Cell growth and division and Cancer Biology Research Literatures

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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 related studies.

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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 related studies.

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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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

Luoh, S. W., et al. (2004). "Overexpression of the amplified Pip4k2beta gene from 17q11-12 in breast cancer cells confers proliferation advantage." Oncogene **23**(7): 1354-1363.

Gene amplification is common in solid tumors and is associated with adverse prognosis, disease progression, and development of drug resistance. A small segment from chromosome 17q11-12 containing the HER-2/Neu gene is amplified in about 25% of breast cancer. HER-2/Neu amplification is associated with adverse prognosis and may predict response to chemotherapy and hormonal manipulation. Moreover, HER-2/Neu amplification may select patients for anti-HER-2/Neu-based therapy with Herceptin. We and others recently described a common sequence element from the HER-2/Neu region that was amplified in breast cancer cells. In addition, most, if not all, of the amplified genes from this region display overexpression. This raises the intriguing possibility that genes immediately adjacent to HER-2/Neu may influence the biological behavior of breast cancer carrying HER-2/Neu amplification and serve as rational targets for therapy. By extracting sequence information from public databases, we have constructed a contig in bacterial artificial chromosomes (BACs) that extends from HER-2/Neu to a phosphotidylinositol phosphate kinase (PIPK), Pip4k2beta from 17q11-12. Although a role of PI-3-kinase and AKT in cancer biology has been previously described, PIPK has not been previously implicated. We show that Pip4k2beta, initially known as Pip5k2beta, is amplified in a subset of breast cancer cell lines and primary breast cancer samples that carry HER-2/Neu amplification. Out of eight breast cancer cell lines with HER-2/Neu amplification, three have concomitant amplification of the Pip4k2beta gene--UACC-812, BT-474 and ZR-75-30. Similarly, two out of four primary breast tumors with HER-2/Neu amplification carry Pip4k2beta gene amplification. Intriguingly, one tumor displays an increase in the gene copy number of Pip4k2beta that is significantly more than that of HER-2/Neu. Moreover, dual color FISH reveals that amplified Pip4k2beta gene may exist in a distinct structure from that of HER-2/Neu in ZR-75-30 cell line. These studies suggest that Pip4k2beta may reside on an amplification maximum distinct from that of HER-2/Neu and serve as an independent target for amplification and selective retention. Pip4k2beta amplification is associated with overexpression at the RNA and protein level in breast cancer cell lines. Stable expression of Pip4k2beta in breast cancer cell lines with and without HER-2/Neu amplification increases cell proliferation and anchorage-independent growth. The above observations implicate Pip4k2beta in the development and/or progression of breast cancer. Our study suggests that Pip4k2beta may be a distinct target for gene amplification and selective retention from 17q11-12.

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.

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.

Omotehara, F., et al. (2003). "Transcriptional activation of cyclin-dependent kinase inhibitor, p21waf1 gene by treatment with a differentiation inducing agent, vesnarinone in a human salivary gland cancer cell line." J Exp Clin Cancer Res **22**(1): 57-60.

Recently, a new concept for cancer therapy termed "tumor dormancy therapy" has been proposed. The concept of this therapy is to prolong the survival time of cancer patients while maintaining their quality of life. We have been developing a differentiation-inducing therapy, which is included in the tumor dormancy therapy, for salivary gland cancer. In this study, we examined the effect of a differentiation-inducing drug, Vesnarinone on the growth of several cancer cells, and examined the molecular mechanism by which Vesnarinone induces the cyclin dependent kinase inhibitor, p21waf1 in the cancer cells. Vesnarinone significantly suppressed the growth of TYS (salivary gland cancer cells), PC3 (prostate cancer cells), and A431 (squamous cell cancer cells). Furthermore, Vesnarinone dose-dependently enhanced the expression of p21waf1 mRNA in TYS cells. Using the luciferase reporter assay it was found that the enhancement of p21waf1 mRNA expression by Vesnarinone was through direct transcriptional activation of the p21waf1 promoter. Thus, analyzing the molecular mechanisms of differentiation inducing drugs may lead to the development of a new therapeutic strategy for several human malignancies, including salivary gland cancer.

Ong, G., et al. (1991). "Inactivation of the retinoblastoma gene does not lead to loss of TGF-beta receptors or response to TGF-beta in breast cancer cell lines." Oncogene **6**(5): 761-763.

Ou, X., et al. (1996). "Mechanism of all-trans-retinoic acid-mediated L-myc gene regulation in small cell lung cancer." Oncogene **13**(9): 1893-1899.

The L-myc oncogene is commonly expressed in small cell lung cancer (SCLC) cells and is associated with SCLC cells with a high level of neuroendocrine differentiation and a relatively low proliferative index. We have previously reported that all-trans-retinoic acid (RA) inhibits the growth of NCI-H82 SCLC cells in association with increased neuroendocrine differentiation, increased L-myc gene expression and decreased c-myc gene expression. In the present report, the mechanism of RA-mediated L-myc up-regulation in NCI-H82 SCLC cells was determined by analysing transcriptional and post-transcriptional control of L-myc gene expression. Increases in steady-state levels of L-myc mRNA occurred in a dose-dependent manner after exposure to RA at a time-point prior to discernible changes in cellular morphology or growth. By nuclear run-on analysis, there was a clear increase in L-myc transcript initiation in NCI-H82 cells treated with 1 microM RA, but no alteration was noted in the baseline degree of transcript attenuation when compared to control cells. L-myc transcript half-life remained unchanged after exposure to 1 microM RA, indicating that post-transcriptional regulation is not a major factor in the control of L-myc gene expression. A marked dose-dependent increase in RARbeta expression was also demonstrated in RA-treated NCI-H82 cells. We conclude that RA-mediated up-regulation of L-myc gene expression occurs through stimulation of transcript initiation and that the biological effects of RA in SCLC cells may be mediated through RARbeta-dependent pathways.

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.

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.

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.

Su, Z. Z., et al. (1998). "The cancer growth suppressor gene mda-7 selectively induces apoptosis in human breast cancer cells and inhibits tumor growth in nude mice." Proc Natl Acad Sci U S A **95**(24): 14400-14405.

A differentiation induction subtraction hybridization strategy is being used to identify and clone genes involved in growth control and terminal differentiation in human cancer cells. This scheme identified melanoma differentiation associated gene-7 (mda-7), whose expression is up-regulated as a consequence of terminal differentiation in human melanoma cells. Forced expression of mda-7 is growth inhibitory toward diverse human tumor cells. The present studies elucidate the mechanism by which mda-7 selectively suppresses the growth of human breast cancer cells and the consequence of ectopic expression of mda-7 on human breast tumor formation in vivo in nude mice. Infection of wild-type, mutant, and null p53 human breast cancer cells with a recombinant type 5 adenovirus expressing mda-7, Ad.mda-7 S, inhibited growth and induced programmed cell death (apoptosis). Induction of apoptosis correlated with an increase in BAX protein,

an established inducer of programmed cell death, and an increase in the ratio of BAX to BCL-2, an established inhibitor of apoptosis. Infection of breast carcinoma cells with Ad.mda-7 S before injection into nude mice inhibited tumor development. In contrast, ectopic expression of mda-7 did not significantly alter cell cycle kinetics, growth rate, or survival in normal human mammary epithelial cells. These data suggest that mda-7 induces its selective anticancer properties in human breast carcinoma cells by promoting apoptosis that occurs independent of p53 status. On the basis of its selective anticancer inhibitory activity and its direct antitumor effects, mda-7 may represent a new class of cancer suppressor genes that could prove useful for the targeted therapy of human cancer.

Subramanian, M., et al. (2009). "Knockdown of IG20 gene expression renders thyroid cancer cells susceptible to apoptosis." J Clin Endocrinol Metab **94**(4): 1467-1471.

AIM: The aim of the study was to investigate the expression and function of the IG20 gene in thyroid cancer cell survival, proliferation, and apoptosis. METHODS: We determined the expression levels of the major isoforms of IG20 by quantitative RT-PCR in normal and thyroid tumor tissues/cell lines. We evaluated the functional consequence of IG20 knockdown in WRO (follicular carcinoma) and FRO (anaplastic carcinoma) thyroid cancer cell lines by measuring spontaneous, TNFalpha-related apoptosis-inducing ligand (TRAIL), and TNFalpha-induced apoptosis. RESULTS: The IG20 gene expression levels were higher in benign and malignant thyroid tumors and in WRO and FRO cells relative to normal tissues. Predominantly, MADD and DENN-SV isoforms of IG20 gene were expressed. IG20 knockdown resulted in increased spontaneous, TRAIL-, and TNFalpha-induced apoptosis in WRO, but not FRO, cells. FRO cell resistance to apoptosis is likely due to caspase-8 deficiency. CONCLUSION: IG20 knockdown renders WRO cells more susceptible to spontaneous, TRAIL-, and TNFalpha-induced apoptosis and thus demonstrates the prosurvival function of the IG20 gene in thyroid cancer. These observations, combined with overexpression of IG20 noted in thyroid tumor tissues, may suggest a potential role in thyroid cancer survival and growth and indicate that IG20 may be targeted either alone or in conjunction with TRAIL or TNFalpha treatment in certain thyroid cancers.

Sugino, T., et al. (1998). "Disorderly CD44 gene expression in human cancer cells can be modulated by growth conditions." J Pathol **186**(1): 17-23.

Disorderly CD44 gene expression is a well-documented characteristic feature of tumour cells from cancers arising in many different organs of the human body. Molecular pathological studies have established that the pattern of the abnormal expression can differ according to the origin and the stage of the tumour. In this investigation it has been demonstrated that in some but not all tumour cell lines, which are undeniably and irreversibly malignant when inoculated in vivo, CD44 gene expression can still be modulated. In two cell lines, the pattern of CD44v expression was found to be affected by cell-to-cell and cell-to-substrate attachment. Expression was up-regulated by cell-substrate interactions, but only until cell-to-cell contact caused subsequent down-regulation of CD44v transcription. This information provides new opportunities for detailed investigation of the mechanisms of abnormal CD44 gene regulation in cancer and for exploring stage-related changes in the expression of this complex gene.

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.

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.

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