced estrogen-mediated growth inhibition of the MDA-MB-231 breast cancer cell line." Biochem Cell Biol 82(2): 335-342.
19. Bateman, A. and H. P. Bennett (2009). "The granulin gene family: from cancer to dementia." Bioessays 31(11): 1245-1254.
20. Bergamaschi, A., et al. (2011). "Reversal of endocrine resistance in breast cancer: interrelationships among 14-3-3zeta, FOXM1, and a gene signature associated with mitosis." Breast Cancer Res 13(3): R70.
21. Boente, M. P., et al. (1998). "Suppression of diacylglycerol levels by antibodies reactive with the c-erbB-2 (HER-2/neu) gene product p185c-erbB-2 in breast and ovarian cancer cell lines." Gynecol Oncol 70(1): 49-55.
22. Bottone, F. G., Jr., et al. (2004). "Gene modulation by Cox-1 and Cox-2 specific inhibitors in human colorectal carcinoma cancer cells." Carcinogenesis 25(3): 349-357.
23. Bougeret, C., et al. (2000). "Cancer gene therapy mediated by CTS1, a p53 derivative: advantage over wild-type p53 in growth inhibition of human tumors overexpressing MDM2." Cancer Gene Ther 7(5): 789-798.
24. Brand, K. (2002). "Cancer gene therapy with tissue inhibitors of metalloproteinases (TIMPs)." Curr Gene Ther 2(2): 255-271.
25. Brauweiler, A., et al. (2007). "RING-dependent tumor suppression and G2/M arrest induced by the TRC8 hereditary kidney cancer gene." Oncogene 26(16): 2263-2271.

26. Burnatowska-Hledin, M. A., et al. (2004). "T47D breast cancer cell growth is inhibited by expression of VACM-1, a cul-5 gene." Biochem Biophys Res Commun 319(3): 817-825.
27. Burney, T. L., et al. (1994). "Partial growth suppression of human prostate cancer cells by the Krev-1 suppressor gene." Prostate 25(4): 177-188.
28. Burns, F. J., et al. (2002). "The action of a dietary retinoid on gene expression and cancer induction in electron-irradiated rat skin." J Radiat Res 43 Suppl: S229-232.
29. Butz, K., et al. (1999). "Induction of the p53-target gene GADD45 in HPV-positive cancer cells." Oncogene 18(14): 2381-2386.
30. Cahlin, C., et al. (2000). "Experimental cancer cachexia: the role of host-derived cytokines interleukin (IL)-6, IL-12, interferon-gamma, and tumor necrosis factor alpha evaluated in gene knockout, tumor-bearing mice on C57 Bl background and eicosanoid-dependent cachexia." Cancer Res 60(19): 5488-5493.
31. Calvo, A., et al. (2002). "Alterations in gene expression profiles during prostate cancer progression: functional correlations to tumorigenicity and down-regulation of selenoprotein-P in mouse and human tumors." Cancer Res 62(18): 5325-5335.
32. Campbell, I., et al. (2000). "Adenovirus-mediated p16INK4 gene transfer significantly suppresses human breast cancer growth." Cancer Gene Ther 7(9): 1270-1278.
33. Cancer Biology. http://www.cancerbio.net. 2019.
34. Cao, G., et al. (2001). "Adenovirus-mediated interferon-beta gene therapy suppresses growth and metastasis of human prostate cancer in nude mice." Cancer Gene Ther 8(7): 497-505.
35. Casey, G., et al. (1991). "Growth suppression of human breast cancer cells by the introduction of a wild-type p53 gene." Oncogene 6(10): 1791-1797.
36. Casey, G., et al. (1993). "Functional evidence for a breast cancer growth suppressor gene on chromosome 17." Hum Mol Genet 2(11): 1921-1927.
37. Celinski, S. A., et al. (2003). "Somatostatin receptor gene transfer inhibits established pancreatic cancer xenografts." J Surg Res 115(1): 41-47.
38. Chan, I., et al. (2008). "Progression elevated gene-3 promoter (PEG-Prom) confers cancer cell selectivity to human polynucleotide phosphorylase (hPNPase (old-35))-mediated growth suppression." J Cell Physiol 215(2): 401-409.
39. Chen, C., et al. (2002). "[The effects of C2 gene on growth of gastric cancer cells]." Zhonghua Nei Ke Za Zhi 41(3): 149-152.
40. Chen, J., et al. (2000). "[The effects of RA538 and antisense c-myc on cervical cancer cell lines with high expression of bcl-2 gene]." Zhonghua Zhong Liu Za Zhi 22(4): 279-282.
41. Chen, L., et al. (1996). "Sensitization of human breast cancer cells to cyclophosphamide and ifosfamide by transfer of a liver cytochrome P450 gene." Cancer Res 56(6): 1331-1340.
42. Chen, M., et al. (2009). "CCDC62/ERAP75 functions as a coactivator to enhance estrogen receptor beta-mediated transactivation and target gene expression in prostate cancer cells." Carcinogenesis 30(5): 841-850.
43. Chen, S. M., et al. (2010). "RNA interference-mediated silencing of UBCH10 gene inhibits colorectal cancer cell growth in vitro and in vivo." Clin Exp Pharmacol Physiol 37(5-6): 525-529.
44. Chen, W., et al. (2003). "Retroviral endostatin gene transfer inhibits human colon cancer cell growth in vivo." Chin Med J (Engl) 116(10): 1582-1584.
45. Chen, Y., et al. (2007). "Homeobox gene HOP has a potential tumor suppressive activity in human lung cancer." Int J Cancer 121(5): 1021-1027.
46. Cheng, Y., et al. (2011). "Evaluation of PPP2R2A as a prostate cancer susceptibility gene: a comprehensive germline and somatic study." Cancer Genet 204(7): 375-381.
47. Chitambar, C. R., et al. (2006). "Expression of the hemochromatosis gene modulates the cytotoxicity of doxorubicin in breast cancer cells." Int J Cancer 119(9): 2200-2204.
48. Cho-Chung, Y. S. (1996). "Protein kinase A-directed antisense restrains cancer growth: sequence-specific inhibition of gene expression." Antisense Nucleic Acid Drug Dev 6(3): 237-244.
49. Chu, I., et al. (2005). "The dual ErbB1/ErbB2 inhibitor, lapatinib (GW572016), cooperates with tamoxifen to inhibit both cell proliferation- and estrogen-dependent gene expression in antiestrogen-resistant breast cancer." Cancer Res 65(1): 18-25.
50. Chung, B. H., et al. (2001). "Effects of docosahexaenoic acid and eicosapentaenoic acid on androgen-mediated cell growth and gene expression in LNCaP prostate cancer cells." Carcinogenesis 22(8): 1201-1206.
51. Church, J. G., et al. (1989). "Activation of the Na+/H+ antiport is not required for epidermal growth factor-dependent gene expression, growth inhibition or proliferation in human breast cancer cells." Biochem J 257(1): 151-157.
52. Cinar, B., et al. (2001). "Androgen receptor mediates the reduced tumor growth, enhanced androgen responsiveness, and selected target gene transactivation in a human prostate cancer cell line." Cancer Res 61(19): 7310-7317.
53. Cogoi, S., et al. (2013). "Guanidino anthrathiophenediones as G-quadruplex binders: uptake, intracellular localization, and anti-Harvey-Ras gene activity in bladder cancer cells." J Med Chem 56(7): 2764-2778.
54. Collinet, P., et al. (2006). "In vivo expression and antitumor activity of p53 gene transfer with naked plasmid DNA in an ovarian cancer xenograft model in nude mice." J Obstet Gynaecol Res 32(5): 449-453.
55. Connolly, J. M. and D. P. Rose (1998). "Enhanced angiogenesis and growth of 12-lipoxygenase gene-transfected MCF-7 human breast cancer cells in athymic nude mice." Cancer Lett 132(1-2): 107-112.
56. Copper, M. P., et al. (1997). "All-trans retinoic acid induced gene expression and growth inhibition in head and neck cancer cell lines." Oral Oncol 33(4): 270-274.
57. Creighton, C., et al. (2003). "Profiling of pathway-specific changes in gene expression following growth of human cancer cell lines transplanted into mice." Genome Biol 4(7): R46.
58. Dai, C., et al. (2015). "Transcriptional activation of human CDCA8 gene regulated by transcription factor NF-Y in embryonic stem cells and cancer cells." J Biol Chem 290(37): 22423-22434.
59. Damon, S. E., et al. (2001). "Transcriptional regulation of insulin-like growth factor-I receptor gene expression in prostate cancer cells." Endocrinology 142(1): 21-27.
60. Davidson, A., et al. (1996). "Regulation of VIP gene expression in general. Human lung cancer cells in particular." J Mol Neurosci 7(2): 99-110.
61. Davies, H., et al. (2002). "Mutations of the BRAF gene in human cancer." Nature 417(6892): 949-954.
62. De Schrijver, E., et al. (2003). "RNA interference-mediated silencing of the fatty acid synthase gene attenuates growth and induces morphological changes and apoptosis of LNCaP prostate cancer cells." Cancer Res 63(13): 3799-3804.
63. Deftos, L. J. (1998). "Granin-A, parathyroid hormone-related protein, and calcitonin gene products in neuroendocrine prostate cancer." Prostate Suppl 8: 23-31.
64. DeYoung, M. P., et al. (2003). "Down's syndrome-associated Single Minded 2 gene as a pancreatic cancer drug therapy target." Cancer Lett 200(1): 25-31.
65. Dirican, E., et al. (2016). "Mutation distributions and clinical correlations of PIK3CA gene mutations in breast cancer." Tumour Biol 37(6): 7033-7045.

66. D'Orazi, G., et al. (2000). "Exogenous wt-p53 protein is active in transformed cells but not in their non-transformed counterparts: implications for cancer gene therapy without tumor targeting." J Gene Med 2(1): 11-21.
67. Draus, J. M., et al. (2001). "p53 gene transfer does not enhance E2F-1-mediated apoptosis in human colon cancer cells." Exp Mol Med 33(4): 209-219.
68. Duan, R., et al. (2002). "Estrogen regulation of c-fos gene expression through phosphatidylinositol-3-kinase-dependent activation of serum response factor in MCF-7 breast cancer cells." Biochem Biophys Res Commun 294(2): 384-394.
69. Duxbury, M. S., et al. (2004). "Systemic siRNA-mediated gene silencing: a new approach to targeted therapy of cancer." Ann Surg 240(4): 667-674; discussion 675-666.
70. Eastham, J. A., et al. (1995). "In vivo gene therapy with p53 or p21 adenovirus for prostate cancer." Cancer Res 55(22): 5151-5155.
71. Edamura, K., et al. (2007). "Adenovirus-mediated REIC/Dkk-3 gene transfer inhibits tumor growth and metastasis in an orthotopic prostate cancer model." Cancer Gene Ther 14(9): 765-772.
72. Eggen, T., et al. (2012). "Increased gene expression of the ABCC5 transporter without distinct changes in the expression of PDE5 in human cervical cancer cells during growth." Anticancer Res 32(8): 3055-3061.
73. Elledge, R. M. and D. C. Allred (1994). "The p53 tumor suppressor gene in breast cancer." Breast Cancer Res Treat 32(1): 39-47.
74. Ellen, T. P., et al. (2008). "NDRG1, a growth and cancer related gene: regulation of gene expression and function in normal and disease states." Carcinogenesis 29(1): 2-8.
75. Elshami, A. A., et al. (1995). "The role of immunosuppression in the efficacy of cancer gene therapy using adenovirus transfer of the herpes simplex thymidine kinase gene." Ann Surg 222(3): 298-307; 307-210.
76. Evoy, D., et al. (1997). "In vivo adenoviral-mediated gene transfer in the treatment of pancreatic cancer." J Surg Res 69(1): 226-231.
77. Ezawa, I., et al. (2016). "Novel p53 target gene FUCA1 encodes a fucosidase and regulates growth and survival of cancer cells." Cancer Sci 107(6): 734-745.
78. Falzon, M. and J. Zong (1998). "The noncalcemic vitamin D analogs EB1089 and 22-oxacalcitriol suppress serum-induced parathyroid hormone-related peptide gene expression in a lung cancer cell line." Endocrinology 139(3): 1046-1053.
79. Fan, M., et al. (2006). "Diverse gene expression and DNA methylation profiles correlate with differential adaptation of breast cancer cells to the antiestrogens tamoxifen and fulvestrant." Cancer Res 66(24): 11954-11966.
80. Fazeli, A., et al. (1997). "Phenotype of mice lacking functional Deleted in colorectal cancer (Dcc) gene." Nature 386(6627): 796-804.
81. Fazzone, W., et al. (2009). "Histone deacetylase inhibitors suppress thymidylate synthase gene expression and synergize with the fluoropyrimidines in colon cancer cells." Int J Cancer 125(2): 463-473.
82. Feldman, A. L., et al. (2000). "Antiangiogenic gene therapy of cancer utilizing a recombinant adenovirus to elevate systemic endostatin levels in mice." Cancer Res 60(6): 1503-1506.
83. Feldman, R. J., et al. (2003). "Pdef expression in human breast cancer is correlated with invasive potential and altered gene expression." Cancer Res 63(15): 4626-4631.
84. Filmus, J., et al. (1985). "MDA-468, a human breast cancer cell line with a high number of epidermal growth factor (EGF) receptors, has an amplified EGF receptor gene and is growth inhibited by EGF." Biochem Biophys Res Commun 128(2): 898-905.
85. Filmus, J., et al. (1987). "Epidermal growth factor receptor gene-amplified MDA-468 breast cancer cell line and its nonamplified variants." Mol Cell Biol 7(1): 251-257.
86. Fisher, W. E., et al. (1996). "Expression of the somatostatin receptor subtype-2 gene predicts response of human pancreatic cancer to octreotide." Surgery 120(2): 234-240; discussion 240-231.
87. Fisher, W. E., et al. (2002). "Somatostatin receptor subtype 2 gene therapy inhibits pancreatic cancer in vitro." J Surg Res 105(1): 58-64.
88. Forsti, A., et al. (2003). "Polymorphisms in the estrogen receptor beta gene and risk of breast cancer: no association." Breast Cancer Res Treat 79(3): 409-413.
89. Franco, O. E., et al. (2003). "Phenylacetate inhibits growth and modulates cell cycle gene expression in renal cancer cell lines." Anticancer Res 23(2B): 1637-1642.
90. Frandsen, T. L., et al. (2001). "Direct evidence of the importance of stromal urokinase plasminogen activator (uPA) in the growth of an experimental human breast cancer using a combined uPA gene-disrupted and immunodeficient xenograft model." Cancer Res 61(2): 532-537.
91. Frasor, J., et al. (2003). "Profiling of estrogen up- and down-regulated gene expression in human breast cancer cells: insights into gene networks and pathways underlying estrogenic control of proliferation and cell phenotype." Endocrinology 144(10): 4562-4574.
92. Frebourg, T., et al. (1992). "Germ-line mutations of the p53 tumor suppressor gene in patients with high risk for cancer inactivate the p53 protein." Proc Natl Acad Sci U S A 89(14): 6413-6417.
93. Fredlund, E., et al. (2012). "The gene expression landscape of breast cancer is shaped by tumor protein p53 status and epithelial-mesenchymal transition." Breast Cancer Res 14(4): R113.
94. Fromigue, O., et al. (2003). "Gene expression profiling of normal human pulmonary fibroblasts following coculture with non-small-cell lung cancer cells reveals alterations related to matrix degradation, angiogenesis, cell growth and survival." Oncogene 22(52): 8487-8497.
95. Fukushima, M., et al. (2007). "Combination of non-viral connexin 43 gene therapy and docetaxel inhibits the growth of human prostate cancer in mice." Int J Oncol 30(1): 225-231.
96. Gao, A. C., et al. (2000). "Enhanced GBX2 expression stimulates growth of human prostate cancer cells via transcriptional up-regulation of the interleukin 6 gene." Clin Cancer Res 6(2): 493-497.
97. Gao, N., et al. (2001). "[Transfection of wild-type p14ARF gene leads to growth inhibition of human lung cancer cell lines]." Zhongguo Fei Ai Za Zhi 4(1): 15-19.
98. Gao, N., et al. (2001). "The exogenous wild-type p14ARF gene induces growth arrest and promotes radiosensitivity in human lung cancer cell lines." J Cancer Res Clin Oncol 127(6): 359-367.
99. Garcia-Olmo, D. C., et al. (2008). "Loss of a reporter gene for green fluorescent protein during tumor progression suggests the recruitment of host cells in rats with experimentally induced colon cancer." Histol Histopathol 23(10): 1205-1211.
100. Gattoni-Celli, S., et al. (1988). "Partial suppression of anchorage-independent growth and tumorigenicity in immunodeficient mice by transfection of the H-2 class I gene H-2Ld into a human colon cancer cell line (HCT)." Proc Natl Acad Sci U S A 85(22): 8543-8547.
101. Ge, Y., et al. (2002). "Effects of adenoviral gene transfer of C. elegans n-3 fatty acid desaturase on the lipid profile and growth of human breast cancer cells." Anticancer Res 22(2A): 537-543.
102. Geisen, C., et al. (2000). "Growth inhibition of cervical cancer cells by the human retinoic acid receptor beta gene." Int J Cancer 85(2): 289-295.

103. Gery, S., et al. (2002). "TMEFF2 is an androgen-regulated gene exhibiting antiproliferative effects in prostate cancer cells." Oncogene 21(31): 4739-4746.
104. Gery, S., et al. (2006). "The circadian gene per1 plays an important role in cell growth and DNA damage control in human cancer cells." Mol Cell 22(3): 375-382.
105. Glondu, M., et al. (2002). "Down-regulation of cathepsin-D expression by antisense gene transfer inhibits tumor growth and experimental lung metastasis of human breast cancer cells." Oncogene 21(33): 5127-5134.
106. Goodison, S., et al. (2007). "Exogenous mycoplasmal p37 protein alters gene expression, growth and morphology of prostate cancer cells." Cytogenet Genome Res 118(2-4): 204-213.
107. Google. http://www.google.com. 2019.
108. Gope, R. and M. L. Gope (1992). "Abundance and state of phosphorylation of the retinoblastoma susceptibility gene product in human colon cancer." Mol Cell Biochem 110(2): 123-133.
109. Gordon, E. M., et al. (2018). "Cell cycle checkpoint control: The cyclin G1/Mdm2/p53 axis emerges as a strategic target for broad-spectrum cancer gene therapy - A review of molecular mechanisms for oncologists." Mol Clin Oncol 9(2): 115-134.
110. Goto, H., et al. (2001). "Gene therapy utilizing the Cre/loxP system selectively suppresses tumor growth of disseminated carcinoembryonic antigen-producing cancer cells." Int J Cancer 94(3): 414-419.
111. Greco, E., et al. (2002). "Retrovirus-mediated herpes simplex virus thymidine kinase gene transfer in pancreatic cancer cell lines: an incomplete antitumor effect." Pancreas 25(2): e21-29.
112. Grzmil, M., et al. (2004). "Up-regulated expression of the MAT-8 gene in prostate cancer and its siRNA-mediated inhibition of expression induces a decrease in proliferation of human prostate carcinoma cells." Int J Oncol 24(1): 97-105.
113. Guan, X., et al. (2012). "[Effect of CDK2-AP1 gene over-expression on proliferation and cell cycle regulation of breast cancer cell line MCF-7]." Zhong Nan Da Xue Xue Bao Yi Xue Ban 37(10): 990-996.
114. Guimaraes, D. A. B., et al. (2017). "Pitaya Extracts Induce Growth Inhibition and Proapoptotic Effects on Human Cell Lines of Breast Cancer via Downregulation of Estrogen Receptor Gene Expression." Oxid Med Cell Longev 2017: 7865073.
115. Guo-Chang, F. and W. Chu-Tse (2000). "Transfer of p14ARF gene in drug-resistant human breast cancer MCF-7/Adr cells inhibits proliferation and reduces doxorubicin resistance." Cancer Lett 158(2): 203-210.
116. Guzey, M., et al. (2004). "Vitamin D3 modulated gene expression patterns in human primary normal and cancer prostate cells." J Cell Biochem 93(2): 271-285.
117. Hamada, K., et al. (1996). "Adenovirus-mediated transfer of a wild-type p53 gene and induction of apoptosis in cervical cancer." Cancer Res 56(13): 3047-3054.
118. Hamaguchi, M., et al. (2002). "DBC2, a candidate for a tumor suppressor gene involved in breast cancer." Proc Natl Acad Sci U S A 99(21): 13647-13652.
119. Hammamieh, R., et al. (2007). "Differential effects of omega-3 and omega-6 Fatty acids on gene expression in breast cancer cells." Breast Cancer Res Treat 101(1): 7-16.
120. Han, Y., et al. (2007). "The zinc finger domain of Wilms' tumor 1 suppressor gene (WT1) behaves as a dominant negative, leading to abrogation of WT1 oncogenic potential in breast cancer cells." Breast Cancer Res 9(4): R43.
121. Hang, Y., et al. (2005). "Suppression of gastric cancer growth by adenovirus-mediated transfer of the PTEN gene." World J Gastroenterol 11(15): 2224-2229.
122. Hausner, P., et al. (1999). "The "comparative growth assay": examining the interplay of anti-cancer agents with cells carrying single gene alterations." Neoplasia 1(4): 356-367.
123. Haussler, O., et al. (1999). "Cell proliferation, apoptosis, oncogene, and tumor suppressor gene status in adenosis with comparison to benign prostatic hyperplasia, prostatic intraepithelial neoplasia, and cancer." Hum Pathol 30(9): 1077-1086.
124. Hayashi, S., et al. (1997). "Inhibition of establishment of hepatic metastasis in mice by combination gene therapy using both herpes simplex virus-thymidine kinase and granulocyte macrophage-colony stimulating factor genes in murine colon cancer." Cancer Gene Ther 4(6): 339-344.
125. Hayashida, Y., et al. (2005). "PPP1R3 gene (protein phosphatase 1) alterations in colorectal cancer and its relationship to metastasis." Oncol Rep 13(6): 1223-1227.
126. He, C., et al. (2002). "[Action of apoptosis-induced ligand gene in relation to tumor necrosis factor on human colon cancer cell line HT29]." Zhonghua Zhong Liu Za Zhi 24(2): 133-136.
127. He, D., et al. (2003). "Overexpression of the promyelocytic leukemia gene suppresses growth of human bladder cancer cells by inducing G1 cell cycle arrest and apoptosis." Chin Med J (Engl) 116(9): 1394-1398.
128. He, X. H., et al. (2003). "[Expression of human novel gene CT120 in lung cancer and its effects on cell growth]." Ai Zheng 22(2): 113-118.
129. Hochscheid, R., et al. (2000). "Transfection of human insulin-like growth factor-binding protein 3 gene inhibits cell growth and tumorigenicity: a cell culture model for lung cancer." J Endocrinol 166(3): 553-563.
130. Hong, L., et al. (2004). "Suppression of the cell proliferation in stomach cancer cells by the ZNRD1 gene." Biochem Biophys Res Commun 321(3): 611-616.
131. Hoshida, T., et al. (2002). "Gene therapy for pancreatic cancer using an adenovirus vector encoding soluble flt-1 vascular endothelial growth factor receptor." Pancreas 25(2): 111-121.
132. Hoshiya, Y., et al. (2003). "Mullerian inhibiting substance promotes interferon gamma-induced gene expression and apoptosis in breast cancer cells." J Biol Chem 278(51): 51703-51712.
133. Hosoi, F., et al. (2009). "N-myc downstream regulated gene 1/Cap43 suppresses tumor growth and angiogenesis of pancreatic cancer through attenuation of inhibitor of kappaB kinase beta expression." Cancer Res 69(12): 4983-4991.
134. Hsieh, T. C. and J. M. Wu (2001). "Cell growth and gene modulatory activities of Yunzhi (Windsor Wunxi) from mushroom Trametes versicolor in androgen-dependent and androgen-insensitive human prostate cancer cells." Int J Oncol 18(1): 81-88.
135. Huang, A., et al. (2014). "Circadian clock gene expression regulates cancer cell growth through glutaminase." Acta Biochim Biophys Sin (Shanghai) 46(5): 409-414.
136. Huang, E. Y., et al. (2001). "Genomic structure, chromosomal localization and expression profile of a novel melanoma differentiation associated (mda-7) gene with cancer specific growth suppressing and apoptosis inducing properties." Oncogene 20(48): 7051-7063.
137. Huang, H. J., et al. (1988). "Suppression of the neoplastic phenotype by replacement of the RB gene in human cancer cells." Science 242(4885): 1563-1566.
138. Huang, Z., et al. (2000). "Impact of liver P450 reductase suppression on cyclophosphamide activation, pharmacokinetics and antitumoral activity in a cytochrome P450-based cancer gene therapy model." Cancer Gene Ther 7(7): 1034-1042.
139. Hwang, J. Y., et al. (1992). "Tamoxifen stimulates human papillomavirus type 16 gene expression and cell proliferation in a cervical cancer cell line." Cancer Res 52(24): 6848-6852.
140. Hwang, R. F., et al. (1998). "Gene therapy for primary and metastatic pancreatic cancer with intraperitoneal retroviral

vector bearing the wild-type p53 gene." Surgery 124(2): 143-150; discussion 150-141.
141. Iejima, D., et al. (2010). "FRS2beta, a potential prognostic gene for non-small cell lung cancer, encodes a feedback inhibitor of EGF receptor family members by ERK binding." Oncogene 29(21): 3087-3099.
142. Inaba, Y., et al. (2003). "Gene transfer of alpha1,3-fucosyltransferase increases tumor growth of the PC-3 human prostate cancer cell line through enhanced adhesion to prostatic stromal cells." Int J Cancer 107(6): 949-957.
143. Inoue, K., et al. (2000). "Gene therapy of human bladder cancer with adenovirus-mediated antisense basic fibroblast growth factor." Clin Cancer Res 6(11): 4422-4431.
144. Inoue, K., et al. (2001). "Adenoviral-mediated gene therapy of human bladder cancer with antisense interleukin-8." Oncol Rep 8(5): 955-964.
145. Inoue, R., et al. (2006). "Gefitinib-related gene signature in bladder cancer cells identified by a cDNA microarray." Anticancer Res 26(6B): 4195-4202.
146. Inui, M., et al. (1996). "Enhanced gene expression of transforming growth factor-alpha and c-met in rat urinary bladder cancer." Urol Res 24(1): 55-60.
147. Ishii, H., et al. (2001). "Effect of adenoviral transduction of the fragile histidine triad gene into esophageal cancer cells." Cancer Res 61(4): 1578-1584.
148. Ishii, H., et al. (2001). "FEZ1/LZTS1 gene at 8p22 suppresses cancer cell growth and regulates mitosis." Proc Natl Acad Sci U S A 98(18): 10374-10379.
149. Itoh, Y., et al. (1995). "Characterization of tumor-necrosis-factor-gene-transduced tumor-infiltrating lymphocytes from ascitic fluid of cancer patients: analysis of cytolytic activity, growth rate, adhesion molecule expression and cytokine production." Cancer Immunol Immunother 40(2): 95-102.
150. Jeng, M. H., et al. (1994). "Paradoxical regulation of estrogen-dependent growth factor gene expression in estrogen receptor (ER)-negative human breast cancer cells stably expressing ER." Cancer Lett 82(2): 123-128.
151. Jiang, H., et al. (1996). "The melanoma differentiation associated gene mda-7 suppresses cancer cell growth." Proc Natl Acad Sci U S A 93(17): 9160-9165.
152. Jin, C., et al. (2016). "Safe engineering of CAR T cells for adoptive cell therapy of cancer using long-term episomal gene transfer." EMBO Mol Med 8(7): 702-711.
153. Johnson, M. D., et al. (1989). "Oestrogenic activity of tamoxifen and its metabolites on gene regulation and cell proliferation in MCF-7 breast cancer cells." Br J Cancer 59(5): 727-738.
154. Joshi, U. S., et al. (1998). "Inhibition of tumor cell growth by p21WAF1 adenoviral gene transfer in lung cancer." Cancer Gene Ther 5(3): 183-191.
155. Jounaidi, Y. and D. J. Waxman (2000). "Combination of the bioreductive drug tirapazamine with the chemotherapeutic prodrug cyclophosphamide for P450/P450-reductase-based cancer gene therapy." Cancer Res 60(14): 3761-3769.
156. Journal of American Science. http://www.jofamericanscience.org. 2019.
157. Ju, Y. H., et al. (2000). "Estrogenic effects of extracts from cabbage, fermented cabbage, and acidified brussels sprouts on growth and gene expression of estrogen-dependent human breast cancer (MCF-7) cells." J Agric Food Chem 48(10): 4628-4634.
158. Kagawa, S., et al. (1999). "Overexpression of the p21 sdi1 gene induces senescence-like state in human cancer cells: implication for senescence-directed molecular therapy for cancer." Cell Death Differ 6(8): 765-772.
159. Kaneko, H., et al. (1998). "Involvement of apoptosis and cyclin D1 gene repression in growth inhibition of T-47D human breast cancer cells by methylglyoxal bis (cyclopentylamidinohydrazone)." Int J Mol Med 1(6): 931-936.
160. Kanemitsu, N., et al. (2001). "Correlation between induction of the mac25 gene and anti-proliferative effects of 1alpha,25(OH)2-D3 on breast cancer and leukemic cells." Int J Mol Med 7(5): 515-520.
161. Kang, S. K., et al. (2001). "Estradiol regulates gonadotropin-releasing hormone (GnRH) and its receptor gene expression and antagonizes the growth inhibitory effects of GnRH in human ovarian surface epithelial and ovarian cancer cells." Endocrinology 142(2): 580-588.
162. Kawabe, S., et al. (2001). "Adenovirus-mediated wild-type p53 gene expression radiosensitizes non-small cell lung cancer cells but not normal lung fibroblasts." Int J Radiat Biol 77(2): 185-194.
163. Kawakami, Y., et al. (2001). "Adenovirus-mediated p16 gene transfer changes the sensitivity to taxanes and Vinca alkaloids of human ovarian cancer cells." Anticancer Res 21(4A): 2537-2545.
164. Kazmi, S. M., et al. (1996). "Comparison of N-(4-hydroxyphenyl)retinamide and all-trans-retinoic acid in the regulation of retinoid receptor-mediated gene expression in human breast cancer cell lines." Cancer Res 56(5): 1056-1062.
165. Kebebew, E., et al. (2004). "Id1 gene expression is up-regulated in hyperplastic and neoplastic thyroid tissue and regulates growth and differentiation in thyroid cancer cells." J Clin Endocrinol Metab 89(12): 6105-6111.
166. Kelley, J. R., et al. (2000). "The cancer-associated Sm-like oncogene: a novel target for the gene therapy of pancreatic cancer." Surgery 128(2): 353-360.
167. Kelley, J. R., et al. (2001). "CaSm/gemcitabine chemo-gene therapy leads to prolonged survival in a murine model of pancreatic cancer." Surgery 130(2): 280-288.
168. Khalighinejad, N., et al. (2008). "Adenoviral gene therapy in gastric cancer: a review." World J Gastroenterol 14(2): 180-184.
169. Kim, C. K., et al. (2003). "Enhanced p53 gene transfer to human ovarian cancer cells using the cationic nonviral vector, DDC." Gynecol Oncol 90(2): 265-272.
170. Kim, I. Y., et al. (1996). "Genetic change in transforming growth factor beta (TGF-beta) receptor type I gene correlates with insensitivity to TGF-beta 1 in human prostate cancer cells." Cancer Res 56(1): 44-48.
171. Kim, M. H., et al. (2009). "C/EBPbeta regulates metastatic gene expression and confers TNF-alpha resistance to prostate cancer cells." Prostate 69(13): 1435-1447.
172. Kim, S. B., et al. (2001). "Growth inhibition and chemosensitivity of poorly differentiated human thyroid cancer cell line (NPA) transfected with p53 gene." Head Neck 23(3): 223-229.
173. Kim, S., et al. (2004). "A screen for genes that suppress loss of contact inhibition: identification of ING4 as a candidate tumor suppressor gene in human cancer." Proc Natl Acad Sci U S A 101(46): 16251-16256.
174. Kimura, M., et al. (1998). "Loss of tumorigenicity of human pancreatic carcinoma cells engineered to produce interleukin-2 or interleukin-4 in nude mice: a potentiality for cancer gene therapy." Cancer Lett 128(1): 47-53.
175. Kito, M., et al. (1999). "Induction of apoptosis in cultured colon cancer cells by transfection with human interferon beta gene." Biochem Biophys Res Commun 257(3): 771-776.
176. Kleinerman, D. I., et al. (1996). "Suppression of human bladder cancer growth by increased expression of C-CAM1 gene in an orthotopic model." Cancer Res 56(15): 3431-3435.
177. Kloth, J. N., et al. (2005). "Substantial changes in gene expression of Wnt, MAPK and TNFalpha pathways induced by TGF-beta1 in cervical cancer cell lines." Carcinogenesis 26(9): 1493-1502.
178. Kochetkova, M., et al. (2002). "CBFA2T3 (MTG16) is a putative breast tumor suppressor gene from the breast cancer loss of heterozygosity region at 16q24.3." Cancer Res 62(16): 4599-4604.

179. Kojima, A., et al. (1998). "Reversal of CPT-11 resistance of lung cancer cells by adenovirus-mediated gene transfer of the human carboxylesterase cDNA." Cancer Res 58(19): 4368-4374.
180. Kondo, M., et al. (2001). "Overexpression of candidate tumor suppressor gene FUS1 isolated from the 3p21.3 homozygous deletion region leads to G1 arrest and growth inhibition of lung cancer cells." Oncogene 20(43): 6258-6262.
181. Kresty, L. A., et al. (2011). "Cranberry proanthocyanidins mediate growth arrest of lung cancer cells through modulation of gene expression and rapid induction of apoptosis." Molecules 16(3): 2375-2390.
182. Krishnan, A. V., et al. (2003). "Inhibition of prostate cancer growth by vitamin D: Regulation of target gene expression." J Cell Biochem 88(2): 363-371.
183. Krishnan, A. V., et al. (2004). "Analysis of vitamin D-regulated gene expression in LNCaP human prostate cancer cells using cDNA microarrays." Prostate 59(3): 243-251.
184. Kruzelock, R. P., et al. (2000). "Functional evidence for an ovarian cancer tumor suppressor gene on chromosome 22 by microcell-mediated chromosome transfer." Oncogene 19(54): 6277-6285.
185. Kubota, Y., et al. (1991). "[Study of c-myc gene transfected T-24 human bladder cancer cells]." Nihon Hinyokika Gakkai Zasshi 82(4): 593-599.
186. Kuhn, H., et al. (2002). "Adenovirus-mediated E2F-1 gene transfer in nonsmall-cell lung cancer induces cell growth arrest and apoptosis." Eur Respir J 20(3): 703-709.
187. Kumagai, T., et al. (1996). "Eradication of Myc-overexpressing small cell lung cancer cells transfected with herpes simplex virus thymidine kinase gene containing Myc-Max response elements." Cancer Res 56(2): 354-358.
188. Kusumoto, M., et al. (1999). "Adenovirus-mediated p53 gene transduction inhibits telomerase activity independent of its effects on cell cycle arrest and apoptosis in human pancreatic cancer cells." Clin Cancer Res 5(8): 2140-2147.
189. Lambert, J. R., et al. (2006). "Prostate derived factor in human prostate cancer cells: gene induction by vitamin D via a p53-dependent mechanism and inhibition of prostate cancer cell growth." J Cell Physiol 208(3): 566-574.
190. Lebedeva, I. V., et al. (2002). "The cancer growth suppressing gene mda-7 induces apoptosis selectively in human melanoma cells." Oncogene 21(5): 708-718.
191. Lee, A. V., et al. (1997). "Activation of estrogen receptor-mediated gene transcription by IGF-I in human breast cancer cells." J Endocrinol 152(1): 39-47.
192. Lee, C. H., et al. (1996). "Prostate-specific antigen promoter driven gene therapy targeting DNA polymerase-alpha and topoisomerase II alpha in prostate cancer." Anticancer Res 16(4A): 1805-1811.
193. Lee, J. H., et al. (1998). "The inhibitory effect of adenovirus-mediated p16INK4a gene transfer on the proliferation of lung cancer cell line." Anticancer Res 18(5A): 3257-3261.
194. Lee, W. H. and E. Y. Lee (1997). "[The retinoblastoma gene: from its basic understanding as a signal mediator for growth and differentiation to its use in the treatment of cancer]." Gan To Kagaku Ryoho 24(11): 1368-1380.
195. Leung, W. K., et al. (2004). "Effect of peroxisome proliferator activated receptor gamma ligands on growth and gene expression profiles of gastric cancer cells." Gut 53(3): 331-338.
196. Life Science Journal. http://www.lifesciencesite.com. 2019.
197. Lilja, J. F., et al. (2001). "Growth suppression activity of the PTEN tumor suppressor gene in human endometrial cancer cells." Anticancer Res 21(3B): 1969-1974.
198. Lindner, D. J., et al. (1997). "Tamoxifen enhances interferon-regulated gene expression in breast cancer cells." Mol Cell Biochem 167(1-2): 169-177.
199. Lipponen, P. K. and T. J. Liukkonen (1995). "Reduced expression of retinoblastoma (Rb) gene protein is related to cell proliferation and prognosis in transitional-cell bladder cancer." J Cancer Res Clin Oncol 121(1): 44-50.
200. Liu, J. C., et al. (2012). "Seventeen-gene signature from enriched Her2/Neu mammary tumor-initiating cells predicts clinical outcome for human HER2+:ERalpha- breast cancer." Proc Natl Acad Sci U S A 109(15): 5832-5837.
201. Lopez-Lazaro, M. (2018). "Cancer etiology: Variation in cancer risk among tissues is poorly explained by the number of gene mutations." Genes Chromosomes Cancer 57(6): 281-293.
202. Luparello, C., et al. (2003). "T47-D cells and type V collagen: a model for the study of apoptotic gene expression by breast cancer cells." Biol Chem 384(6): 965-975.
203. Ma H, Chen G. Stem cell. The Journal of American Science 2005;1(2):90-92. doi:10.7537/marsjas010205.14. http://www.jofamericanscience.org/journals/am-sci/0102/14-mahongbao.pdf.
204. Ma H, Cherng S. Eternal Life and Stem Cell. Nature and Science. 2007;5(1):81-96. doi:10.7537/marsnsj050107.10. http://www.sciencepub.net/nature/0501/10-0247-mahongbao-eternal-ns.pdf.
205. Ma H, Cherng S. Nature of Life. Life Science Journal 2005;2(1):7-15. doi:10.7537/marslsj020105.03. http://www.lifesciencesite.com/lsj/life0201/life-0201-03.pdf.
206. Ma H, Yang Y. Turritopsis nutricula. Nature and Science 2010;8(2):15-20. doi:10.7537/marsnsj080210.03. http://www.sciencepub.net/nature/ns0802/03_1279_hongbao_turritopsis_ns0802_15_20.pdf.
207. Ma H. The Nature of Time and Space. Nature and science 2003;1(1):1-11. doi:10.7537/marsnsj010103.01. http://www.sciencepub.net/nature/0101/01-ma.pdf.
208. Maemondo, M., et al. (2004). "Gene therapy with secretory leukoprotease inhibitor promoter-controlled replication-competent adenovirus for non-small cell lung cancer." Cancer Res 64(13): 4611-4620.
209. Manabe, T., et al. (2003). "Cell-based protein delivery system for the inhibition of the growth of pancreatic cancer: NK4 gene-transduced oral mucosal epithelial cell sheet." Clin Cancer Res 9(8): 3158-3166.
210. Manjeshwar, S., et al. (2003). "Tumor suppression by the prohibitin gene 3'untranslated region RNA in human breast cancer." Cancer Res 63(17): 5251-5256.
211. Margueron, R., et al. (2003). "Oestrogen receptor alpha increases p21(WAF1/CIP1) gene expression and the antiproliferative activity of histone deacetylase inhibitors in human breast cancer cells." J Endocrinol 179(1): 41-53.
212. Markowitz, S. D., et al. (1994). "A benign cultured colon adenoma bears three genetically altered colon cancer oncogenes, but progresses to tumorigenicity and transforming growth factor-beta independence without inactivating the p53 tumor suppressor gene." J Clin Invest 93(3): 1005-1013.
213. Marsland Press. http://www.sciencepub.net. 2019; http://www.sciencepub.org. 2019.
214. Matozaki, T., et al. (1992). "p53 gene mutations in human gastric cancer: wild-type p53 but not mutant p53 suppresses growth of human gastric cancer cells." Cancer Res 52(16): 4335-4341.
215. Matsushima-Nishiu, M., et al. (2001). "Growth and gene expression profile analyses of endometrial cancer cells expressing exogenous PTEN." Cancer Res 61(9): 3741-3749.
216. Maurice-Duelli, A., et al. (2004). "Enhanced cell growth inhibition following PTEN nonviral gene transfer using polyethylenimine and photochemical internalization in endometrial cancer cells." Technol Cancer Res Treat 3(5): 459-465.
217. Mhashilkar, A. M., et al. (2001). "Melanoma differentiation associated gene-7 (mda-7): a novel anti-tumor gene for cancer gene therapy." Mol Med 7(4): 271-282.
218. Miki, K., et al. (2001). "Demethylation by 5-aza-2'-deoxycytidine (5-azadC) of p16INK4A gene results in

downregulation of vascular endothelial growth factor expression in human lung cancer cell lines." Oncol Res 12(8): 335-342.
219. Minaguchi, T., et al. (1999). "Growth suppression of human ovarian cancer cells by adenovirus-mediated transfer of the PTEN gene." Cancer Res 59(24): 6063-6067.
220. Mittal, R. D., et al. (2007). "Role of an androgen receptor gene polymorphism in development of hormone refractory prostate cancer in Indian population." Asian Pac J Cancer Prev 8(2): 275-278.
221. Miyake, H., et al. (2000). "Synergistic chemosensitization and inhibition of tumor growth and metastasis by adenovirus-mediated P53 gene transfer in human bladder cancer model." Urology 56(2): 332-336.
222. Morioka, C. Y., et al. (2005). "Suppression of invasion of a hamster pancreatic cancer cell line by antisense oligonucleotides mutation-matched to K-ras gene." In Vivo 19(3): 535-538.
223. Mu, Y. M., et al. (2003). "Human pituitary tumor transforming gene (hPTTG) inhibits human lung cancer A549 cell growth through activation of p21(WAF1/CIP1)." Endocr J 50(6): 771-781.
224. Muramaki, M., et al. (2003). "Introduction of midkine gene into human bladder cancer cells enhances their malignant phenotype but increases their sensitivity to antiangiogenic therapy." Clin Cancer Res 9(14): 5152-5160.
225. Murphy, L. C., et al. (1994). "Regulation of gene expression in T-47D human breast cancer cells by progestins and antiprogestins." Hum Reprod 9 Suppl 1: 174-180.
226. Nakano, K., et al. (1997). "Butyrate activates the WAF1/Cip1 gene promoter through Sp1 sites in a p53-negative human colon cancer cell line." J Biol Chem 272(35): 22199-22206.
227. Naruse, I., et al. (1998). "High concentrations of recombinant adenovirus expressing p16 gene induces apoptosis in lung cancer cell lines." Anticancer Res 18(6A): 4275-4282.
228. National Center for Biotechnology Information, U.S. National Library of Medicine. http://www.ncbi.nlm.nih.gov/pubmed. 2019.
229. Nature and Science. http://www.sciencepub.net/nature. 2019.
230. Nawa, A., et al. (2000). "Tumor metastasis-associated human MTA1 gene: its deduced protein sequence, localization, and association with breast cancer cell proliferation using antisense phosphorothioate oligonucleotides." J Cell Biochem 79(2): 202-212.
231. Ndisang, D., et al. (1999). "The Brn-3a transcription factor plays a critical role in regulating human papilloma virus gene expression and determining the growth characteristics of cervical cancer cells." J Biol Chem 274(40): 28521-28527.
232. Nesaretnam, K., et al. (2000). "Effect of a carotene concentrate on the growth of human breast cancer cells and pS2 gene expression." Toxicology 151(1-3): 117-126.
233. Nguyen, D. M., et al. (1997). "Delivery of the p53 tumor suppressor gene into lung cancer cells by an adenovirus/DNA complex." Cancer Gene Ther 4(3): 191-198.
234. Nicolson, G. L., et al. (2003). "Tumor metastasis-associated human MTA1 gene and its MTA1 protein product: role in epithelial cancer cell invasion, proliferation and nuclear regulation." Clin Exp Metastasis 20(1): 19-24.
235. Nielsen, L. L., et al. (1997). "Efficacy of p53 adenovirus-mediated gene therapy against human breast cancer xenografts." Cancer Gene Ther 4(2): 129-138.
236. Nishida, K., et al. (1996). "Introduction of the c-kit gene leads to growth suppression of a breast cancer cell line, MCF-7." Anticancer Res 16(6B): 3397-3402.
237. Nishino, K., et al. (2001). "Adenovirus-mediated gene therapy specific for small cell lung cancer cells using a Myc-Max binding motif." Int J Cancer 91(6): 851-856.
238. Nishioka, M., et al. (2002). "MYO18B, a candidate tumor suppressor gene at chromosome 22q12.1, deleted, mutated, and methylated in human lung cancer." Proc Natl Acad Sci U S A 99(19): 12269-12274.
239. Nishizaki, M., et al. (2001). "Synergistic inhibition of human lung cancer cell growth by adenovirus-mediated wild-type p53 gene transfer in combination with docetaxel and radiation therapeutics in vitro and in vivo." Clin Cancer Res 7(9): 2887-2897.
240. Noda, D., et al. (2006). "ELAC2, a putative prostate cancer susceptibility gene product, potentiates TGF-beta/Smad-induced growth arrest of prostate cells." Oncogene 25(41): 5591-5600.
241. Oakley, R., et al. (2002). "A preclinical model of minimal residual cancer in the muscle highlights challenges associated with adenovirus-mediated p53 gene transfer." Clin Cancer Res 8(6): 1984-1994.
242. O'Flanagan, C. H., et al. (2015). "The Parkinson's gene PINK1 regulates cell cycle progression and promotes cancer-associated phenotypes." Oncogene 34(11): 1363-1374.
243. Oh, J. J., et al. (2002). "A candidate tumor suppressor gene, H37, from the human lung cancer tumor suppressor locus 3p21.3." Cancer Res 62(11): 3207-3213.
244. Oki, T., et al. (2004). "Genistein induces Gadd45 gene and G2/M cell cycle arrest in the DU145 human prostate cancer cell line." FEBS Lett 577(1-2): 55-59.
245. Pagliuca, A., et al. (2013). "Analysis of the combined action of miR-143 and miR-145 on oncogenic pathways in colorectal cancer cells reveals a coordinate program of gene repression." Oncogene 32(40): 4806-4813.
246. Park, J., et al. (2001). "Msx1 gene overexpression induces G1 phase cell arrest in human ovarian cancer cell line OVCAR3." Biochem Biophys Res Commun 281(5): 1234-1240.
247. Pei, L. J., et al. (2017). "[Effect of triptolide on human oral cancer cell proliferation and PTEN gene mRNA expression in oral cancer]." Zhonghua Kou Qiang Yi Xue Za Zhi 52(1): 44-47.
248. Peterson, G. and S. Barnes (1991). "Genistein inhibition of the growth of human breast cancer cells: independence from estrogen receptors and the multi-drug resistance gene." Biochem Biophys Res Commun 179(1): 661-667.
249. Petrovics, G., et al. (2004). "Elevated expression of PCGEM1, a prostate-specific gene with cell growth-promoting function, is associated with high-risk prostate cancer patients." Oncogene 23(2): 605-611.
250. Pio, R., et al. (2004). "Alpha CP-4, encoded by a putative tumor suppressor gene at 3p21, but not its alternative splice variant alpha CP-4a, is underexpressed in lung cancer." Cancer Res 64(12): 4171-4179.
251. Prasad, K. A. and J. G. Church (1991). "EGF-dependent growth inhibition in MDA-468 human breast cancer cells is characterized by late G1 arrest and altered gene expression." Exp Cell Res 195(1): 20-26.
252. Qiu, S., et al. (2002). "[Study on the effects of combined IL-12 and GM-CSF gene therapy for murine liver cancer]." Zhonghua Gan Zang Bing Za Zhi 10(6): 413-416.
253. Qiu, Z., et al. (2007). "RNA interference-mediated signal transducers and activators of transcription 3 gene silencing inhibits invasion and metastasis of human pancreatic cancer cells." Cancer Sci 98(7): 1099-1106.
254. Ralhan, R., et al. (2000). "Association between polymorphism in p21(Waf1/Cip1) cyclin-dependent kinase inhibitor gene and human oral cancer." Clin Cancer Res 6(6): 2440-2447.
255. Ram, T. G., et al. (2000). "Blocking HER-2/HER-3 function with a dominant negative form of HER-3 in cells stimulated by heregulin and in breast cancer cells with HER-2 gene amplification." Cell Growth Differ 11(3): 173-183.
256. Ramondetta, L., et al. (2000). "Adenovirus-mediated expression of p53 or p21 in a papillary serous endometrial carcinoma cell line (SPEC-2) results in both growth inhibition and apoptotic cell death: potential application of gene therapy to endometrial cancer." Clin Cancer Res 6(1): 278-284.

257. Ranzani, G. N., et al. (1995). "p53 gene mutations and protein nuclear accumulation are early events in intestinal type gastric cancer but late events in diffuse type." Cancer Epidemiol Biomarkers Prev 4(3): 223-231.
258. Rao, G., et al. (2004). "Facilitating role of preprotachykinin-I gene in the integration of breast cancer cells within the stromal compartment of the bone marrow: a model of early cancer progression." Cancer Res 64(8): 2874-2881.
259. Reddy, M. K., et al. (1995). "Inhibitors of angiotensin-converting enzyme modulate mitosis and gene expression in pancreatic cancer cells." Proc Soc Exp Biol Med 210(3): 221-226.
260. Reinholz, M. M., et al. (2010). "Expression profiling of formalin-fixed paraffin-embedded primary breast tumors using cancer-specific and whole genome gene panels on the DASL (R) platform." BMC Med Genomics 3: 60.
261. Reyes, N., et al. (2004). "Effect of linoleic acid on proliferation and gene expression in the breast cancer cell line T47D." Cancer Lett 209(1): 25-35.
262. Rizk, N. P., et al. (1999). "The evaluation of adenoviral p53-mediated bystander effect in gene therapy of cancer." Cancer Gene Ther 6(4): 291-301.
263. Rocco, J. W., et al. (1998). "p16INK4A adenovirus-mediated gene therapy for human head and neck squamous cell cancer." Clin Cancer Res 4(7): 1697-1704.
264. Rohr, U. P., et al. (2003). "Non-small lung cancer cells are prime targets for p53 gene transfer mediated by a recombinant adeno-associated virus type-2 vector." Cancer Gene Ther 10(12): 898-906.
265. Rosell, R., et al. (1995). "Mutated K-ras gene analysis in a randomized trial of preoperative chemotherapy plus surgery versus surgery in stage IIIA non-small cell lung cancer." Lung Cancer 12 Suppl 1: S59-70.
266. Roth, J. A. (1998). "Gene replacement strategies for lung cancer." Curr Opin Oncol 10(2): 127-132.
267. Sadanandam, A., et al. (2010). "High gene expression of semaphorin 5A in pancreatic cancer is associated with tumor growth, invasion and metastasis." Int J Cancer 127(6): 1373-1383.
268. Saeki, T., et al. (2002). "Inhibition of human lung cancer growth following adenovirus-mediated mda-7 gene expression in vivo." Oncogene 21(29): 4558-4566.
269. Saimura, M., et al. (2002). "Intraperitoneal injection of adenovirus-mediated NK4 gene suppresses peritoneal dissemination of pancreatic cancer cell line AsPC-1 in nude mice." Cancer Gene Ther 9(10): 799-806.
270. Saito, Y., et al. (2003). "Adenovirus-mediated transfer of the PTEN gene inhibits human colorectal cancer growth in vitro and in vivo." Gene Ther 10(23): 1961-1969.
271. Sakakura, C., et al. (1995). "Inhibition of colon cancer cell proliferation by antisense oligonucleotides targeting the messenger RNA of the Ki-ras gene." Anticancer Drugs 6(4): 553-561.
272. Sakurada, A., et al. (1999). "Adenovirus-mediated delivery of the PTEN gene inhibits cell growth by induction of apoptosis in endometrial cancer." Int J Oncol 15(6): 1069-1074.
273. Sato, M., et al. (1997). "Induction of cyclin-dependent kinase inhibitor, p21WAF1, by treatment with 3,4-dihydro-6-[4-(3,4)-dimethoxybenzoyl)-1-piperazinyl]-2(1H)-quinoline (vesnarinone) in a human salivary cancer cell line with mutant p53 gene." Cancer Lett 112(2): 181-189.
274. Sauer, M. K. and I. L. Andrulis (2005). "Identification and characterization of missense alterations in the BRCA1 associated RING domain (BARD1) gene in breast and ovarian cancer." J Med Genet 42(8): 633-638.
275. Sazawa, A., et al. (2002). "Adenovirus mediated gelsolin gene therapy for orthotopic human bladder cancer in nude mice." J Urol 168(3): 1182-1187.
276. Schmidt, L. J., et al. (2009). "Effects of the 5 alpha-reductase inhibitor dutasteride on gene expression in prostate cancer xenografts." Prostate 69(16): 1730-1743.
277. Seki, T., et al. (2001). "Mechanism of growth-inhibitory effect of cisplatin on human pancreatic cancer cells and status of p53 gene." Anticancer Res 21(3B): 1919-1924.
278. Sekine, S., et al. (2002). "Target disruption of the mutant beta-catenin gene in colon cancer cell line HCT116: preservation of its malignant phenotype." Oncogene 21(38): 5906-5911.
279. Sheikh, M. S., et al. (1993). "Estrogen receptor-negative breast cancer cells transfected with the estrogen receptor exhibit increased RAR alpha gene expression and sensitivity to growth inhibition by retinoic acid." J Cell Biochem 53(4): 394-404.
280. Shi, Y. E., et al. (1997). "Antitumor activity of the novel human breast cancer growth inhibitor, mammary-derived growth inhibitor-related gene, MRG." Cancer Res 57(15): 3084-3091.
281. Shiau, A. L., et al. (2001). "Retrovirus-mediated transfer of prothymosin gene inhibits tumor growth and prolongs survival in murine bladder cancer." Gene Ther 8(21): 1609-1617.
282. Shimizu, M., et al. (1998). "Effect on colon cancer cells of human interferon-beta gene entrapped in cationic multilamellar liposomes." Biochem Mol Biol Int 44(6): 1235-1243.
283. Shinderman-Maman, E., et al. (2016). "The thyroid hormone-alphavbeta3 integrin axis in ovarian cancer: regulation of gene transcription and MAPK-dependent proliferation." Oncogene 35(15): 1977-1987.
284. Sica, G., et al. (1999). "Effect of leuprorelin acetate on cell growth and prostate-specific antigen gene expression in human prostatic cancer cells." Eur Urol 35 Suppl 1: 2-8.
285. Siemens, D. R., et al. (2000). "Viral vector delivery in solid-state vehicles: gene expression in a murine prostate cancer model." J Natl Cancer Inst 92(5): 403-412.
286. Singh, P., et al. (1996). "Gastrin gene expression is required for the proliferation and tumorigenicity of human colon cancer cells." Cancer Res 56(18): 4111-4115.
287. Soler, M. N., et al. (2000). "Gene therapy of rat medullary thyroid cancer by naked nitric oxide synthase II DNA injection." J Gene Med 2(5): 344-352.
288. Sorrells, D. L., et al. (1999). "Competitive PCR to detect eIF4E gene amplification in head and neck cancer." Head Neck 21(1): 60-65.
289. Spitz, F. R., et al. (1996). "In vivo adenovirus-mediated p53 tumor suppressor gene therapy for colorectal cancer." Anticancer Res 16(6B): 3415-3422.
290. Srivastava, M., et al. (2001). "ANX7, a candidate tumor suppressor gene for prostate cancer." Proc Natl Acad Sci U S A 98(8): 4575-4580.
291. Stem Cell. http://www.sciencepub.net/stem. 2019.
292. Stoll, V., et al. (2005). "Dominant negative inhibitors of signalling through the phosphoinositol 3-kinase pathway for gene therapy of pancreatic cancer." Gut 54(1): 109-116.
293. Sumitomo, K., et al. (2000). "Expression of a TGF-beta1 inducible gene, TSC-36, causes growth inhibition in human lung cancer cell lines." Cancer Lett 155(1): 37-46.
294. Sumitomo, M., et al. (1999). "Overexpression of IL-1ra gene up-regulates interleukin-1beta converting enzyme (ICE) gene expression: possible mechanism underlying IL-1beta-resistance of cancer cells." Br J Cancer 81(2): 277-286.
295. Suzuki, S., et al. (2001). "Coexpression of the partial androgen receptor enhances the efficacy of prostate-specific antigen promoter-driven suicide gene therapy for prostate cancer cells at low testosterone concentrations." Cancer Res 61(4): 1276-1279.

296. Takaoka, A., et al. (1998). "Suppression of invasive properties of colon cancer cells by a metastasis suppressor KAI1 gene." Oncogene 16(11): 1443-1453.
297. Takei, Y., et al. (1998). "Isolation of a novel TP53 target gene from a colon cancer cell line carrying a highly regulated wild-type TP53 expression system." Genes Chromosomes Cancer 23(1): 1-9.
298. Takeyama, Y., et al. (2010). "Knockdown of ZEB1, a master epithelial-to-mesenchymal transition (EMT) gene, suppresses anchorage-independent cell growth of lung cancer cells." Cancer Lett 296(2): 216-224.
299. Tanaka, M. and H. B. Grossman (2001). "Connexin 26 gene therapy of human bladder cancer: induction of growth suppression, apoptosis, and synergy with Cisplatin." Hum Gene Ther 12(18): 2225-2236.
300. Tayeb, M. T., et al. (2003). "CYP3A4 and VDR gene polymorphisms and the risk of prostate cancer in men with benign prostate hyperplasia." Br J Cancer 88(6): 928-932.
301. Tekmal, R. R. and V. R. Durgam (1997). "A novel in vitro and in vivo breast cancer model for testing inhibitors of estrogen biosynthesis and its action using mammary tumor cells with an activated int-5/aromatase gene." Cancer Lett 118(1): 21-28.
302. Thompson, A. M., et al. (1990). "Gene expression in oestrogen-dependent human breast cancer xenograft tumours." Br J Cancer 62(1): 78-84.
303. Tomida, S., et al. (2004). "Gene expression-based, individualized outcome prediction for surgically treated lung cancer patients." Oncogene 23(31): 5360-5370.
304. Tomizawa, Y., et al. (2001). "Inhibition of lung cancer cell growth and induction of apoptosis after reexpression of 3p21.3 candidate tumor suppressor gene SEMA3B." Proc Natl Acad Sci U S A 98(24): 13954-13959.
305. Tomoda, K., et al. (1998). "Cationic multilamellar liposome-mediated human interferon-beta gene transfer into cervical cancer cell." Anticancer Res 18(3A): 1367-1371.
306. Tong, Q., et al. (2003). "Growth inhibiting effects of antisense eukaryotic expression vector of proliferating cell nuclear antigen gene on human bladder cancer cells." Chin Med J (Engl) 116(8): 1203-1206.
307. Trapasso, F., et al. (2006). "Genetic ablation of Ptprj, a mouse cancer susceptibility gene, results in normal growth and development and does not predispose to spontaneous tumorigenesis." DNA Cell Biol 25(6): 376-382.
308. Trougakos, I. P., et al. (2004). "Silencing expression of the clusterin/apolipoprotein j gene in human cancer cells using small interfering RNA induces spontaneous apoptosis, reduced growth ability, and cell sensitization to genotoxic and oxidative stress." Cancer Res 64(5): 1834-1842.
309. Tsai, L. C., et al. (1997). "Effects of tamoxifen and retinoic acid on cell growth and c-myc gene expression in human breast and cervical cancer cells." Anticancer Res 17(6D): 4557-4562.
310. Tsao, Y. P., et al. (1999). "Adenovirus-mediated p21((WAF1/SDII/CIP1)) gene transfer induces apoptosis of human cervical cancer cell lines." J Virol 73(6): 4983-4990.
311. Tsunemitsu, Y., et al. (2004). "Molecular therapy for peritoneal dissemination of xenotransplanted human MKN-45 gastric cancer cells with adenovirus mediated Bax gene transfer." Gut 53(4): 554-560.
312. Tsunoo, H., et al. (2002). "Effect of transfection with human interferon-beta gene entrapped in cationic multilamellar liposomes in combination with 5-fluorouracil on the growth of human esophageal cancer cells in vitro." Anticancer Res 22(3): 1537-1543.
313. Tu, S. P., et al. (2005). "Gene therapy for colon cancer by adeno-associated viral vector-mediated transfer of survivin Cys84Ala mutant." Gastroenterology 128(2): 361-375.
314. Ueki, T., et al. (2001). "Silencing of the caspase-1 gene occurs in murine and human renal cancer cells and causes solid tumor growth in vivo." Int J Cancer 91(5): 673-679.
315. Ueno, M., et al. (2001). "Tumor-specific chemo-radio-gene therapy for colorectal cancer cells using adenovirus vector expressing the cytosine deaminase gene." Anticancer Res 21(4A): 2601-2608.
316. Unni, E., et al. (2004). "Osteopontin is a potential target gene in mouse mammary cancer chemoprevention by Se-methylselenocysteine." Breast Cancer Res 6(5): R586-592.
317. Vernejoul, F., et al. (2002). "Antitumor effect of in vivo somatostatin receptor subtype 2 gene transfer in primary and metastatic pancreatic cancer models." Cancer Res 62(21): 6124-6131.
318. Vikhanskaya, F., et al. (1993). "Effects of DNA damaging agents on gene expression in two human cancer cell lines." Cell Mol Biol (Noisy-le-grand) 39(8): 855-862.
319. Viney, J. L. (1995). "Transgenic and gene knockout mice in cancer research." Cancer Metastasis Rev 14(2): 77-90.
320. von Gruenigen, V. E., et al. (1998). "In vivo studies of adenovirus-based p53 gene therapy for ovarian cancer." Gynecol Oncol 69(3): 197-204.
321. von Knebel Doeberitz, M., et al. (1990). "Growth-regulating functions of human papillomavirus early gene products in cervical cancer cells acting dominant over enhanced epidermal growth factor receptor expression." Cancer Res 50(12): 3730-3736.
322. Wada, Y., et al. (2001). "Gene therapy for bladder cancer using adenoviral vector." Mol Urol 5(2): 47-52.
323. Wallqvist, A., et al. (2003). "Linking the growth inhibition response from the National Cancer Institute's anticancer screen to gene expression levels and other molecular target data." Bioinformatics 19(17): 2212-2224.
324. Watts, C. K., et al. (1994). "Antiestrogen regulation of cell cycle progression and cyclin D1 gene expression in MCF-7 human breast cancer cells." Breast Cancer Res Treat 31(1): 95-105.
325. Werner, H. and R. Sarfstein (2014). "Transcriptional and epigenetic control of IGF1R gene expression: implications in metabolism and cancer." Growth Horm IGF Res 24(4): 112-118.
326. Wiechen, K., et al. (1999). "Suppression of the c-erbB-2 gene product decreases transformation abilities but not the proliferation and secretion of proteases of SK-OV-3 ovarian cancer cells." Br J Cancer 81(5): 790-795.
327. Wierer, M., et al. (2013). "PLK1 signaling in breast cancer cells cooperates with estrogen receptor-dependent gene transcription." Cell Rep 3(6): 2021-2032.
328. Wikipedia. The free encyclopedia. http://en.wikipedia.org. 2019.
329. Wilczynska, U., et al. (2001). "Combined delivery of an antiangiogenic protein (angiostatin) and an immunomodulatory gene (interleukin-12) in the treatment of murine cancer." Acta Biochim Pol 48(4): 1077-1084.
330. Wilson, G. D., et al. (2014). "Gene expression changes during repopulation in a head and neck cancer xenograft." Radiother Oncol 113(1): 139-145.
331. Wilson, L. C., et al. (2003). "Nonsteroidal anti-inflammatory drug-activated gene (NAG-1) is induced by genistein through the expression of p53 in colorectal cancer cells." Int J Cancer 105(6): 747-753.
332. Wolf, J. K., et al. (1999). "Growth suppression of human ovarian cancer cell lines by the introduction of a p16 gene via a recombinant adenovirus." Gynecol Oncol 73(1): 27-34.
333. Wosikowski, K., et al. (1997). "Altered gene expression in drug-resistant human breast cancer cells." Clin Cancer Res 3(12 Pt 1): 2405-2414.
334. Yamato, H., et al. (1995). "In vivo evidence for progressive activation of parathyroid hormone-related peptide gene transcription with tumor growth and stimulation of

osteoblastic bone formation at an early stage of humoral hypercalcemia of cancer." J Bone Miner Res 10(1): 36-44.
335. Yan, R. L., et al. (2002). "[Experimental study of anti-VEGF hairpin ribozyme gene inhibiting expression of VEGF and proliferation of ovarian cancer cells]." Ai Zheng 21(1): 39-44.
336. Yanagie, H., et al. (2009). "Tumor growth suppression by adenovirus-mediated introduction of a cell-growth-suppressing gene tob in a pancreatic cancer model." Biomed Pharmacother 63(4): 275-286.
337. Yang, G., et al. (2003). "Silencing of H-ras gene expression by retrovirus-mediated siRNA decreases transformation efficiency and tumorgrowth in a model of human ovarian cancer." Oncogene 22(36): 5694-5701.
338. Yang, Q., et al. (2002). "[Gene transfer of murine Flt3 ligand mediated by adenoviral vector efficiently induces growth inhibition of murine liver cancer]." Zhonghua Yi Xue Za Zhi 82(11): 775-779.
339. Yang, W. M., et al. (2006). "LRIG1, a candidate tumour-suppressor gene in human bladder cancer cell line BIU87." BJU Int 98(4): 898-902.
340. Yang, W., et al. (2001). "sFlt-1 gene-transfected fibroblasts: a wound-specific gene therapy inhibits local cancer recurrence." Cancer Res 61(21): 7840-7845.
341. Yang, Y. J., et al. (2003). "[Expression of osm gene driven by hTERT gene promoter inhibits cancer cells proliferation in vitro]." Ai Zheng 22(6): 575-578.
342. Ye, D., et al. (2001). "Growth inhibition of interleukin-2 receptor gene-transduced peripheral blood lymphocytes on human ovarian cancer cells." Chin Med J (Engl) 114(3): 303-307.
343. Ye, X. and M. Wu (1992). "Retrovirus mediated transfer of antisense human c-myc gene into human esophageal cancer cells suppressed cell proliferation and malignancy." Sci China B 35(1): 76-83.
344. Yeap, B. B., et al. (1999). "Differential posttranscriptional regulation of androgen receptor gene expression by androgen in prostate and breast cancer cells." Endocrinology 140(7): 3282-3291.
345. Yoshioka, N., et al. (2002). "Suppression of anchorage-independent growth of human cancer cell lines by the TRIF52/periostin/OSF-2 gene." Exp Cell Res 279(1): 91-99.
346. Yu, J. X., et al. (2007). "Pathway analysis of gene signatures predicting metastasis of node-negative primary breast cancer." BMC Cancer 7: 182.
347. Yuan, B. Z., et al. (2003). "DLC-1 gene inhibits human breast cancer cell growth and in vivo tumorigenicity." Oncogene 22(3): 445-450.
348. Yuan, F., et al. (1999). "Altered growth and viral gene expression in human papillomavirus type 16-containing cancer cell lines treated with progesterone." Cancer Invest 17(1): 19-29.
349. Zerbini, L. F., et al. (2006). "A novel pathway involving melanoma differentiation associated gene-7/interleukin-24 mediates nonsteroidal anti-inflammatory drug-induced apoptosis and growth arrest of cancer cells." Cancer Res 66(24): 11922-11931.
350. Zha, Y., et al. (2000). "[The use of gene gun in cancer gene therapy]." Zhonghua Yi Xue Za Zhi 80(7): 522-525.
351. Zhang, F., et al. (2002). "Tumor-infiltrating macrophages are involved in suppressing growth and metastasis of human prostate cancer cells by INF-beta gene therapy in nude mice." Clin Cancer Res 8(9): 2942-2951.
352. Zhang, H., et al. (2016). "Integrated analysis of the miRNA, gene and pathway regulatory network in gastric cancer." Oncol Rep 35(2): 1135-1146.
353. Zhang, J. F., et al. (1996). "Treatment of a human breast cancer xenograft with an adenovirus vector containing an interferon gene results in rapid regression due to viral oncolysis and gene therapy." Proc Natl Acad Sci U S A 93(9): 4513-4518.
354. Zhang, J., et al. (2000). "[The growth inhibitory effects by transfection of p16 gene on human pancreatic cancer cell line]." Zhonghua Wai Ke Za Zhi 38(6): 457-459.
355. Zhang, J., et al. (2004). "Cystatin m: a novel candidate tumor suppressor gene for breast cancer." Cancer Res 64(19): 6957-6964.
356. Zhang, L., et al. (2010). "The impact of C-MYC gene expression on gastric cancer cell." Mol Cell Biochem 344(1-2): 125-135.
357. Zhang, T., et al. (2010). "PIM-1 gene RNA interference induces growth inhibition and apoptosis of prostate cancer cells and suppresses tumor progression in vivo." J Surg Oncol 101(6): 513-519.
358. Zhang, X., et al. (2001). "Apoptosis induction in prostate cancer cells by a novel gene product, pHyde, involves caspase-3." Oncogene 20(42): 5982-5990.
359. Zhang, X., et al. (2007). "Effects of exogenous p16(ink4a) gene on biological behaviors of human lung cancer cells." J Huazhong Univ Sci Technolog Med Sci 27(1): 37-40.
360. Zhang, Y. M., et al. (2003). "Effect of ZNRD1 gene antisense RNA on drug resistant gastric cancer cells." World J Gastroenterol 9(5): 894-898.
361. Zhang, Y., et al. (2005). "The Human mineral dust-induced gene, mdig, is a cell growth regulating gene associated with lung cancer." Oncogene 24(31): 4873-4882.
362. Zhang, Z., et al. (2003). "Antisense therapy targeting MDM2 oncogene in prostate cancer: Effects on proliferation, apoptosis, multiple gene expression, and chemotherapy." Proc Natl Acad Sci U S A 100(20): 11636-11641.
363. Zhao, A. G., et al. (2002). "Effects of Chinese Jianpi herbs on cell apoptosis and related gene expression in human gastric cancer grafted onto nude mice." World J Gastroenterol 8(5): 792-796.
364. Zhao, R., et al. (2017). "Screening of potential therapy targets for prostate cancer using integrated analysis of two gene expression profiles." Oncol Lett 14(5): 5361-5369.
365. Zheng, A. Q., et al. (2005). "Liposome transfected to plasmid-encoding endostatin gene combined with radiotherapy inhibits liver cancer growth in nude mice." World J Gastroenterol 11(28): 4439-4442.

8/25/2020