Cancer and Hereditary 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.

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

Abbosh, P. H., et al. (2006). "Dominant-negative histone H3 lysine 27 mutant derepresses silenced tumor suppressor genes and reverses the drug-resistant phenotype in cancer cells." Cancer Res **66**(11): 5582-5591.

Histone modifications and DNA methylation are epigenetic phenomena that play a critical role in many neoplastic processes, including silencing of tumor suppressor genes. One such histone modification, particularly at H3 and H4, is methylation at specific lysine (K) residues. Whereas histone methylation of H3-K9 has been linked to DNA methylation and aberrant gene silencing in cancer cells, no such studies of H3-K27 have been reported. Here, we generated a stable cell line overexpressing a dominant-negative point mutant, H3-K27R, to examine the role of that specific lysine in ovarian cancer. Expression of this construct resulted in loss of methylation at H3-K27, global reduction of DNA methylation, and increased expression of tumor suppressor genes. One of the affected genes, RASSF1, was shown to be a direct target of H3-K27 methylation-mediated silencing. By increasing DNA-platinum adduct formation, indicating increased access of the drug to target DNA sequences, removal of H3-K27 methylation resensitized drug-resistant ovarian cancer cells to the chemotherapeutic agent cisplatin. This increased platinum-DNA access was likely due to relaxation of condensed chromatin. Our results show that overexpression of mutant H3-K27 in mammalian cells represents a novel tool for studying epigenetic mechanisms and the Histone Code Hypothesis in human cancer. Such findings show the significance of H3-K27 methylation as a promising target for epigenetic-based cancer therapies.

Afgar, A., et al. (2016). "MiR-339 and especially miR-766 reactivate the expression of tumor suppressor genes in colorectal cancer cell lines through DNA methyltransferase 3B gene inhibition." Cancer Biol Ther **17**(11): 1126-1138.

It is observed that upregulation of DNMT3B enzyme in some cancers, including colon cancer, could lead to silencing of tumor suppressor genes. MiR-339 and miR-766 have been predicted to target 3'UTR of DNMT3B gene. Luciferase reporter assay validated that individual and co-transfection of miR-766 and miR-339 into the HEK293T cell reduced luciferase activity to 26% +/- 0.41%, 43% +/- 0.42 and 64% +/- 0.52%, respectively, compared to the control ($P < 0.05$). Furthermore, transduction of miR-339 and miR-766 expressing viruses into colon cancer cell lines (SW480 and HCT116) decreased DNMT3B expression (1.5, 3-fold) and (3, 4-fold), respectively. In addition, DNA methylation of some tumor suppressor genes decreased. Expression of these genes such as SFRP1 (2 and 1.6-fold), SFRP2 (0.07 and 4-fold), WIF1 (0.05 and 4-fold), and DKK2 (2 and 4-fold) increased in SW-339 and SW-766 cell lines; besides, expression increments for these genes in HCT-339 and HCT-766 cell lines were (2.8, 4-fold), (0.005, 1.5-fold), (1.7 and 3-fold) and (0.04, 1.7-fold), respectively. Also, while in SW-766, cell proliferation

reduced to 2.8% and 21.7% after 24 and 48 hours, respectively, SW-339 showed no reduced proliferation. Meanwhile, HCT-766 and HCT-339 showed (3.5%, 12.8%) and (18.8%, 33.9%) reduced proliferation after 24 and 48 hours, respectively. Finally, targeting DNMT3B by these miRs, decreased methylation of tumor suppressor genes such as SFRP1, SFRP2, WIF1 and DKK2 in the mentioned cell lines, and returned the expression of these tumor suppressor genes which can contribute to lethal effect on colon cancer cells and reducing tumorigenicity of these cells.

Agajanian, S., et al. (2018). "Machine Learning Classification and Structure-Functional Analysis of Cancer Mutations Reveal Unique Dynamic and Network Signatures of Driver Sites in Oncogenes and Tumor Suppressor Genes." J Chem Inf Model.

In this study, we developed two cancer-specific machine learning classifiers for prediction of driver mutations in cancer-associated genes that were validated on canonical data sets of functionally validated mutations and applied to a large cancer genomics data set. By examining sequence, structure, and ensemble-based integrated features, we have shown that evolutionary conservation scores play a critical role in classification of cancer drivers and provide the strongest signal in the machine learning prediction. Through extensive comparative analysis with structure-functional experiments and multicenter mutational calling data from Pan Cancer Atlas studies, we have demonstrated the robustness of our models and addressed the validity of computational predictions. To address the interpretability of cancer-specific classification models and obtain novel insights about molecular signatures of driver mutations, we have complemented machine learning predictions with structure-functional analysis of cancer driver mutations in several important oncogenes and tumor suppressor genes. By examining structural and dynamic signatures of known mutational hotspots and the predicted driver mutations, we have shown that the greater flexibility of specific functional regions targeted by driver mutations in oncogenes may facilitate activating conformational changes, while loss-of-function driver mutations in tumor suppressor genes can preferentially target structurally rigid positions that mediate allosteric communications in residue interaction networks and modulate protein binding interfaces. By revealing molecular signatures of cancer driver mutations, our results highlighted limitations of the binary driver/passenger classification, suggesting that functionally relevant cancer mutations may span a continuum spectrum of driverlike effects. Based on this analysis, we propose for experimental testing a group of novel potential driver mutations that can act by altering structure, global dynamics, and allosteric interaction networks in important cancer genes.

Aguirre, E., et al. (2014). "Genetic Modeling of PIM Proteins in Cancer: Proviral Tagging and Cooperation with Oncogenes, Tumor Suppressor Genes, and Carcinogens." Front Oncol **4**: 109.

The PIM proteins, which were initially discovered as proviral insertion sites in Moloney-murine leukemia virus infection, are a family of highly homologous serine/threonine kinases that have been reported to be overexpressed in hematological malignancies and solid tumors. The PIM proteins have also been associated with metastasis and overall treatment responses and implicated in the regulation of apoptosis, metabolism, the cell cycle, and homing and migration, which makes these proteins interesting targets for anti-cancer drug discovery. The use of retroviral insertional mutagenesis and refined approaches such as complementation tagging has allowed the identification of myc, pim, and a third group of genes (including bmi1 and gfi1) as complementing genes in lymphomagenesis. Moreover, mouse modeling of human cancer has provided an understanding of the molecular pathways that are involved in tumor initiation and progression at the physiological level. In particular, genetically modified mice have allowed researchers to further elucidate the role of each of the Pim isoforms in various tumor types. PIM kinases have been identified as weak oncogenes because experimental overexpression in lymphoid tissue, prostate, and liver induces tumors at a relatively low incidence and with a long latency. However, very strong synergistic tumorigenicity between Pim1/2 and c-Myc and other oncogenes has been observed in lymphoid tissues. Mouse models have also been used to study whether the inhibition of specific PIM isoforms is required to prevent carcinogen-induced sarcomas, indicating that the absence of Pim2 and Pim3 greatly reduces sarcoma growth and bone invasion; the extent of this effect is similar to that observed in the absence of all three isoforms. This review will summarize some of the animal models that have been used to understand the isoform-specific contribution of PIM kinases to tumorigenesis.

Al-Ansari, M. M. and A. Aboussekhra (2014). "Caffeine mediates sustained inactivation of breast cancer-associated myofibroblasts via up-regulation of tumor suppressor genes." PLoS One **9**(3): e90907.

BACKGROUND: Active cancer-associated fibroblasts (CAFs) or myofibroblasts play important roles not only in the development and progression of breast carcinomas, but also in their prognosis and treatment. Therefore, targeting these cells through

suppressing their supportive procarcinogenic paracrine effects is mandatory for improving the current therapies that are mainly targeting tumor cells. To this end, we investigated the effect of the natural and pharmacologically safe molecule, caffeine, on CAF cells and their various procarcinogenic effects. METHODOLOGY/PRINCIPAL FINDINGS: We have shown here that caffeine up-regulates the tumor suppressor proteins p16, p21, p53 and Cav-1, and reduces the expression/secretion of various cytokines (IL-6, TGF-beta, SDF-1 and MMP-2), and down-regulates alpha-SMA. Furthermore, caffeine suppressed the migratory/invasiveness abilities of CAF cells through PTEN-dependent Akt/Erk1/2 inactivation. Moreover, caffeine reduced the paracrine pro-invasion/-migration effects of CAF cells on breast cancer cells. These results indicate that caffeine can inactivate breast stromal myofibroblasts. This has been confirmed by showing that caffeine also suppresses the paracrine pro-angiogenic effect of CAF cells through down-regulating HIF-1alphaand its downstream effector VEGF-A. Interestingly, these effects were sustained in absence of caffeine. CONCLUSION/SIGNIFICANCE: The present findings provide a proof of principle that breast cancer myofibroblasts can be inactivated, and thereby caffeine may provide a safe and effective prevention against breast tumor growth/recurrence through inhibition of the procarcinogenic effects of active stromal fibroblasts.

Alhosin, M., et al. (2016). "Signalling pathways in UHRF1-dependent regulation of tumor suppressor genes in cancer." J Exp Clin Cancer Res **35**(1): 174.

Epigenetic silencing of tumor suppressor genes (TSGs) through DNA methylation and histone changes is a main hallmark of cancer. Ubiquitin-like with PHD and RING Finger domains 1 (UHRF1) is a potent oncogene overexpressed in various solid and haematological tumors and its high expression levels are associated with decreased expression of several TSGs including p16 (INK4A), BRCA1, PPARG and KiSS1. Using its several functional domains, UHRF1 creates a strong coordinated dialogue between DNA methylation and histone post-translation modification changes causing the epigenetic silencing of TSGs which allows cancer cells to escape apoptosis. To ensure the silencing of TSGs during cell division, UHRF1 recruits several enzymes including histone deacetylase 1 (HDAC1), DNA methyltransferase 1 (DNMT1) and histone lysine methyltransferases G9a and Suv39H1 to the right place at the right moment. Several in vitro and in vivo works have reported the direct implication of the epigenetic player UHRF1 in tumorigenesis through the repression of TSGs expression and suggested UHRF1 as a promising target for cancer treatment. This review describes the molecular mechanisms underlying UHRF1 regulation in cancer and discusses its importance as a therapeutic target to induce the reactivation of TSGs and subsequent apoptosis.

Alhosin, M., et al. (2011). "Down-regulation of UHRF1, associated with re-expression of tumor suppressor genes, is a common feature of natural compounds exhibiting anti-cancer properties." J Exp Clin Cancer Res **30**: 41.

Over-expressed in numerous cancers, Ubiquitin-like containing PHD Ring Finger 1 (UHRF1, also known as ICBP90 or Np95) is characterized by a SRA domain (Set and Ring Associated) which is found only in the UHRF family. UHRF1 constitutes a complex with histone deacetylase 1 (HDAC1) and DNA methyltransferase 1 (DNMT1) via its SRA domain and represses the expression of several tumour suppressor genes (TSGs) including p16INK4A, hMLH1, BRCA1 and RB1. Conversely, UHRF1 is regulated by other TSGs such as p53 and p73. UHRF1 is hypothetically involved in a macro-molecular protein complex called "ECREM" for "Epigenetic Code Replication Machinery". This complex would be able to duplicate the epigenetic code by acting at the DNA replication fork and by activating the right enzymatic activity at the right moment. There are increasing evidence that UHRF1 is the conductor of this replication process by ensuring the crosstalk between DNA methylation and histone modifications via the SRA and Tandem Tudor Domains, respectively. This cross-talk allows cancer cells to maintain the repression of TSGs during cell proliferation. Several studies showed that down-regulation of UHRF1 expression in cancer cells by natural pharmacological active compounds, favors enhanced expression or re-expression of TSGs, suppresses cell growth and induces apoptosis. This suggests that hindering UHRF1 to exert its role in the duplication of the methylation patterns (DNA + histones) is responsible for inducing apoptosis. In this review, we present UHRF1 expression as a target of several natural products and we discuss their underlying molecular mechanisms and benefits for chemoprevention and chemotherapy.

Ali Khan, M., et al. (2015). "Sulforaphane Reverses the Expression of Various Tumor Suppressor Genes by Targeting DNMT3B and HDAC1 in Human Cervical Cancer Cells." Evid Based Complement Alternat Med **2015**: 412149.

Sulforaphane (SFN) may hinder carcinogenesis by altering epigenetic events in the cells; however, its molecular mechanisms are unclear. The present study investigates the role of SFN in modifying epigenetic events in human cervical cancer cells, HeLa. HeLa

cells were treated with SFN (2.5 microM) for a period of 0, 24, 48, and 72 hours for all experiments. After treatment, expressions of DNMT3B, HDAC1, RARbeta, CDH1, DAPK1, and GSTP1 were studied using RT-PCR while promoter DNA methylation of tumor suppressor genes (TSGs) was studied using MS-PCR. Inhibition assays of DNA methyl transferases (DNMTs) and histone deacetylases (HDACs) were performed at varying time points. Molecular modeling and docking studies were performed to explore the possible interaction of SFN with HDAC1 and DNMT3B. Time-dependent exposure to SFN decreases the expression of DNMT3B and HDAC1 and significantly reduces the enzymatic activity of DNMTs and HDACs. Molecular modeling data suggests that SFN may interact directly with DNMT3B and HDAC1 which may explain the inhibitory action of SFN. Interestingly, time-dependent reactivation of the studied TSGs via reversal of methylation in SFN treated cells correlates well with its impact on the epigenetic alterations accumulated during cancer development. Thus, SFN may have significant implications for epigenetic based therapy.

Alsiary, R., et al. (2018). "Expression analysis of the MCPH1/BRIT1 and BRCA1 tumor suppressor genes and telomerase splice variants in epithelial ovarian cancer." Gene **672**: 34-44.

AIMS: The aim of this study was to explore the correlation of hTERT splice variant expression with MCPH1/BRIT1 and BRCA1 expression in epithelial ovarian cancer (EOC) samples. BACKGROUND: Telomerase activation can contribute to the progression of tumors and the development of cancer. However, the regulation of telomerase activity remains unclear. MCPH1 (also known as BRIT1, BRCT-repeat inhibitor of hTERT expression) and BRCA1 are tumor suppressor genes that have been linked to telomerase expression. METHODS: qPCR was used to investigate telomerase splice variants, MCPH1/BRIT1 and BRCA1 expression in EOC tissue and primary cultures. RESULTS: The wild type alpha+/beta+ hTERT variant was the most common splice variant in the EOC samples, followed by alpha+/beta- hTERT, a dominant negative regulator of telomerase activity. EOC samples expressing high total hTERT demonstrated significantly lower MCPH1/BRIT1 expression in both tissue (p=0.05) and primary cultures (p=0.03). We identified a negative correlation between MCPH1/BRIT1 and alpha+/beta+ hTERT (p=0.04), and a strong positive association between MCPH1/BRIT1 and both alpha-/beta+ hTERT and alpha-/beta- hTERT (both p=0.02). A positive association was observed between BRCA1 and alpha-/beta+ hTERT and alpha-/beta- hTERT expression (p=0.003 and p=0.04, respectively). CONCLUSIONS: These findings support a regulatory effect of MCPH1/BRIT1 and BRCA1 on telomerase activity, particularly the negative association between MCPH1/BRIT1 and the functional form of hTERT (alpha+/beta+).

Alvarez, C., et al. (2013). "Silencing of tumor suppressor genes RASSF1A, SLIT2, and WIF1 by promoter hypermethylation in hereditary breast cancer." Mol Carcinog **52**(6): 475-487.

Promoter hypermethylation is gaining strength as one of the main mechanisms through which tumor suppressor genes are silenced during tumor progression. Three tumor suppressor genes are frequently found methylated in their promoter, in concordance with absence of expression, RASSF1A, SLIT2, and WIF1. In addition, a previous array-CGH analysis from our group showed that these genes are found in deleted genomic regions observed in hereditary breast cancer tumors. In the present work we analyzed the methylation status of these three tumor suppressor gene promoters in 47 hereditary breast cancer tumors. Promoter methylation status analysis of hereditary breast tumors revealed high methylation frequencies for the three genes (67% RASSF1A, 80% SLIT2, and 72% WIF1). Additionally, the presence of methylated PCR products was associated with absence of protein expression for the three genes and statistically significant for RASSF1A and WIF1. Interestingly, methylation of all the three genes was found in 4 out of 6 grade I invasive ductal carcinoma tumors. Association between RASSF1A methylation and DCIS tumors was found. These results suggest that silencing of these tumor suppressor genes is an early event in hereditary breast cancer, and could be a marker for pre-malignant phenotypes.

An, J., et al. (2010). "Messenger RNA expression and methylation of candidate tumor-suppressor genes and risk of ovarian cancer-a case-control analysis." Int J Mol Epidemiol Genet **1**(1): 1-10.

To investigate the association of expression and promoter methylation of tumor-suppressor genes with risk of ovarian cancer, we conducted a case-control study of 102 patients with serous epithelial ovarian cancer and 100 patients without ovarian cancers. We measured mRNA expression levels (by real-time reverse transcription polymerase chain reaction) and methylation status (by methylation-specific polymerase chain reaction) of five candidate genes (BRCA1, BRCA2, hMLH1, MGMT, and DNMT3B) in tumors from the cases and normal ovaries from the controls. We found that mRNA expression levels of the five genes were decreased in tumors than in normal ovaries with 0.39-fold for BRCA1, 0.25-fold for BRCA2, 0.42-fold for hMLH1, 0.45-fold for MGMT,

and 0.87-fold for DNMT3B, calculated by the 2(-DeltaDeltaCT) method. Ovarian cancer risk (odds ratios, ORs) was associated with low expression of all genes (2.95 [95% confidence interval (CI), 1.51 - 5.78] for BRCA1, 3.65 (95% CI, 1.82 - 7.30) for BRCA2, 5.25 (95% CI, 2.52 - 10.96) for hMLH1, and 4.72 (95% CI, 2.32 - 9.62) for MGMT) but not DNMT3B. However, methylation status was not associated with gene expression levels in the tumors, except for hMLH1 whose mean (+/- SD) gene expression was significantly lower in methylated (13.0 +/- 7.6) than in unmethylated (31.2 +/- 44.8) tumors ($P < 0.001$). We concluded that low mRNA expression of these tumor-suppressor genes, likely due to molecular mechanisms in addition to the promoter methylation in some instances, may be a biomarker for ovarian cancer risk in this study population. Larger studies are needed to validate our findings.

An, Q., et al. (2002). "Deletion of tumor suppressor genes in Chinese non-small cell lung cancer." Cancer Lett **184**(2): 189-195.

In the present study, we used 22 microsatellite markers flanking to or within 13 known or candidate tumor suppressor genes (TSGs) to detect loss of heterozygosity (LOH) in these chromosomal regions among 41 cases of non-small cell lung cancer, including 28 squamous cell carcinoma (SCC) and 13 adenocarcinoma (ADC). The studied TSGs comprised FHIT, VHL, APC, PRLTS, p16, IFNA, PTEN, p57, ATM, p53, BRCA1, DPC4 and DCC. Our data demonstrated frequent allelic losses of FHIT, p53, IFNA, VHL and p16 in both SCC and ADC. PTEN and ATM showed the least frequency of LOH, while no deletion of BRCA1 was detected in all tumor samples. LOH analysis of PRLTS was extended to 26 cases of ADC, which demonstrated significantly higher frequency of LOH than SCC. Our data indicated a possible correlation between specific TSG (s) and either histological type of lung cancer, and more attention should be paid to the PRLTS gene, which might play an important role in the development of ADC.

Aunoble, B., et al. (2000). "Major oncogenes and tumor suppressor genes involved in epithelial ovarian cancer (review)." Int J Oncol **16**(3): 567-576.

Ovarian cancer remains the leading cause of death from gynecologic malignancy in Western countries. This cancer results from a succession of genetic alterations involving oncogenes and tumor suppressor genes which have a critical role in normal cell growth regulation. Mutations and/or overexpression of three oncogenes, HER-2/neu, c-myc and K-ras, and of the tumor suppressor gene p53, have frequently been observed in sporadic ovarian cancer. In the context of high risk families, the most frequently involved genes are BRCA1 and BRCA2. We review the function of these different proteins, the incidence of mutations in their genes in carcinogenesis and as potential prognostic factors in sporadic and hereditary ovarian cancer.

Azizi, M., et al. (2014). "MicroRNA-148b and microRNA-152 reactivate tumor suppressor genes through suppression of DNA methyltransferase-1 gene in pancreatic cancer cell lines." Cancer Biol Ther **15**(4): 419-427.

Overexpression of DNA methyltransferase 1 (DNMT-1) is observed mostly in pancreatic cancer and it can cause tumor suppressor genes silencing in this disease. Recent studies suggest that abnormal expressions of microRNAs (miRs) are involved in pathogenesis of different types of human cancers including pancreatic cancer. In this study we aimed to investigate the effect of miR-148b and -152 on reverting the tumorigenic phenotype of pancreatic cancer cell lines. In order to investigate whether miR-148b and -152 are involved in the regulation of DNMT-1, luciferase reporter assay was used and confirmed that the DNMT-1 mRNA could be a target for miR-148b and miR-152. Furthermore, overexpression of miR-148b and -152 in pancreatic cancer cell lines (MIA PaCa-2 and AsPC-1) decreased DNMT-1 expression (53% and 59% respectively), returned DNA methylation to normal patterns and induced re-expression of tumor suppressor genes, like BNIP3 (4.7- and 3.8-fold) and SPARC (5.3- and 2.9-fold) for miR-148b and -152 respectively. Moreover, the introduced miR-148b and -152 could inhibit the proliferation of MIA PaCa-2 (35% and 37% respectively) and AsPC-1 (39% and 40% respectively) cell lines. The apoptosis rates of MIA PaCa-1 after treatment with miR-148b and -152 were 10% and 8% respectively; while these rates in AsPC-1 were 16% and 11% respectively. Conclusively these findings mean that miRs that are targeting DNMT-1 and modifying methylation status of tumor suppressor genes such as BNIP3 and SPARC can be applied in killing the pancreatic cancer cells and decreasing the tumorigenicity of these cells.

Badawi, A. F. (1996). "Molecular and genetic events in schistosomiasis-associated human bladder cancer: role of oncogenes and tumor suppressor genes." Cancer Lett **105**(2): 123-138.

Carcinoma of the urinary bladder is the most common malignancy in many tropical and subtropical countries and is mainly due to endemic schistosomal infection. Schistosomiasis-associated bladder cancer defines a characteristic pathology and cellular and molecular biology that differs from urothelial

carcinoma of non-schistosomal origin. N-Nitroso compounds are suspected etiologic agents in the process of bladder cancer induction during schistosomiasis. Elevated levels of DNA alkylation damage have been detected in schistosome-infected bladders and are accompanied by an inefficient capacity of DNA repair mechanisms. Consequently, high frequency of G --> A transition mutations were observed in the H-ras gene and at the CpG sequences of the p53 tumor suppressor gene. Genetic changes have also been detected in the c-erbB-1 and c-erbB-2 oncogenes and in the cdkn2 and Rb tumor suppressor genes. The potential application of these mutational patterns in providing a biological marker suitable for the biomonitoring and early detection of this neoplasm could indicate new avenues of approach that might alleviate the problem in the future. It can also assist in elucidating the mechanisms by which schistosomiasis augments human bladder cancers.

Bagci, B., et al. (2016). "KRAS, BRAF oncogene mutations and tissue specific promoter hypermethylation of tumor suppressor SFRP2, DAPK1, MGMT, HIC1 and p16 genes in colorectal cancer patients." Cancer Biomark **17**(2): 133-143.

BACKGROUND: Colorectal cancer is a serious disease that causes significant morbidity and mortality in developed countries. Genetic changes, such as mutations in proto-oncogenes and DNA repair genes, and loss of function in the tumor suppressor genes cause colorectal cancer development. Abnormal DNA methylation is also known to play a crucial role in colorectal carcinogenesis. OBJECTIVE: In this study, frequencies of KRAS and BRAF mutations, promoter hypermethylation profiles of SFRP2, DAPK1, MGMT, HIC1 and p16 genes, and possible associations between hypermethylation of these genes and KRAS and BRAF mutations were aimed to find out. METHODS: Ninety three colorectal cancer tissues and 14 normal colon mucosas were included in the study. Common twelve KRAS gene mutation were investigated with using reverse-hybridization strip assay method. BRAF V600E mutations were investigated with RFLP method. Hypermethylation status of five tumor suppressor genes were detected by using reverse-hybridization strip assay method after bisulfite modification of DNA. RESULTS: KRAS and BRAF mutation frequencies were determined as 54.84% and 12.9%, respectively. Promoter hypermethylation frequencies of tumor suppressor genes SFRP2, DAPK1, MGMT, HIC1 and p16 were determined as 66.7%, 45.2%, 40.9%, 40.9% and 15.1%, respectively. Statistically significant associations were found between BRAF mutation and SFRP2 and p16 tumor suppressor genes hypermethylation (SFRP2; p= 0.005, p16; p= 0.016). Compared to rectum, SFRP2 (p= 0.017) and MGMT (p= 0.013) genes have statistically significantly higher promoter hypermethylation in colon. CONCLUSIONS: Results of the current study have confirmed that KRAS mutations and SFRP2 hypermethylation can be used as genetic markers in colorectal cancer.

Bamberger, A. M., et al. (2002). "Expression of the adhesion molecule CEACAM1 (CD66a, BGP, C-CAM) in breast cancer is associated with the expression of the tumor-suppressor genes Rb, Rb2, and p27." Virchows Arch **440**(2): 139-144.

The adhesion molecule CEACAM1 (CD66a, BGP, C-CAM) is not only involved in maintaining normal tissue architecture, but also acts as a tumor suppressor in several experimental systems where loss of CEACAM1 expression results in enhanced tumor-cell growth and tumorigenicity. In order to further analyze the role of CEACAM1 in the development of breast cancer, we performed Western-blot analysis and immunohistochemistry with highly specific monoclonal antibodies in a cohort of 68 mammary carcinomas which had also been analyzed for expression of cell-cycle regulatory proteins cyclin D1, cyclin E, p16, p21, p27, Rb, and Rb2, as well as for steroid hormone receptor status, Ki67, and HER2/neu immunoreactivity. High CEACAM1 protein expression as found using both methods correlated significantly with expression of the retinoblastoma proteins Rb (P=0.004 and 0.013) and Rb2/p130 (P=0.003 and 0.007). In addition, we found a weak association of CEACAM1 expression with p27 protein levels (P=0.087 and 0.039), but with none of the other analyzed parameters. These results indicate the possibility of a functional link between cell-adhesion molecules and cell-cycle regulation that might play an important role in the development of mammary carcinomas.

Battagli, C., et al. (2003). "Promoter hypermethylation of tumor suppressor genes in urine from kidney cancer patients." Cancer Res **63**(24): 8695-8699.

Kidney cancer confined by the renal capsule can be surgically cured in the majority of cases, whereas the prognosis for patients with advanced disease at presentation remains poor. Novel strategies for early detection are therefore needed. Molecular DNA-based tests have successfully used the genetic alterations that initiate and drive tumorigenesis as targets for the early detection of several types of cancer in bodily fluids, including urine. Using sensitive methylation-specific PCR, we screened matched tumor DNA and sediment DNA from preoperative urine specimens obtained in 50 patients with kidney tumors, representing all major histological types, for hypermethylation status of a

panel of six normally unmethylated tumor suppressor genes VHL, p16/CDKN2a, p14ARF, APC, RASSF1A, and Timp-3. Hypermethylation of at least one gene was found in all 50 tumor DNAs (100% diagnostic coverage) and an identical pattern of gene hypermethylation found in the matched urine DNA from 44 of 50 patients (88% sensitivity), including 27/30 cases of stage I disease. In contrast, hypermethylation of the genes in the panel was not observed in normal kidney tissue or in urine from normal healthy individuals and patients with benign kidney disease (100% specificity). Hypermethylation of VHL was found only in clear cell, whereas hypermethylation of p14ARF, APC, or RASSF1A was more frequent in nonclear cell tumors, which suggested that the panel might facilitate differential diagnosis. We conclude that promoter hypermethylation is a common and early event in kidney tumorigenesis and can be detected in the urine DNA from patients with organ-confined renal cancers of all histological types. Methylation-specific PCR may enhance early detection of renal cancer using a noninvasive urine test.

Bersanelli, M., et al. (2018). "Loss of heterozygosity of key tumor suppressor genes in advanced renal cancer patients treated with nivolumab." Immunotherapy **10**(9): 743-752.

AIM: We studied the possible clinical significance of loss of heterozygosity (LOH) at key tumor suppressor genes loci in advanced renal cancer patients treated with nivolumab. METHODS: LOH study was performed on 3p14.2 (FHIT gene); 3p21.3-21.2; 9p21 (BDMF gene); 9p22 (SH3GL2 gene). RESULTS: Of 12 patients, 8 (67%) had LOH. The most affected gene was FHIT. All five patients with LOH at FHIT locus had good outcome, mean progression free survival of 6.8 months. The patients LOH negative at FHIT locus had mean progression free survival of 4 months, 67% were treatment refractory. Overall, 75% of patients with LOH of at least one gene had benefit; 75% of LOH negative cases were refractory. CONCLUSION: LOH at key tumor suppressor genes should be further investigated as predictive for immunotherapy.

Bock, J., et al. (2018). "Single CpG hypermethylation, allele methylation errors, and decreased expression of multiple tumor suppressor genes in normal body cells of mutation-negative early-onset and high-risk breast cancer patients." Int J Cancer **143**(6): 1416-1425.

To evaluate the role of constitutive epigenetic changes in normal body cells of BRCA1/BRCA2-mutation negative patients, we have developed a deep bisulfite sequencing assay targeting the promoter regions of 8 tumor suppressor (TS) genes (BRCA1, BRCA2, RAD51C, ATM, PTEN, TP53, MLH1, RB1) and the estrogene receptor gene (ESR1), which plays a role in tumor progression. We analyzed blood samples of two breast cancer (BC) cohorts with early onset (EO) and high risk (HR) for a heterozygous mutation, respectively, along with age-matched controls. Methylation analysis of up to 50,000 individual DNA molecules per gene and sample allowed quantification of epimutations (alleles with >50% methylated CpGs), which are associated with epigenetic silencing. Compared to ESR1, which is representative for an average promoter, TS genes were characterized by a very low (< 1%) average methylation level and a very low mean epimutation rate (EMR; < 0.0001% to 0.1%). With exception of BRCA1, which showed an increased EMR in BC (0.31% vs. 0.06%), there was no significant difference between patients and controls. One of 36 HR BC patients exhibited a dramatically increased EMR (14.7%) in BRCA1, consistent with a disease-causing epimutation. Approximately one third (15 of 44) EO BC patients exhibited increased rates of single CpG methylation errors in multiple TS genes. Both EO and HR BC patients exhibited global underexpression of blood TS genes. We propose that epigenetic abnormalities in normal body cells are indicative of disturbed mechanisms for maintaining low methylation and appropriate expression levels and may be associated with an increased BC risk.

Brechot, J. M., et al. (2002). "[2000 Standards, Options and Recommendations for prognostic value of oncogenes and tumor suppressor genes in non small cell lung cancer]." Bull Cancer **89**(10): 857-867.

CONTEXT: The "Standards, Options and Recommendations" (SOR) project, started in 1993, is a collaboration between the Federation of French cancer centers (FNCLCC), the 20 French cancer centers, and specialists from French public universities, general hospitals and private clinics. The main objective is the development of clinical practice guidelines to improve the quality of health care and the outcome of cancer patients. The methodology is based on a literature review and critical appraisal by a multidisciplinary group of experts, with feedback from specialists in cancer care delivery. OBJECTIVES: To develop clinical practice guidelines for non small cell lung cancer patients according to the definitions of the Standards, Options and Recommendations project. METHODS: Data were identified by searching Medline, web sites, and using the personal reference lists of members of the expert groups. Once the guidelines were defined, the document was submitted for review to independent reviewers. RESULTS: This article presents the chapter "Prognosis significance of oncogenes and tumor suppressor genes" from the full

report "Standards, Options and Recommendation for non small cell lung cancer" validated in August 2000. The main recommendations are: 1) No clear clinical prognostic value of oncogenes and tumor suppressor genes (p53, bcl-2, Ki-ras, c-erbB-2, Rb, p16) in non small cell lung cancer, can be established from the available evidences (standard, level of evidence C). 2) Prospective multicenter studies should be performed to assess prognostic significance of oncogenes and tumor suppressor genes in non small cell lung cancer.

Christoph, F., et al. (2007). "A gene expression profile of tumor suppressor genes commonly methylated in bladder cancer." J Cancer Res Clin Oncol **133**(6): 343-349.

PURPOSE: The functional relationship between promoter hypermethylation and gene inactivation has been demonstrated for few genes only. We examined the promoter methylation status of two important tumor suppressor genes APAF-1 and DAPK-1 in bladder cancer as well as the mRNA expression pattern of these two genes for possible correlation between promoter hypermethylation and transcriptional repression. METHODS: The methylation status and mRNA expression levels were related to clinicopathological features in 34 patients with transitional cell carcinoma (TCC) of the bladder with a median clinical follow-up of more than 45 months. Tissue from ten patients with nonmalignant disease served as a control group. Quantitative real-time PCR-based detection methods were used for determination of the normalized index of methylation (NIM) as well as the mRNA expression level. RESULTS: APAF-1 and DAPK-1 methylation and mRNA expression was observed in all tumor and normal control samples investigated. Methylation (NIM) levels were significantly higher in tumor tissue for APAF-1 and DAPK-1, but median mRNA expression levels did not differ significantly comparing tumorous and non tumorous tissue. No correlation between expression levels of APAF-1 and DAPK-1 mRNA and tumor stage or grade was observed. However, in superficial TCC a strong correlation between higher NIM levels and lower mRNA expression of the APAF-1 gene was observed (P = 0.014). CONCLUSIONS: Our results, although preliminary, provide first time in vivo expression analysis of the APAF-1 gene in bladder cancer specimen, suggesting expression control by promoter methylation in early stage tumor disease of the bladder.

Diamandis, E. P. (1997). "Clinical applications of tumor suppressor genes and oncogenes in cancer." Clin Chim Acta **257**(2): 157-180.

In the search for new ways to better diagnose and monitor cancer, scientists have turned to oncogenes and tumor suppressor genes. These genes are involved in cell differentiation, communication and proliferation and their alteration is frequently associated with cancer. Such alterations include mutations, translocations, amplifications and deletions. In this review, I give examples of using the detection of such alterations for patient diagnosis and monitoring. The practical examples are restricted to a few cancer types, but the identification of new tumor suppressor genes, like BRCA-1 and BRCA-2, is creating new possibilities for determining cancer risk of individual family members. There is no doubt that the cloning of new genes which predispose to sporadic cancer will lead to the introduction of widespread testing to assess risk and to the application of preventive measures.

Dietrich, P. Y. and J. P. Droz (1992). "[Renal cell cancer: oncogenes and tumor suppressor genes]." Rev Prat **42**(10): 1236-1240.

Recently, there has been a significant increase in the knowledge about the molecular basis of renal cell carcinoma. Its sporadic form is characterized by a deletion on the short arm of chromosome 3, even for localized stages. The same genetic abnormality is found in renal cell carcinomas associated with the von Hippel-Lindau disease. Such findings strongly suggest the presence of a common tumor suppressor gene which seems involved in the genesis of sporadic renal cell carcinoma and in the predisposition of hereditary cancers. Other tumor suppressor genes located on other chromosomes, as well as over expression of growth factors, seem to be related to the progression of this malignancy. Further studies of the molecular events associated with cellular transformation should help to better understand the behavior of such cancers and to find new therapeutical approaches.

Dogan, S., et al. (2015). "Detection of G-type density in promoter sequence of colon cancer oncogenes and tumor suppressor genes." Bioinformation **11**(6): 290-295.

The guanine rich locations are present in human genome. Previous studies have shown that the presence of G rich sequences and motifs may be significant for gene activity and function. We decided to focus our interest to identify G rich motifs in promoters of oncogenes and tumor suppressor genes. We used a set of 100 most common oncogenes and tumor suppressor genes (TSG) for this analysis. We collected 600nt long promoters with -500 and +100 TSS (transcription start site) from the oncogenes and TSG set. Using a computer program, we calculated the G densities using numbers and locations of G forms with 100nt moving widow. We included G numbers from 2 to 7 guanines. Analysis shows that G density

increases from -500 to +100 and more from TSS. G density is found to be maximum within -/+100 of TSS. The results of G densities were compared with the expression data of the selected oncogenes and tumor suppressor genes in patients with colon cancer (n=174).

Dong, J. T. (2001). "Chromosomal deletions and tumor suppressor genes in prostate cancer." Cancer Metastasis Rev **20**(3-4): 173-193.

Chromosomal deletion appears to be the earliest as well as the most frequent somatic genetic alteration during carcinogenesis. It inactivates a tumor suppressor gene in three ways, that is, revealing a gene mutation through loss of heterozygosity as proposed in the two-hit theory, inducing haploinsufficiency through quantitative hemizygous deletion and associated loss of expression, and truncating a genome by homozygous deletion. Whereas the two-hit theory has guided the isolation of many tumor suppressor genes, the haploinsufficiency hypothesis seems to be also useful in identifying target genes of chromosomal deletions, especially for the deletions detected by comparative genomic hybridization (CGH). At present, a number of chromosomal regions have been identified for their frequent deletions in prostate cancer, including 2q13-q33, 5q14-q23, 6q16-q22, 7q22-q32, 8p21-p22, 9p21-p22, 10q23-q24, 12p12-13, 13q14-q21, 16q22-24, and 18q21-q24. Strong candidate genes have been identified for some of these regions, including NKX3.1 from 8p21, PTEN from 10q23, p27/Kip1 from 12p13, and KLF5 from 13q21. In addition to their location in a region with frequent deletion, there are functional and/or genetic evidence supporting the candidacy of these genes. Thus far PTEN is the most frequently mutated gene in prostate cancer, and KLF5 showed the most frequent hemizygous deletion and loss of expression. A tumor suppressor role has been demonstrated for NKX3.1, PTEN, and p27/Kip1 in knockout mice models. Such genes are important targets of investigation for the development of biomarkers and therapeutic regimens.

Drusco, A., et al. (2011). "Common fragile site tumor suppressor genes and corresponding mouse models of cancer." J Biomed Biotechnol **2011**: 984505.

Chromosomal common fragile sites (CFSs) are specific mammalian genomic regions that show an increased frequency of gaps and breaks when cells are exposed to replication stress in vitro. CFSs are also consistently involved in chromosomal abnormalities in vivo related to cancer. Interestingly, several CFSs contain one or more tumor suppressor genes whose structure and function are often affected by chromosomal fragility. The two most active fragile sites in the human genome are FRA3B and FRA16D where the tumor suppressor genes FHIT and WWOX are located, respectively. The best approach to study tumorigenic effects of altered tumor suppressors located at CFSs in vivo is to generate mouse models in which these genes are inactivated. This paper summarizes our present knowledge on mouse models of cancer generated by knocking out tumor suppressors of CFS.

Dunford, A., et al. (2017). "Tumor-suppressor genes that escape from X-inactivation contribute to cancer sex bias." Nat Genet **49**(1): 10-16.

There is a striking and unexplained male predominance across many cancer types. A subset of X-chromosome genes can escape X-inactivation, which would protect females from complete functional loss by a single mutation. To identify putative 'escape from X-inactivation tumor-suppressor' (EXITS) genes, we examined somatic alterations from >4,100 cancers across 21 tumor types for sex bias. Six of 783 non-pseudoautosomal region (PAR) X-chromosome genes (ATRX, CNKSR2, DDX3X, KDM5C, KDM6A, and MAGEC3) harbored loss-of-function mutations more frequently in males (based on a false discovery rate < 0.1), in comparison to zero of 18,055 autosomal and PAR genes (Fisher's exact $P < 0.0001$). Male-biased mutations in genes that escape X-inactivation were observed in combined analysis across many cancers and in several individual tumor types, suggesting a generalized phenomenon. We conclude that biallelic expression of EXITS genes in females explains a portion of the reduced cancer incidence in females as compared to males across a variety of tumor types.

El Ouar, I., et al. (2017). "Effect of Helix aspersa extract on TNFalpha, NF-kappaB and some tumor suppressor genes in breast cancer cell line Hs578T." Pharmacogn Mag **13**(50): 281-285.

BACKGROUND: The garden snail, Helix aspersa, is a big land snail widely found in the Mediterranean countries. It is one of the most consumed species and widely used in zootherapy. OBJECTIVE: The present study was carried out to investigate for the first time the first time the antitumor activity of an aqueous extract from Helix aspersa. MATERIALS AND METHODS: The effect of H. aspersa extract was studied on a triple negative breast cancer cell line Hs578T. Firstly, the morphological changes and the mode of cell death induced by the extract have been evaluated by microscopy and acridine orange/ethidium bromide staining. The effect of the extract at dilution 0.1% and 1% was then tested on some genes, regulators of cell death and proliferation like tumor necrosis factor alpha (TNFalpha), NF- kappaB, and the tumor suppressor genes P53 and PTEN. RESULTS: Data demonstrate that the extract induces necrosis in tumor cells. It

enhances significantly the expression of TNFalpha; mRNA levels were 20 and 10 times more important in treated cells compared to nontreated cells. NF-kappaB and PTEN were inhibited with the dilution 1% after 8 and 24 hours of treatment. P53 expression was further inhibited but only with the highest dose, after 4, 8, and 24 hours. CONCLUSION: Our results show that H. aspersa extract has an antitumor activity against Hs578T cells; it is a potent stimulator for TNFalpha and a good inhibitor for NF-kappaB. Abbreviations used: AO: acridine orange; Bcl-2: B cell lymphoma 2. cDNA: complementary DNA; ELISA: enzyme linked immunosorbent assay; EB: ethidium bromide; IC50: the half maximal inhibitory concentration; mRNA: messenger RNA. MAPK: mitogen-activated protein kinase; NF-kappaB: nuclearfactorkappa B; PBS: phosphate buffered saline. PI3K: phospho-inositol 3 kinase; PTEN: phosphatase and tensin homolog; ROS: reactive oxygen species. RT-PCR: reverse transcription polymerase chain reaction; TNFalpha: tumor necrosis factor alpha. TNFR1: TNF receptor-1; TP53: tumor protein 53.

Esposito, V., et al. (1996). "Altered expression of p53 and Rb tumor suppressor genes in lung cancer." Int J Oncol **9**(3): 439-443.

The aim of this study was to evaluate the frequency of altered expression of pRb and p53, two well known tumor suppressor genes, in lung cancer and to relate it to the prognosis of the patients affected by this type of neoplasm. We evaluated 68 specimens from patients with surgically resected lung cancer. Of the 68 neoplasms investigated, 29 (42.6%) displayed a positive nuclear staining for p53. Ten (15.7%) of the investigated tumors showed absence of pRb nuclear immunoreactivity. p53 overexpression correlated statistically with short-term survival. On the other hand no statistically significant difference (p=ns) in survival was detected between pRb producers and nonproducer patients. In addition, we divided our specimens into two groups according to the p53 and pRb status. The first group consisted of all the p53(+) pRb specimens. The second group contained all the remaining specimens. Comparison between these two groups did not reveal any significant difference in overall survival time. These findings confirm that only p53 overexpression can be considered an independent prognostic factor in lung cancer.

Esteller, M., et al. (1999). "Detection of aberrant promoter hypermethylation of tumor suppressor genes in serum DNA from non-small cell lung cancer patients." Cancer Res **59**(1): 67-70.

Recent evidence suggests that tumor cells may release DNA into the circulation, which is enriched in the serum and plasma, allowing detection of ras and p53 mutations and microsatellite alterations in the serum DNA of cancer patients. We examined whether aberrant DNA methylation might also be found in the serum of patients with non-small cell lung cancer. We tested 22 patients with non-small cell lung cancer using methylation-specific PCR, searching for promoter hypermethylation of the tumor suppressor gene p16, the putative metastasis suppressor gene death-associated protein kinase, the detoxification gene glutathione S-transferase P1, and the DNA repair gene O6-methylguanine-DNA-methyltransferase. Aberrant methylation of at least one of these genes was detected in 15 of 22 (68%) NSCLC tumors but not in any paired normal lung tissue. In these primary tumors with methylation, 11 of 15 (73%) samples also had abnormal methylated DNA in the matched serum samples. Moreover, none of the sera from patients with tumors not demonstrating methylation was positive. Abnormal promoter methylation in serum DNA was found in all tumor stages. Although these results need to be confirmed in larger studies and in other tumor types, detection of aberrant promoter hypermethylation of cancer-related genes in serum may be useful for cancer diagnosis or the detection of recurrence.

Fukushige, S., et al. (2009). "Methyl-CpG targeted recruitment of p300 reactivates tumor suppressor genes in human cancer cells." Biochem Biophys Res Commun **379**(4): 1021-1026.

Aberrant hypermethylation of gene promoters is a major mechanism associated with inactivation of tumor suppressor genes (TSGs) in cancer. We have previously shown that the methyl-CpG targeted transcriptional activation (MeTA) that allows re-expression of TSGs in human cancer cells is accomplished by combining a methyl-CpG binding domain (MBD) with a NFkappaB transcriptional activation domain (AD), accompanied by histone H3K9/K14 acetylation. Herein we demonstrate that p300 histone acetyltransferase (HAT), one of the NFkappaB (AD)-associated coactivators, reactivates epigenetically silenced MLH1 in 293T cells. Interestingly, the HAT domain of p300 is not essential for the reactivation of MLH1; instead, the C-terminal transactivation domain (C-TAD) but not the N-terminal one (N-TAD) reactivates MLH1. Furthermore, all ten of the cancer-related genes analyzed in three types of cancer cells were reactivated by the effect of p300 linked to MBD. These results demonstrate that it is possible to reactivate epigenetically silenced TSGs in human cancer cells by direct targeting of a transcriptional coactivator at highly methylated promoters.

Furukawa, T. and A. Horii (2004). "Molecular pathology of pancreatic cancer: in quest of tumor suppressor genes." Pancreas **28**(3): 253-256.

To find molecular clues useful for early detection and effective therapy for pancreatic cancer, we first carried out genomic analysis by means of comparative genomic hybridization and micro-satellite analysis. We found very complicated molecular alterations in multiple chromosomal regions, including 1p, 6q, 9p, 12q, 17p, 18q, and 21q for losses and 8q and 20q for gains. These diverse changes are very characteristic of pancreatic cancer, and from this information, we developed a method for detecting the aberrant copy numbers of specific chromosomal regions by fluorescence in situ hybridization in cells collected from pancreatic juice for early diagnosis of pancreatic neoplasms. The regions of losses suggest the existence of tumor suppressor genes (TSGs). We identified DUSP6/MKP-3 at 12q21-q22 as a strong candidate TSG; it showed epigenetic inactivation in some fractions of invasive pancreatic cancer and growth suppression and apoptosis by overexpression in vitro. To determine the pathologic roles of 18q, we introduced a normal copy of chromosome 18 into cultured pancreatic cancer cells. The introduction induced marked suppressions of tumor formation and metastasis formation in vivo. We continue work to more completely understand the complex molecular mechanisms of pancreatic carcinogenesis and to apply the information gained to the clinical treatment of pancreatic cancer.

Gao, X., et al. (1997). "Involvement of the multiple tumor suppressor genes and 12-lipoxygenase in human prostate cancer. Therapeutic implications." Adv Exp Med Biol **407**: 41-53.

We performed a detailed and comprehensive study of the involvement of tumor suppressor genes in human prostate cancer. We utilized primers flanking either the restriction fragment length polymorphism (RFLP) or variable number of tandem repeat [VNTR; microsatellite or simple repeat site (SRS)] polymorphic sites to polymerase chain reaction (PCR) amplify the genomic DNA and detect loss of heterozygosity of the target genes. Quantitative reverse transcription (RT)-PCR was performed to measure the mRNA expression levels and PCR/single strand conformational polymorphism (SSCP) and DNA sequencing carried out to detect mutation of the tumor suppressor genes. We found that multiple tumor suppressor genes (e.g., p53, DCC, APC, MCC, BRCA1, and WAF1/CIP1) were inactivated at different frequencies via various mechanisms [e.g., loss of heterozygosity (LOH), loss of expression (LOE), mutation, and inactivation by cellular binding protein]. Several important and novel findings are as following: LOH and LOE of the DCC gene, LOH, LOE, and possible mutation of the APC/MCC genes, LOH of the BRCA1 locus, and mutation of the WAF1/CIP1 gene. For p53 tumor suppressor gene alone, multiple inactivation mechanisms (i.e., LOH, LOE, mutation, and amplification of the cellular inactivating protein MDM2) were identified. A possible involvement of genomic instability or mutator phenotype in human prostate cancer was investigated by microsatellite typing using PCR. A high frequency of microsatellite instability was detected and the microsatellite instability found to correlate with advanced stage and poor differentiation of prostate cancer, suggesting that genes functioning in DNA mismatch repair or general stabilization of the genome may be involved in prostate cancer. The results obtained in this study suggested that multiple tumor suppressor genes (both known and unknown genes) may share the role in prostate cancer; a pattern which has been found in a number of human malignancies such as cancers of the esophagus, colon and breast. In fact, we performed deletion studies aimed at localizing potential tumor suppressor loci on various chromosomal regions. A number of chromosomal regions (i.e., 6p12-24 and 17q21) were found to potentially harbor unidentified tumor suppressor genes. Detailed deletion mapping has localized the potential tumor suppressor loci to a < 2 Mb region centromeric to the BRCA1 gene on chromosome 17q. In addition, we identified a number of novel mechanisms of tumor suppressor gene inactivation, in prostate cancer such as loss of mRNA expression of the DCC, APC, MCC and p53 gene, and mutator phenotype. And for the very first time, we identified somatic mutations of the WAF1/CIP1 gene in primary human malignancy-human prostate cancer. This finding provides the first evidence in primary tumor that the WAF1/CIP1 gene may be a tumor suppressor gene and may be involved in prostate cancer. We identified 12-lipoxygenase (12-LOX) as a potential prognostic marker for human prostate cancer. mRNA expression levels of the 12-LOX gene was measured by quantitative reverse transcription-polymerase chain reaction (RT-PCR) and semi-quantitative in situ hybridization (ISH) in 122 pairs of matched normal and tumor tissues from prostate cancer patients. We found that 12-LOX expression levels were elevated in approximately half of the patients analyzed and the 12-LOX elevation correlates with advanced stage, poor differentiation, and surgical margin positivity. Our data suggest that 12-LOX may serve as a correlative marker for a more aggressive phenotype of prostate cancer and therefore for poor prognosis. We are currently refining our assays for possible clinical applicability. Since not all patients with loss of expression of the DCC gene showed LOH of the DCC locus, there must be other

mechanism (s) responsible for loss of expression of the DCC gene. When we analyzed the relationship between DCC loss of expression and 12-LOX elevation in prostate cancer pati

Garcia-Baquero, R., et al. (2013). "Methylation of a novel panel of tumor suppressor genes in urine moves forward noninvasive diagnosis and prognosis of bladder cancer: a 2-center prospective study." J Urol **190**(2): 723-730.

PURPOSE: Changes in DNA methylation of tumor suppressor genes early in carcinogenesis represent potential indicators of cancer detection and disease evolution. We examined the diagnostic, stratification and prognostic biomarker roles in urine of the methylation of a novel panel of tumor suppressor genes in bladder cancer. MATERIAL AND METHODS: We evaluated the methylation of 18 tumor suppressor genes in 2 prospective, independent sets of urine samples (training set of 120 preparations and validation set of 128) from patients with bladder cancer (170) and controls (78) using methylation specific multiplex ligation-dependent probe amplification. Diagnostic performance was evaluated with ROC curves. Recurrence, progression and disease specific survival were analyzed using univariate and multivariate Cox models. RESULTS: PRDM2, HLTF, ID4, DLC1, BNIP3, H2AFX, CACNA1G, TGIF and CACNA1A were methylated in bladder cancer. CCND2, SCGB3A1, BNIP3, ID4 and RUNX3 were the most frequently methylated tumor suppressor genes in each urine set. Methylation of several tumor suppressor genes correlated with clinicopathological variables, such as stage, tumor grade, focality or age. ROC analysis revealed significant diagnostic accuracy for RUNX3 and CACNA1A in the training set, and for RUNX3 and ID4 in the validation set. On univariate and multivariate analysis CACNA1A methylation correlated with recurrence in the training set, while in the validation set PRDM2 and BNIP3 were significantly associated with recurrence and disease specific survival, respectively. CONCLUSIONS: Tumor suppressor gene methylation allowed for histopathological and clinical stratification. Urine methylation has noninvasive usefulness not only for diagnostic assessment but also as independent bladder cancer prognosticators.

Giordano, A., et al. (1998). "Cell cycle: molecular targets for diagnosis and therapy: tumor suppressor genes and cell cycle progression in cancer." J Cell Biochem **70**(1): 1-7.

A significant portion of published literature is dedicated to describing the cloning and the characterization of proteins involved in the progression of the cell cycle, which govern cell growth both in cancer and normal ontogenesis. With this abundance of information, the cascading pathways of molecular events that occur in the cell cycle are proving to be exceedingly complicated. The purpose of this conference was to attract the leading clinical and basic science investigators in the growth control field with a final goal to determine how this current wealth of knowledge can be used to impact upon patient care and management by the design of novel adjuvant therapeutics specifically targeted at tumor cells and the identification of molecular diagnostic and/or prognostic markers in an efficient and cost effective manner.

Goonesekere, N. C. W., et al. (2018). "Identification of genes highly downregulated in pancreatic cancer through a meta-analysis of microarray datasets: implications for discovery of novel tumor-suppressor genes and therapeutic targets." J Cancer Res Clin Oncol **144**(2): 309-320.

PURPOSE: The lack of specific symptoms at early tumor stages, together with a high biological aggressiveness of the tumor contribute to the high mortality rate for pancreatic cancer (PC), which has a 5-year survival rate of about 7%. Recent failures of targeted therapies inhibiting kinase activity in clinical trials have highlighted the need for new approaches towards combating this deadly disease. METHODS: In this study, we have identified genes that are significantly downregulated in PC, through a meta-analysis of large number of microarray datasets. We have used qRT-PCR to confirm the downregulation of selected genes in a panel of PC cell lines. RESULTS: This study has yielded several novel candidate tumor-suppressor genes (TSGs) including GNMT, CEL, PLA2G1B and SERPINI2. We highlight the role of GNMT, a methyl transferase associated with the methylation potential of the cell, and CEL, a lipase, as potential therapeutic targets. We have uncovered genetic links to risk factors associated with PC such as smoking and obesity. Genes important for patient survival and prognosis are also discussed, and we confirm the dysregulation of metabolic pathways previously observed in PC. CONCLUSIONS: While many of the genes downregulated in our dataset are associated with protein products normally produced by the pancreas for excretion, we have uncovered some genes whose downregulation appear to play a more causal role in PC. These genes will assist in providing a better understanding of the disease etiology of PC, and in the search for new therapeutic targets and biomarkers.

Grander, D. (1998). "How do mutated oncogenes and tumor suppressor genes cause cancer?" Med Oncol **15**(1): 20-26.

In recent decades we have been given insight into the process that transforms a normal cell into a malignant cancer cell. It has been recognised that malignant transformation occurs through successive mutations in specific cellular genes, leading to the activation of oncogenes and inactivation of tumor suppressor genes. The further study of these genes has generated much of its excitement from the convergence of experiments addressing the genetic basis of cancer, together with cellular pathways that normally control important cellular regulatory programmes. In the present review the context in which oncogenes and tumor suppressor genes normally function as key regulators of physiological processes such as proliferation, cell death/apoptosis, differentiation and senescence will be described, as well as how these cellular programmes become deregulated in cancer due to mutations.

Grimm, M. O., et al. (1995). "Inactivation of tumor suppressor genes and deregulation of the c-myc gene in urothelial cancer cell lines." Urol Res **23**(5): 293-300.

Recent investigations have demonstrated p53 and Rb alterations in a subset of transitional cell carcinoma (TCC). Further genetic changes during tumor progression include overexpression of the c-myc gene in a significant number of mainly invasive bladder tumors. To study the possible interactions between these genes in TCC, urothelial cancer cell lines were chosen as an in vitro model. Expression and mutation of p53 was studied in 15 bladder cancer cell lines by immunocytochemistry, Western blot, polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) analysis and direct sequencing of double stranded PCR products of exons 4, 5, 7 and 8 of genomic DNA. C-myc expression and gene structure were studied using Northern and Southern blot techniques Rb protein expression was analyzed by Western blot. Twelve of 15 cell lines showed either p53 mutations or abnormal protein expression. Consistent with previous studies, five cell lines did not express Rb protein. None of the cell lines studied retained both tumor suppressor genes in a functional form. The c-myc gene appeared to be intact in all cell lines and copy numbers were close to normal. Northern analysis demonstrated that all cell lines expressed c-myc mRNA but evidence for altered regulation was found in at least two cell lines. Our data suggest that amplification or translocation are not the underlying mechanism for c-myc overexpression in urothelial tumors. No correlation between loss of Rb protein and c-myc expression was observed. The results presented here for the cell lines match well those obtained in vivo. Thus, these cell lines may provide a suitable model for further analysis of molecular alterations in urothelial cancer.

Hadziavdic, V., et al. (2008). "Microsatellite instability and loss of heterozygosity of tumor suppressor genes in Bosnian patients with sporadic colorectal cancer." Bosn J Basic Med Sci **8**(4): 313-321.

Considering its frequency, high mortality rate as well as many etiological mysteries colorectal cancer is a challenge to contemporary science. In our study we analyzed RER + and RER--phenotypes and their relations with clinical-pathological characteristics of sporadic colorectal cancers. We also analyzed genetic alterations of tumor suppressor genes as well as their relation with microsatellite instability. The study was based on 54 tumor samples and 54 samples of the surrounding healthy tissue of patients with colorectal cancer. According to Amsterdam Criteria and Bethesda Criteria 35/54 or 64,81% belonged in the group of sporadic colorectal cancer. Mononucleotide marker Bat 25 showed instability in 48,57%; Bat 26 in 45,71% and Bat 40 in 29/35 82,86% of tumor samples. Considering dinucleotide markers, TP 53 showed instability in 54,29% and DS123 in 37,14% of tumor samples. Genetic alterations in tumor suppressor genes were found in tumor tissue: NM 23 in 54,29% samples, p53 in 51,43%, APC in 51,43%, DCC2 in 34,29%, RB1 in 22, 86% and DCC 1 in 28,57%. Our studies confirmed that genetic instability had an important role in the development of tumor type. Our results showed that mononucleotide marker Bat 40 might be used for an easy and fast screening procedure in Bosnian population, because it exhibited high percent of microsatellite instability and was in relation with RER+ phenotype. This investigation showed that different genetic alterations may occur during cancer development in each individual patient's tumor. These changes result in MMR inactivation, which causes RER+ phenotype. Our results suggest a connection between alteration in some tumor suppressor genes and MSI phenotype of sporadic colorectal cancer in Bosnian population.

Han, S. Y., et al. (2003). "Candidate tumor suppressor genes at FRA7G are coamplified with MET and do not suppress malignancy in a gastric cancer." Genomics **81**(2): 105-107.

Common fragile sites predispose to specific chromosomal breakage associated with deletion, amplification, and/or translocation in certain forms of cancer. Chromosomal fragile sites not only are susceptible to DNA instability in cancer cells, but may also be associated with genes that contribute to the neoplastic process. FRA7G is a common fragile site containing the candidate tumor suppressor genes

CAV1, CAV2, and TESTIN (TES). The human gastric cancer cell line GTL-16 has an amplification of this genomic region and was used to seek evidence for the suppressor candidacy of one of these genes. Our results demonstrate that CAV1, CAV2, and TESTIN are coamplified with the MET oncogene and overexpressed in GTL-16. Somatic mutation was not detected in the coding regions of these genes, although they were each overexpressed. The results show that CAV1, CAV2, and TESTIN are not tumor suppressor genes in this gastric cancer.

Hedan, B., et al. (2011). "Molecular cytogenetic characterization of canine histiocytic sarcoma: A spontaneous model for human histiocytic cancer identifies deletion of tumor suppressor genes and highlights influence of genetic background on tumor behavior." BMC Cancer **11**: 201.

BACKGROUND: Histiocytic malignancies in both humans and dogs are rare and poorly understood. While canine histiocytic sarcoma (HS) is uncommon in the general domestic dog population, there is a strikingly high incidence in a subset of breeds, suggesting heritable predisposition. Molecular cytogenetic profiling of canine HS in these breeds would serve to reveal recurrent DNA copy number aberrations (CNAs) that are breed and/or tumor associated, as well as defining those shared with human HS. This process would identify evolutionarily conserved cytogenetic changes to highlight regions of particular importance to HS biology. METHODS: Using genome wide array comparative genomic hybridization we assessed CNAs in 104 spontaneously occurring HS from two breeds of dog exhibiting a particularly elevated incidence of this tumor, the Bernese Mountain Dog and Flat-Coated Retriever. Recurrent CNAs were evaluated further by multicolor fluorescence in situ hybridization and loss of heterozygosity analyses. Statistical analyses were performed to identify CNAs associated with tumor location and breed. RESULTS: Almost all recurrent CNAs identified in this study were shared between the two breeds, suggesting that they are associated more with the cancer phenotype than with breed. A subset of recurrent genomic imbalances suggested involvement of known cancer associated genes in HS pathogenesis, including deletions of the tumor suppressor genes CDKN2A/B, RB1 and PTEN. A small number of aberrations were unique to each breed, implying that they may contribute to the major differences in tumor location evident in these two breeds. The most highly recurrent canine CNAs revealed in this study are evolutionarily conserved with those reported in human histiocytic proliferations, suggesting that human and dog HS share a conserved pathogenesis. CONCLUSIONS: The breed associated clinical features and DNA copy number aberrations exhibited by canine HS offer a valuable model for the human counterpart, providing additional evidence towards elucidation of the pathophysiological and genetic mechanisms associated with histiocytic malignancies. Extrapolation of data derived from canine histiocytic disorders to human histiocytic proliferation may help to further our understanding of the propagation and cancerization of histiocytic cells, contributing to development of new and effective therapeutic modalities for both species.

Heidenreich, B., et al. (2000). "Aneuploidy of chromosome 9 and the tumor suppressor genes p16(INK4) and p15(INK4B) detected by in situ hybridization in locally advanced prostate cancer." Eur Urol **38**(4): 475-482.

INTRODUCTION AND OBJECTIVES: The linked p16(INK4)/MTS1 and p15(INK4B)/MTS2 genes on chromosome 9p21 encode proteins that inhibit the cyclinD dependent kinases CDK4/6. Biallelic homozygous deletions involving this locus have been identified in a wide range of tumor cell lines, but in a lower frequency of primary tumors. As PCR based approaches analyzing for homozygous deletions could be confounded by unavoidable contributions of normal cells in microdissected tissue, we performed in situ hybridization (ISH) on primary prostate carcinomas to accurately evaluate p16 and p15 copy numbers on a cell-by-cell basis. MATERIAL AND METHODS: p16 and p15 loci were evaluated in 28 pT3N0M0 prostate cancer specimens. Of 28 patients, 15 (53%) were ascertained showing no recurrence (mean follow-up 61+/-17 months), 13 (47%) developed recurrences within 27+/-19 months. Tissues were provided for ISH analysis in a blinded fashion. Isolated DNA derived from P1 clone 1063 compromising p16 and p15 as well as a centromeric probe for chromosome 9 were used for hybridization. Signals were enumerated within 300 interphase nuclei per tumor specimen, and in 100 nuclei derived from 18 benign prostate tissues and 7 adjacent PIN regions. RESULTS: ISH detected aneuploid tumors in 12/13 (92%) patients with recurrence and in 5/15 (33%) without recurrence ($p<0.0014$). Whereas 3/7 PIN specimens associated with nonrecurrent PCA demonstrated euploidy, all 4/7 PIN associated with recurrent disease demonstrated the same aneuploidy for chr9 as the primary tumor. All benign tissues evaluated exhibited euploidy for chr9, p16 and p15. None of the PCA and PIN samples revealed homozygous deletions for p16(INK4)/MTS1/p15(INK4B)/MTS2; 2/28 (7.1%) PCA exhibited partial deletion for p16(INK4)/MTS1/p15(INK4B)/MTS2 and aneuploidy for chr9; both PCA derived from the recurrent group.

CONCLUSIONS: Deletion of 9p21 was rare and therefore such genetic alterations may not play an important role in the pathogenesis of PCA. Analysis of the limited number of PCA examined suggest a strong association between chr9 aneuploidy and recurrenct disease. Aneuploidy in both PIN and PCA suggests that the clinical outcome of PCA might already be determined in the preinvasive PIN.

Herman, J. G. (1999). "Hypermethylation of tumor suppressor genes in cancer." Semin Cancer Biol **9**(5): 359-367.

Hypermethylation of tumor suppressor genes and other genes functionally important in the neoplastic process is a recently recognized process. This epigenetic process is characterized by loss of function of these genes associated with transcriptional loss in the absence of structural alterations. The growing list of genes inactivated by promoter region hypermethylation provide an opportunity to examine the patterns of inactivation of such genes and provide clues as to the roles these events play in the neoplastic process.

Hernandez-Rosas, F., et al. (2018). "Histone deacetylase inhibitors induce the expression of tumor suppressor genes Per1 and Per2 in human gastric cancer cells." Oncol Lett **16**(2): 1981-1990.

Period circadian regulator (Per)1 and Per2 genes are involved in the molecular mechanism of the circadian clock, and exhibit tumor suppressor properties. Several studies have reported a decreased expression of Per1, Per2 and Per3 genes in different types of cancer and cancer cell lines. Promoter methylation downregulates Per1, Per2 or Per3 expression in myeloid leukemia, breast, lung, and other cancer cells; whereas histone deacetylase inhibitors (HDACi) upregulate Per1 or Per3 expression in certain cancer cell lines. However, the transcriptional regulation of Per1 and Per2 in cancer cells by chromatin modifications is not fully understood. The present study aimed to determine whether HDACi regulate Per1 and Per2 expression in gastric cancer cell lines, and to investigate changes in chromatin modifications in response to HDACi. Treatment of KATO III and NCI-N87 human gastric cancer cells with sodium butyrate (NaB) or Trichostatin A (TSA) induced Per1 and Per2 mRNA expression in a dose-dependent manner. Chromatin immunoprecipitaion assays revealed that NaB and TSA decreased lysine 9 trimethylation on histone H3 (H3K9me3) at the Per1 promoter. TSA, but not NaB increased H3K9 acetylation at the Per2 promoter. It was also observed that binding of Sp1 and Sp3 to the Per1 promoter decreased following NaB treatment, whereas Sp1 binding increased at the Per2 promoter of NaB- and TSA-treated cells. In addition, Per1 promoter is not methylated in KATO III cells, while Per2 promoter was methylated, although NaB, TSA, and 5-Azacytidine do not change the methylated CpGs analyzed. In conclusion, HDACi induce Per1 and Per2 expression, in part, through mechanisms involving chromatin remodeling at the proximal promoter of these genes; however, other indirect mechanisms triggered by these HDACi cannot be ruled out. These findings reveal a previously unappreciated regulatory pathway between silencing of Per1 gene by H3K9me3 and upregulation of Per2 by HDACi in cancer cells.

Ho, W. L., et al. (2002). "Loss of heterozygosity at loci of candidate tumor suppressor genes in microdissected primary non-small cell lung cancer." Cancer Detect Prev **26**(5): 343-349.

To investigate the etiological association of allelic loss at chromosomal regions containing tumor suppressor genes (TSGs) in non-small cell lung cancer (NSCLC) in Taiwan, we examined 48 microdissected NSCLC samples for loss of heterozygosity (LOH) at nine loci where TSGs are localized nearby. The associations of LOH at each locus with clinicoparameters and prognosis were also examined. The frequent LOH was observed using markers, D3S1285 near the FHIT gene (58.3%), D17S938 near the p53 gene (56.7%), D9S925 near the p16 gene (54.5%), and D13S153 near the RB gene (47.6%). The occurrence of LOH at each TSG locus was compared with the patients' clinicoparameters. The incidence of LOH at D17S938 (p53 gene) and D3S4545 (VHL gene) was significantly higher in squamous carcinoma tumors than in adenocarcinoma tumors ($P = 0.003$ and 0.024, respectively). LOH of these two loci also occurred frequently in tumors from smoker patients compared to that from nonsmoker patients ($P = 0.013$ and 0.025, respectively). LOH at D13S153 (RB gene) was also associated with smoking ($P = 0.008$). In addition, the prognostic analyses indicated that the patients with LOH at D18S535 (18q21, near the SMAD2/4 gene) had significantly longer post-operative survival time compared to those without LOH ($P = 0.03$). Our results suggested that LOH at FHIT, p53, and p16 genes may occur frequently in NSCLC patients in Taiwan. In addition, LOH at p53, RB, and VHL may associate with smoking or squamous carcinoma patients and LOH at SMAD2/4 may be correlated with better prognosis.

Hollingsworth, R. E. and W. H. Lee (1991). "Tumor suppressor genes: new prospects for cancer research." J Natl Cancer Inst **83**(2): 91-96.

Cancer is thought to arise from the accumulation of several genetic mutations in a single cell. Until recently, the only tumorigenic mutations that have

been studied in detail are those that activate oncogenes. The discovery of tumor suppressor genes, for which inactivating mutations elicit tumorigenesis, has added a new dimension to our understanding of neoplasia. The retinoblastoma susceptibility gene RB is the prototype tumor suppressor gene and has been shown to suppress the transformed phenotype for several different cancers. Additional studies have revealed other tumor suppressor genes that may operate in a variety of tissues through a variety of mechanisms. These mechanisms may regulate the choice between cellular proliferation and differentiation and appear to involve such processes as the initiation of DNA replication, regulation of expression of certain genes, intercellular communication and adhesion, and the transduction of external signals to intracellular effectors. The elucidation of these mechanisms will enhance our understanding of both oncogenesis and the fundamental operations of the cell.

Ivanov, I., et al. (2007). "Identifying candidate colon cancer tumor suppressor genes using inhibition of nonsense-mediated mRNA decay in colon cancer cells." Oncogene **26**(20): 2873-2884.

Inhibition of the nonsense-mediated decay (NMD) mechanism in cells results in stabilization of transcripts carrying premature translation termination codons. A strategy referred to as gene identification by NMD inhibition (GINI) has been proposed to identify genes carrying nonsense mutations. Genes containing frameshift mutations in colon cancer cell line have been identified using a modified version of GINI. To increase the efficiency of identifying mutant genes using GINI, we have now further improved the strategy. In this approach, inhibition of NMD with emetine is complemented with inhibiting NMD by blocking the phosphorylation of the hUpf1 protein with caffeine. In addition, to enhance the GINI strategy, comparing mRNA level alterations produced by inhibiting transcription alone or inhibiting transcription together with NMD following caffeine pretreatment were used for the efficient identification of false positives produced as a result of stress response to NMD inhibition. To demonstrate the improved efficiency of this approach, we analysed colon cancer cell lines showing microsatellite instability. Bi-allelic inactivating mutations were found in the FXR1, SEC31L1, NCOR1, BAT3, PHF14, ZNF294, C19ORF5 genes as well as genes coding for proteins with yet unknown functions.

Jha, A. K., et al. (2016). "A Comparative Analysis of Methylation Status of Tumor Suppressor Genes in Paired Biopsy and Serum Samples from Cervical Cancer Patients among North Indian Population." Genetika **52**(2): 255-259.

Tumor-specific genetic or epigenetic alterations have been detected in serum DNA in case of various types of cancers. In breast cancer, the detection of tumor suppressor gene hypermethylation has been reported in several body fluids. Promoter hypermethylation of some genes like MYOD1, CALCA, hTERT etc. has also been detected in serum samples from cervical cancer. The present study is the first report on the comparison of promoter hypermethylation of tumor suppressor genes likep14, p15, p16, p21, p27, p57, p53, p73, RARbeta2, FHIT, DAPK, STAT1 and-RB1 genes in paired biopsy and serum samples from cervical cancer patients among north Indian population. This is also the first report on the hypermethylation of these genes in serum samples from cervical cancer patients among north Indian population. According to the results of the present study, promoter hypermethylation of these genes can also be detected in serum samples of cervical cancer patients. The sensitivity of detection of promoter hypermethylation in serum samples of cervical cancer patients as compared to paired biopsy samples was found to be around 83.3%. It was observed that promoter hypermethylation was mainly observed in the serum samples in the higher stages and very rarely in the lower stages. The present study clearly showed that serum of patients with cervical cancer can also be used to study methylated genes as biomarkers.

Kagan, J., et al. (1995). "Homozygous deletions at 8p22 and 8p21 in prostate cancer implicate these regions as the sites for candidate tumor suppressor genes." Oncogene **11**(10): 2121-2126.

Frequent loss of an allele at specific chromosomal regions implicates these regions as sites of tumor suppressor genes (TSG) that become inactivated during tumor progression. We have studied chromosome 8p allele losses in 32 primary human prostate carcinomas with 16 polymorphic microsatellite sequences. Overall, 22 of 32 (69%) informative specimens showed loss of allele in at least one locus. The most frequent losses of heterozygosity (LOH) occurred at the LPL locus (46%) on chromosome 8p22 and at the D8S360 (45%) and NEFL (43%) loci on chromosome 8p21. Homozygous deletions were detected at the LPL and NEFL loci at 8p22 and 8p21, respectively. The minimal region with frequent LOH and homozygous deletion, around the LPL locus, was restricted between the MSR locus and the D8S258 marker, separated by less than 9 cM. The second region was restricted between markers D8S1128 and D8S131 separated by 12 cM. The results suggest the existence of two chromosome 8p sites for candidate TSGs in prostate cancer.

Kaino, M. (1997). "Alterations in the tumor suppressor genes p53, RB, p16/MTS1, and p15/MTS2 in human pancreatic cancer and hepatoma cell lines." J Gastroenterol **32**(1): 40-46.

The tumor suppressor genes p53, retinoblastoma (RB), p16, and p15 encode proteins that regulate the cell cycle cooperatively by controlling the transition from G1 to S phase and may play an important role in cell growth and differentiation. To screen for abnormalities in these genes in cancer, we performed genetic analysis in six human pancreatic cancer and five hepatoma cell lines, by single-strand conformation polymorphism (SSCP) analysis, direct sequencing, and the reverse transcriptase-polymerase chain reaction (RT-PCR). All six pancreatic cancer cell lines had p53 mutations, with the concomitant loss of the other normal allele, encoding wild-type p53. Frequent homozygous deletions were found in p16 and p15, but the RB gene was expressed. Four of the five hepatoma cell lines had p53 mutations with loss of the normal allele and aberrant RB. There were no deletions of p16 and p15 in any of the hepatoma cell lines. These findings suggest that alterations in the p53, p16, and p15 genes are common in human pancreatic cancer cell lines, while p53 or RB mutations are common in hepatoma cell lines. Alterations of these tumor suppressor genes may thus be important features in organ-specific carcinogenesis.

Kaise, M., et al. (2008). "CpG island hypermethylation of tumor-suppressor genes in H. pylori-infected non-neoplastic gastric mucosa is linked with gastric cancer risk." Helicobacter **13**(1): 35-41.

BACKGROUND AND AIM: Gastric carcinogenesis involves CpG island hypermethylation (CIHM) of tumor-suppressor genes. Although the CIHM of these genes occurs in non-neoplastic gastric cells, it is unclear whether this epigenetic alteration is linked with aging and/or gastric cancer risk. We investigated this linkage in noncancerous gastric mucosa infected with H. pylori. SUBJECTS AND METHODS: Noncancerous corpus mucosa was endoscopically obtained from H. pylori-positive gastric cancer patients (n = 34), and age-matched H. pylori-positive noncancerous controls (n = 68). Genomic DNA retrieved from the mucosa was subjected to methylation-specific polymerase chain reaction for p16, Ecad, and DAPK genes. Linkage between CIHM and clinicopathologic factors was evaluated. RESULTS: CIHM rates of DAPK, Ecad, and p16 promoters were significantly higher in noncancerous gastric mucosa of gastric cancer patients (91, 88, and 68%, respectively) than in noncancerous controls (71, 53, and 25%, respectively). Multivariate regression analysis showed a significant linkage between CIHM in noncancerous mucosa and coexistence of gastric cancer. Significant linkage between polymorphoneutrophil infiltration and CIHM was observed except for CIHM of p16. No linkage was observed between CIHM and other parameters, including age. High CIHM status (all three tested genes methylated) was associated with an increased risk of gastric cancer, with an odds ratio of 9.8 (95% confidence interval, 3.8-25.3). CONCLUSIONS: In a subset of the H. pylori-infected population, CIHM of tumor-suppressor genes in noncancerous gastric mucosa is linked with the risk of gastric cancer and polymorphoneutrophil infiltration, but not aging. CIHM is a potential marker of gastric cancer risk.

Kashuba, V. I., et al. (2009). "High mutability of the tumor suppressor genes RASSF1 and RBSP3 (CTDSPL) in cancer." PLoS One **4**(5): e5231.

BACKGROUND: Many different genetic alterations are observed in cancer cells. Individual cancer genes display point mutations such as base changes, insertions and deletions that initiate and promote cancer growth and spread. Somatic hypermutation is a powerful mechanism for generation of different mutations. It was shown previously that somatic hypermutability of proto-oncogenes can induce development of lymphomas. METHODOLOGY/PRINCIPAL FINDINGS: We found an exceptionally high incidence of single-base mutations in the tumor suppressor genes RASSF1 and RBSP3 (CTDSPL) both located in 3p21.3 regions, LUCA and AP20 respectively. These regions contain clusters of tumor suppressor genes involved in multiple cancer types such as lung, kidney, breast, cervical, head and neck, nasopharyngeal, prostate and other carcinomas. Altogether in 144 sequenced RASSF1A clones (exons 1-2), 129 mutations were detected (mutation frequency, MF = 0.23 per 100 bp) and in 98 clones of exons 3-5 we found 146 mutations (MF = 0.29). In 85 sequenced RBSP3 clones, 89 mutations were found (MF = 0.10). The mutations were not cytidine-specific, as would be expected from alterations generated by AID/APOBEC family enzymes, and appeared de novo during cell proliferation. They diminished the ability of corresponding transgenes to suppress cell and tumor growth implying a loss of function. These high levels of somatic mutations were found both in cancer biopsies and cancer cell lines. CONCLUSIONS/SIGNIFICANCE: This is the first report of high frequencies of somatic mutations in RASSF1 and RBSP3 in different cancers suggesting it may underlay the mutator phenotype of cancer. Somatic hypermutations in tumor suppressor genes involved in major human malignancies offer a novel insight in cancer development, progression and spread.

Kawakami, T., et al. (2003). "Multipoint methylation and expression analysis of tumor suppressor genes in human renal cancer cells." Urology **61**(1): 226-230.

OBJECTIVES: To analyze the methylation status and expression profiles of multiple tumor suppressor genes in renal cell carcinoma-derived cell lines. Aberrant promoter methylation is commonly found in human cancers. Nonetheless, it is challenging to demonstrate that methylation of a specific gene results in gene inactivation. METHODS: We simultaneously analyzed methylation and expression profiles of five putative tumor suppressor genes (p15, p16, Rb, BRCA1, and E-cadherin) in 14 different cell lines using bisulfite genomic sequencing and reverse transcriptase-polymerase chain reaction. We also used multiplex polymerase chain reaction to identify homozygous deletions at the p15 and p16 loci. RESULTS: Expression of p16, BRCA1, and E-cadherin was maintained in 4 (29%) of 14 cell lines, regardless of the presence of methylation. Aberrant methylation of p16 was observed in 2 (14%), of BRCA1 in 1 (7%), and of E-cadherin in 9 (64%) of 14 cell lines. Concurrent methylation was observed among p16 and BRCA1 (1 [7%] of 14 cell lines) and among p16 and E-cadherin (1 [7%] of 14 cell lines). We detected homozygous deletion of p16 and p15 in 11 (78%) and 6 (43%) cell lines, respectively. CONCLUSIONS: The present data shows the presence of methylation does not always contribute to the loss of expression of tumor suppressor genes. Therefore, we must be cautious in interpreting the results of methylation assays--in particular, detection of methylation by nonquantitative methods. The data also demonstrated that multiple tumor suppressor genes are simultaneously inactivated in renal cell carcinoma-derived cell lines by distinctive mechanisms.

Khan, M. A., et al. (2015). "(-)-Epigallocatechin-3-gallate reverses the expression of various tumor-suppressor genes by inhibiting DNA methyltransferases and histone deacetylases in human cervical cancer cells." Oncol Rep **33**(4): 1976-1984.

There has been increasing evidence that numerous bioactive dietary agents can hamper the process of carcinogenesis by targeting epigenetic alterations including DNA methylation. This therapeutic approach is considered as a significant goal for cancer therapy due to the reversible nature of epigenetic-mediated gene silencing and warrants further attention. One such dietary agent, green tea catechin, (-)-epigallocatechin-3-gallate (EGCG) has been shown to modulate many cancer-related pathways. Thus, the present study was designed to investigate the role of EGCG as an epigenetic modifier in HeLa cells. DNA methyltransferase (DNMT) and histone deacetylase (HDAC) inhibition assays were conducted, and the transcription levels of DNMT3B and HDAC1 were assessed by enzymatic activity assay and RT-PCR, respectively. Furthermore, we studied the binding interaction of EGCG with DNMT3B and HDAC1 by molecular modeling as well as promoter DNA methylation and expression of retinoic acid receptor-beta (RARbeta), cadherin 1 (CDH1) and death-associated protein kinase-1 (DAPK1) in EGCG-treated HeLa cells by RT-PCR and MS-PCR. In the present study, time-dependent EGCG-treated HeLa cells were found to have a significant reduction in the enzymatic activity of DNMT and HDAC. However, the expression of DNMT3B was significantly decreased in a time-dependent manner whereas there was no significant change in HDAC1 expression. Molecular modeling data also supported the EGCG-mediated DNMT3B and HDAC1 activity inhibition. Furthermore, time-dependent exposure to EGCG resulted in reactivation of known tumor-suppressor genes (TSGs) in HeLa cells due to marked changes in the methylation of the promoter regions of these genes. Overall, the present study suggests that EGCG may have a significant impact on the development of novel epigenetic-based therapy.

Khatami, F., et al. (2017). "Meta-analysis of promoter methylation in eight tumor-suppressor genes and its association with the risk of thyroid cancer." PLoS One **12**(9): e0184892.

Promoter methylation in a number of tumor-suppressor genes (TSGs) can play crucial roles in the development of thyroid carcinogenesis. The focus of the current meta-analysis was to determine the impact of promoter methylation of eight selected candidate TSGs on thyroid cancer and to identify the most important molecules in this carcinogenesis pathway. A comprehensive search was performed using Pub Med, Scopus, and ISI Web of Knowledge databases, and eligible studies were included. The methodological quality of the included studies was evaluated according to the Newcastle Ottawa scale table and pooled odds ratios (ORs); 95% confidence intervals (CIs) were used to estimate the strength of the associations with Stata 12.0 software. Egger's and Begg's tests were applied to detect publication bias, in addition to the "Metatrim" method. A total of 55 articles were selected, and 135 genes with altered promoter methylation were found. Finally, we included eight TSGs that were found in more than four studies (RASSF1, TSHR, PTEN, SLC5A, DAPK, P16, RARbeta2, and CDH1). The order of the pooled ORs for these eight TSGs from more to less significant was CDH1 (OR = 6.73), SLC5 (OR = 6.15), RASSF1 (OR

= 4.16), PTEN (OR = 3.61), DAPK (OR = 3.51), P16 (OR = 3.31), TSHR (OR = 2.93), and RARbeta2 (OR = 1.50). Analyses of publication bias and sensitivity confirmed that there was very little bias. Thus, our findings showed that CDH1 and SCL5A8 genes were associated with the risk of thyroid tumor genesis.

Kikuno, N., et al. (2008). "Genistein mediated histone acetylation and demethylation activates tumor suppressor genes in prostate cancer cells." Int J Cancer **123**(3): 552-560.

Genistein is a phytoestrogen that has been reported to suppress the AKT signaling pathway in several malignancies. However, the molecular mechanism of genistein action is not known. We tested the hypothesis that genistein activates expression of several aberrantly silenced tumor suppressor genes (TSGs) that have unmethylated promoters such as PTEN, CYLD, p53 and FOXO3a. We report here that genistein activates TSGs through remodeling of the heterochromatic domains at promoters in prostate cancer cells by modulating histone H3-Lysine 9 (H3-K9) methylation and deacetylation. Genistein activation involved demethylation and acetylation of H3-K9 at the PTEN and the CYLD promoter, while acetylation of H3-K9 at the p53 and the FOXO3a promoter occurred through reduction of endogenous SIRT1 activity. There was a decrease of SIRT1 expression and accumulation of SIRT1 in the cytoplasm from the nucleus. Increased expression of these TSGs was also reciprocally related to attenuation of phosphorylated-AKT and NF-kappaB binding activity in prostate cancer cells. This is the first report describing a novel epigenetic pathway that activates TSGs by modulating either histone H3-Lysine 9 (H3-K9) methylation or deacetylation at gene promoters leading to inhibition of the AKT signaling pathway. These findings strengthen the understanding of how genistein may be chemoprotective in prostate cancer.

Kim, M. S., et al. (1993). "State of p53, Rb and DCC tumor suppressor genes in human oral cancer cell lines." Anticancer Res **13**(5A): 1405-1413.

The tumor suppressor genes p53, Rb, and DCC were studied in five human oral cancer cell lines (FaDu, SCC-4, HEp-2, 1483, and OEC-M1) and in primary normal human oral keratinocytes (NHOK). All tested cancer lines had similar amount of p53 messages to normal cells, but the cancer lines FaDu and SCC-4 contained significantly higher p53 protein levels than did the normal counterpart. Sequencing p53 cDNA for these cancer cells showed point mutations: In the FaDu cell line, a mutation of CGG to CTG occurred at codon 248; and in the SCC-4 cell line, a mutation of CCC to TCC occurred at codon 151. The HEp-2 and 1483 cancer lines translated very low levels of p53 protein compared to the normal counterpart. Sequencing of p53 cDNA for HEp-2 and 1483 lines showed no mutations. Southern and Northern analyses revealed that these cell lines harbored HPV-18 DNA and expressed the viral E6/E7 protein. The OEC-M1 line showed different restriction fragment length polymorphism for the p53 gene compared with other cells, and did not express p53. All oral cancer cell lines except the OEC-M1 cells expressed both phosphorylated and hypophosphorylated Rb proteins. Further, the OEC-M1 line expressed smaller sized hypophosphorylated Rb proteins compared with normal cells. Unlike the other cancer lines, the HEp-2 and OEC-M1 lines also did not contain DCC mRNAs. These data indicate that "high risk" HPV infections and mutations of p53, Rb, and DCC genes are frequently found in oral cancer cells and may be associated with oral cancer.

Kohno, T., et al. (2010). "A catalog of genes homozygously deleted in human lung cancer and the candidacy of PTPRD as a tumor suppressor gene." Genes Chromosomes Cancer **49**(4): 342-352.

A total of 176 genes homozygously deleted in human lung cancer were identified by DNA array-based whole genome scanning of 52 lung cancer cell lines and subsequent genomic PCR in 74 cell lines, including the 52 cell lines scanned. One or more exons of these genes were homozygously deleted in one (1%) to 20 (27%) cell lines. These genes included known tumor suppressor genes, e.g., CDKN2A/p16, RB1, and SMAD4, and candidate tumor suppressor genes whose hemizygous or homozygous deletions were reported in several types of human cancers, such as FHIT, KEAP1, and LRP1B/LRP-DIP. CDKN2A/p16 and p14ARF located in 9p21 were most frequently deleted (20/74, 27%). The PTPRD gene was most frequently deleted (8/74, 11%) among genes mapping to regions other than 9p21. Somatic mutations, including a nonsense mutation, of the PTPRD gene were detected in 8/74 (11%) of cell lines and 4/95 (4%) of surgical specimens of lung cancer. Reduced PTPRD expression was observed in the majority (>80%) of cell lines and surgical specimens of lung cancer. Therefore, PTPRD is a candidate tumor suppressor gene in lung cancer. Microarray-based expression profiling of 19 lung cancer cell lines also indicated that some of the 176 genes, such as KANK and ADAMTS1, are preferentially inactivated by epigenetic alterations. Genetic/epigenetic as well as functional studies of these 176 genes will increase our understanding of molecular mechanisms behind lung carcinogenesis.

Komarova, N. L., et al. (2003). "Mutation-selection networks of cancer initiation: tumor

suppressor genes and chromosomal instability." J Theor Biol **223**(4): 433-450.

In this paper, we derive analytic solutions of stochastic mutation-selection networks that describe early events of cancer formation. A main assumption is that cancer is initiated in tissue compartments, where only a relatively small number of cells are at risk of mutating into cells that escape from homeostatic regulation. In this case, the evolutionary dynamics can be approximated by a low-dimensional stochastic process with a linear Kolmogorov forward equation that can be solved analytically. Most of the time, the cell population is homogeneous with respect to relevant mutations. Occasionally, such homogeneous states are connected by 'stochastic tunnels'. We give a precise analysis of the existence of tunnels and calculate the rate of tunneling. Finally, we calculate the conditions for chromosomal instability (CIN) to precede inactivation of the first tumor suppressor gene. In this case, CIN is an early event and a driving force of cancer progression. The techniques developed in this paper can be used to study arbitrarily complex mutation-selection networks of the somatic evolution of cancer.

Komarova, N. L. and D. Wodarz (2004). "The optimal rate of chromosome loss for the inactivation of tumor suppressor genes in cancer." Proc Natl Acad Sci U S A **101**(18): 7017-7021.

Many cancers are characterized by chromosomal instability (CIN). This phenotype involves the deletion and duplication of chromosomes or chromosome parts and results in a high degree of aneuploidy. The role of CIN for cancer progression is a very important, yet unresolved question. It has been argued that CIN contributes to cancer initiation because chromosome loss can unmask a mutated tumor suppressor (TSP) gene. At the same time, CIN is costly for the cell because it destroys the genome and therefore compromises clonal expansion. Here, we use mathematical models to determine whether CIN can accelerate the generation and expansion of TSP (-/-) cells in the context of this tradeoff. Comparing cells with different degrees of CIN, we find that the emergence and growth of TSP (-/-) cells is optimized if the rate of chromosome loss is of the order of 10(-3) to 10(-2). This result is very robust, is independent of parameter values, and coincides with experimental measures using colon cancer cell lines. However, if we consider all of the steps in the pathway, including the generation of the CIN phenotype from stable cells, then it turns out that the emergence and growth of TSP (-/-) cells is never accelerated by CIN. Therefore, CIN does not arise because it accelerates the accumulation of adaptive mutations. Instead, it arises for other reasons, such as environmental factors, and is subsequently fine-tuned by selection to minimize the time to further cancer progression by means of the inactivation of TSP genes.

Krawczyk, B., et al. (2007). "The effects of nucleoside analogues on promoter methylation of selected tumor suppressor genes in MCF-7 and MDA-MB-231 breast cancer cell lines." Nucleosides Nucleotides Nucleic Acids **26**(8-9): 1043-1046.

The effects of 2-chloro-2'-deoxyadenosine, 9-beta-D-arabinofuranosyl-2-fluoroadenine, and 5-aza-2'-deoxycytidine on promoter methylation of the selected tumor suppressor genes (i.e., ERalpha, BRCA1, RARbeta2, E-cadherin, PTEN, and APC) were estimated using methylation-sensitive restriction analysis. The studies were carried out in hormone-responsive, low-invasive cell line MCF-7 and hormone-insensitive, highly invasive cell line MDA-MB-231. The results demonstrate an implication of the tested adenosine analogues and 5-aza-dCyd in regulation of DNA methylation process. Moreover, the effects of nucleoside analogues on PTEN promoter methylation suggest distinct mechanism of regulation of the epigenetic DNA modification in low-invasive compared to highly invasive breast cancer cells.

Lomberk, G. A. (2011). "Epigenetic silencing of tumor suppressor genes in pancreatic cancer." J Gastrointest Cancer **42**(2): 93-99.

INTRODUCTION: Without any alteration of DNA sequence, heritable changes in gene expression, caused by epigenetic pathways, are gaining a spotlight in research of diseases, and in particular, cancer. Although the dominant paradigm in cancer research, proposed by Vogelstein, suggested that cancer progression was caused by a sequential accumulation of genetic aberrations, basic science studies in epigenetics have now advanced our knowledge enough to apply its concepts and methodology to the study of cancer. In fact, chromatin dynamics and small RNAs are altered far more prevalently in cancer than genetic alterations and most important, can be reversible, lending themselves as attractive therapeutic targets. CONCLUDING REMARKS: In the current review, the inactivation of p16 will be utilized as the most prominent example of epigenetic silencing of a tumor suppressor gene in pancreatic cancer. In addition, fundamental insight will be given into why and how epigenetics can be targeted for therapeutic purposes. This knowledge will help the reader in determining the breadth and depth of this field of study with potentially high impact to oncology.

Lopes, E. C., et al. (2008). "Kaiso contributes to DNA methylation-dependent silencing of tumor

suppressor genes in colon cancer cell lines." Cancer Res **68**(18): 7258-7263.

Aberrant CpG methylation of tumor suppressor gene regulatory elements is associated with transcriptional silencing and contributes to malignant transformation of different tissues. It is presumed that methylated DNA sequences recruit repressor machinery to actively shutdown gene expression. The Kaiso protein is a transcriptional repressor expressed in human and murine colorectal tumors that can bind to methylated clusters of CpG dinucleotides. We show here that Kaiso represses methylated tumor suppressor genes and can bind in a methylation-dependent manner to the CDKN2A in human colon cancer cell lines. The contribution of Kaiso to epigenetic silencing was underlined by the fact that Kaiso depletion induced tumor suppressor gene expression without affecting DNA methylation levels. As a consequence, colon cancer cells became susceptible to cell cycle arrest and cell death mediated by chemotherapy. The data suggest that Kaiso is a methylation-dependent "opportunistic" oncogene that silences tumor suppressor genes when they become hypermethylated. Because Kaiso inactivation sensitized colon cancer cell lines to chemotherapy, it is possible that therapeutic targeting of Kaiso could improve the efficacy of current treatment regimens.

Lopez-Serra, L., et al. (2006). "A profile of methyl-CpG binding domain protein occupancy of hypermethylated promoter CpG islands of tumor suppressor genes in human cancer." Cancer Res **66**(17): 8342-8346.

Methyl-CpG binding domain (MBD) proteins have been shown to couple DNA methylation to transcriptional repression. This biological property suggests a role for MBD proteins in the silencing of tumor suppressor genes that are hypermethylated at their promoter CpG islands in cancer cells. Despite the demonstration of the presence of MBDs in the methylated promoter of several genes, we still ignore how general and specific is this association. Here, we investigate the profile of MBD occupancy in a large panel of tumor suppressor gene promoters and cancer cell lines. Our study shows that most hypermethylated promoters are occupied by MBD proteins, whereas unmethylated promoters are generally devoid of MBDs, with the exception of MBD1. Treatment of cancer cells with the demethylating agent 5-aza-2'-deoxycytidine results in CpG island hypomethylation, MBD release, and gene reexpression, reinforcing the notion that association of MBDs with methylated promoters is methylation-dependent. Whereas several promoters are highly specific in recruiting a particular set of MBDs, other promoters seem to be less exclusive. Our results indicate that MBDs have a great affinity in vivo for binding hypermethylated promoter CpG islands of tumor suppressor genes, with a specific profile of MBD occupancy that it is gene and tumor type specific.

Lu, H., et al. (2014). "CK2 phosphorylates and inhibits TAp73 tumor suppressor function to promote expression of cancer stem cell genes and phenotype in head and neck cancer." Neoplasia **16**(10): 789-800.

Cancer stem cells (CSC) and genes have been linked to cancer development and therapeutic resistance, but the signaling mechanisms regulating CSC genes and phenotype are incompletely understood. CK2 has emerged as a key signal serine/threonine kinase that modulates diverse signal cascades regulating cell fate and growth. We previously showed that CK2 is often aberrantly expressed and activated in head and neck squamous cell carcinomas (HNSCC), concomitantly with mutant (mt) tumor suppressor TP53, and inactivation of its family member, TAp73. Unexpectedly, we observed that classical stem cell genes Nanog, Sox2, and Oct4, are overexpressed in HNSCC with inactivated TAp73 and mtTP53. However, the potential relationship between CK2, TAp73 inactivation, and CSC phenotype is unknown. We reveal that inhibition of CK2 by pharmacologic inhibitors or siRNA inhibits the expression of CSC genes and side population (SP), while enhancing TAp73 mRNA and protein expression. Conversely, CK2 inhibitor attenuation of CSC protein expression and the SP by was abrogated by TAp73 siRNA. Bioinformatic analysis uncovered a single predicted CK2 threonine phosphorylation site (T27) within the N-terminal transactivation domain of TAp73. Nuclear CK2 and TAp73 interaction, confirmed by co-immunoprecipitation, was attenuated by CK2 inhibitor, or a T27A point-mutation of this predicted CK2 threonine phospho-acceptor site of TAp73. Further, T27A mutation attenuated phosphorylation, while enhancing TAp73 function in repressing CSC gene expression and SP cells. A new CK2 inhibitor, CX-4945, inhibited CSC related SP cells, clonogenic survival, and spheroid formation. Our study unveils a novel regulatory mechanism whereby aberrant CK2 signaling inhibits TAp73 to promote the expression of CSC genes and phenotype.

Majid, S., et al. (2010). "MicroRNA-205-directed transcriptional activation of tumor suppressor genes in prostate cancer." Cancer **116**(24): 5637-5649.

BACKGROUND: MicroRNAs (miRNAs) are small noncoding RNAs that regulate the expression of approximately 60% of all human genes. They play important roles in numerous cellular processes, including development, proliferation, and apoptosis. Currently, it is believed that miRNAs elicit their effect

by silencing the expression of target genes. In this study, the authors demonstrated that miRNA-205 (miR-205) induced the expression the interleukin (IL) tumor suppressor genes IL24 and IL32 by targeting specific sites in their promoters. METHODS: The methods used in this study included transfection of small RNAs; quantitative real-time polymerase chain reaction; in situ hybridization; fluorescence-labeled in situ hybridization; cell cycle, apoptosis, cell viability, migratory, clonability, and invasion assays; immunoblotting; and luciferase reporter, nuclear run-on, and chromatin immunoprecipitation assays. RESULTS: The results revealed that miR-205 was silenced in prostate cancer. Its re-expression induced apoptosis and cell cycle arrest. It also impaired cell growth, migration, clonability, and invasiveness of prostate cancer cells. Micro-RNA-205 induced the expression of tumor suppressor genes IL24 and IL32 at both the messenger RNA and protein levels. The induction of in vitro transcription and enrichment of markers for transcriptionally active promoters in the IL24 and IL32 genes was observed in response to miR-205. CONCLUSIONS: In this study, a new function for miR-205 was identified that specifically activated tumor suppressor genes by targeting specific sites in their promoters. These results corroborate a newly identified function that miRNAs have in regulating gene expression at the transcriptional level. The specific activation of tumor suppressor genes (eg, IL24, IL32) or other dysregulated genes by miRNA may contribute to a novel therapeutic approach for the treatment of prostate cancer.

Majid, S., et al. (2008). "Genistein induces the p21WAF1/CIP1 and p16INK4a tumor suppressor genes in prostate cancer cells by epigenetic mechanisms involving active chromatin modification." Cancer Res **68**(8): 2736-2744.

Genistein (4',5,7-trihydroxyisoflavone) is the most abundant isoflavone found in the soybean. The effects of genistein on various cancer cell lines have been extensively studied but the precise molecular mechanisms are not known. We report here the epigenetic mechanism of the action of genistein on androgen-sensitive (LNCaP) and androgen-insensitive (DuPro) human prostate cancer cell lines. Genistein induced the expression of tumor suppressor genes p21 (WAF1/CIP1/KIP1) and p16 (INK4a) with a concomitant decrease in cyclins. There was a G (0)-G (1) cell cycle arrest in LNCaP cells and a G (2)-M arrest in DuPro cells after genistein treatment. Genistein also induced apoptosis in DuPro cells. DNA methylation analysis revealed the absence of p21 promoter methylation in both cell lines. The effect of genistein on chromatin remodeling has not been previously reported. We found that genistein increased acetylated histones 3, 4, and H3/K4 at the p21 and p16 transcription start sites. Furthermore, we found that genistein treatment also increased the expression of histone acetyl transferases that function in transcriptional activation. This is the first report on epigenetic regulation of various genes by genistein through chromatin remodeling in prostate cancer. Altogether, our data provide new insights into the epigenetic mechanism of the action of genistein that may contribute to the chemopreventive activity of this dietary isoflavone and have important implications for epigenetic therapy.

Manderson, E. N., et al. (2009). "Molecular genetic analysis of a cell adhesion molecule with homology to L1CAM, contactin 6, and contactin 4 candidate chromosome 3p26pter tumor suppressor genes in ovarian cancer." Int J Gynecol Cancer **19**(4): 513-525.

Loss of heterozygosity (LOH) analyses of epithelial ovarian cancers (EOCs) previously identified a candidate tumor suppressor gene (TSG) locus within the chromosomal region 3p25.3-pter. Loss of heterozygosity analysis was performed to define the locus and identify candidates for further study. Loss of heterozygosity analysis of 124 malignant EOC samples of different histopathologic subtypes using 12 polymorphic microsatellite repeat markers identified a 330-kilobase minimal region of overlapping deletions at 3p26.3 that contained contactin 4 (CNTN4) as the only known TSG candidate. However, evaluation of the LOH patterns in the serous EOC samples, the most common subtype, enabled the identification of a second, broader region of LOH also included the cell adhesion molecule with homology to L1CAM (CHL1) and CNTN6 as candidates. Gene expression by reverse transcription polymerase chain reaction was not detectable in primary cultures of normal ovarian surface epithelial cells for any of these candidates. CNTN6 expression was also not detectable in serous EOC samples. In contrast, gene expression of CNTN4 and CHL1, particularly overexpression of CHL1, was observed in serous EOC samples. Mutation and gene expression analyses of well-defined EOC cell lines (OV-90, TOV-112D, TOV-21G, and TOV-81D) that differ in their tumorigenic potential and chromosome 3p26-pter genomic content revealed CNTN4 expression and a novel mutation only in the tumorigenic EOC cell line TOV-21G. This mutation was neither observed in controls (n = 105) nor detected by sequencing analysis of complementary DNA. Taken together, these results do not support the candidacy of CHL1, CNTN6, and CNTN4 as TSGs in the 3p26-pter region. However, the overexpression of CHL1, a member of the L1 cell adhesion molecule (L1CAM) family, warrants further investigation.

Manvikar, V., et al. (2016). "Role of human papillomavirus and tumor suppressor genes in oral cancer." J Oral Maxillofac Pathol **20**(1): 106-110.

The incidence of oral cancer remains high and is associated with many deaths in both Western and Asian countries. Several risk factors for the development of oral cancer are now well known, including smoking, drinking and consumption of smokeless tobacco products. Genetic predisposition to oral cancer has been found in certain cases, but its components are not yet entirely clear. In accordance with the multi-step theory of carcinogenesis, the natural history of oral cancer seems to gradually evolve through transitional precursor lesions from normal epithelium to a full-blown metastatic phenotype. A number of genomic lesions accompany this transformation and a wealth of related results has appeared in recent literature and is being summarized here. Furthermore, several key genes have been implicated, especially well-known tumor suppressors such as the cyclin-dependent kinase inhibitors, TP53 and RB1 and oncogenes such as the cyclin family, epidermal growth factor receptor and RAS. Viral infections, particularly oncogenic human papillomavirus subtypes and Epstein-Barr virus, can have a tumorigenic effect on oral epithelia and their role is discussed, along with potential therapeutic interventions. A brief explanatory theoretical model of oral carcinogenesis is provided and potential avenues for further research are highlighted.

Marsit, C. J., et al. (2005). "Hypermethylation of RASSF1A and BLU tumor suppressor genes in non-small cell lung cancer: implications for tobacco smoking during adolescence." Int J Cancer **114**(2): 219-223.

The putative tumor suppressors RASSF1A and BLU are mapped adjacent to one another on chromosome 3p21.3, a region frequently deleted in lung cancer. These genes are often inactivated by promoter hypermethylation, but the association of this inactivation with clinical features of the disease or with carcinogen exposure has been poorly studied. Early age starting smoking has been hypothesized as an independent risk factor for lung cancer, and mechanistically, adolescence may constitute a critical period for tobacco carcinogen exposure. To study the relationship of tobacco smoke exposure with hypermethylation of RASSF1A and BLU, methylation-specific PCR was performed on a case series study of incident, surgically resected non-small cell lung cancer (NSCLC), and the prevalence of this alteration was examined in relation to clinical and exposure information collected on the patients. Hypermethylation of the RASSF1A promoter occurred in 47% (83/178) and of the BLU promoter in 43% (68/160) of NSCLC tumors examined. There was no significant association between methylation of these 2 genes, but methylation of either of these genes tended to occur more often in the adenocarcinoma (AC) histology compared to squamous cell carcinoma (SCC). Controlling for pack-years smoked, age, gender and histology, starting smoking under age 18 was significantly related to RASSF1A methylation [prevalence ratio (PR) = 1.6, 95% confidence interval [CI] = 1.1-2.3]. These results indicate that starting smoking under age 18 is an independent risk for RASSF1A hypermethylation, thus identifying a molecular alteration related to the epidemiologic effect of teenage smoking as a lung cancer risk.

Murria, R., et al. (2015). "Methylation of tumor suppressor genes is related with copy number aberrations in breast cancer." Am J Cancer Res **5**(1): 375-385.

This study investigates the relationship of promoter methylation in tumor suppressor genes with copy-number aberrations (CNA) and with tumor markers in breast cancer (BCs). The study includes 98 formalin fixed paraffin-embedded BCs in which promoter methylation of 24 tumour suppressor genes were assessed by Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA), CNA of 20 BC related genes by MLPA and ER, PR, HER2, CK5/6, CK18, EGFR, Cadherin-E, P53, Ki-67 and PARP expression by immunohistochemistry (IHC). Cluster analysis classed BCs in two groups according to promoter methylation percentage: the highly-methylated group (16 BCs), containing mostly hyper-methylated genes, and the sparsely-methylated group (82 BCs) with hypo-methylated genes. ATM, CDKN2A, VHL, CHFR and CDKN2B showed the greatest differences in the mean methylation percentage between these groups. We found no relationship of the IHC parameters or pathological features with methylation status, except for Catherin-E ($p = 0.008$). However the highly methylated BCs showed higher CNA proportion than the sparsely methylated BCs ($p < 0.001$, OR = 1.62; IC 95% [1.26, 2.07]). CDC6, MAPT, MED1, PRMD14 and AURKA showed the major differences in the CNA percentage between the two groups, exceeding the 22%. Methylation in RASSF1, CASP8, DAPK1 and GSTP1 conferred the highest probability of harboring CNA. Our results show a new link between promoter methylation and CNA giving support to the importance of methylation events to establish new BCs subtypes. Our findings may be also of relevance in personalized therapy assessment, which could benefit the hyper methylated BC patients group.

Nagaoka, S., et al. (2003). "Poor prognosis of colorectal cancer in patients over 80 years old is associated with down-regulation of tumor suppressor genes." J Clin Gastroenterol **37**(1): 48-54.

UNLABELLED: GOALS, BACKGROUND: The elderly population has been increasing during the last half a century and it would be important to know how aging influences the occurrence and biologic behavior of cancers. STUDY: We investigated clinicopathologic characteristics of colorectal cancer in 1354 patients who underwent colorectal cancer resection and compared the results between extremely elderly patients (over 80 years old) and middle-aged/elderly patients (40 to less than 80 years old). Furthermore, we also examined expression of tumor suppressor genes and Cox-2 using frozen samples of colorectal cancer obtained from 62 patients ranging in age from 45 to 87 years. RESULTS: The results obtained in the extremely aged patients were: (1) higher ratio of women, (2) higher incidence at the proximal site, (3) higher incidence of cases with deeper invasion, (4) higher incidence of cases with lymph node metastasis (5) poorer survival rate as compared with middle-aged/elderly patients, and (6) lower mRNA expression levels of p27 and p53. CONCLUSIONS: These findings taken together suggest that poor prognosis of colorectal cancer in patients over 80 years is associated with down-regulation of mRNA expression of some tumor suppressor genes.

Nandakumar, V., et al. (2011). "(-)-Epigallocatechin-3-gallate reactivates silenced tumor suppressor genes, Cip1/p21 and p16INK4a, by reducing DNA methylation and increasing histones acetylation in human skin cancer cells." Carcinogenesis **32**(4): 537-544.

The anti-skin carcinogenic effects of green tea catechins have been studied extensively in vitro and in vivo models but the precise epigenetic molecular mechanisms are still unclear. Accumulating data suggest that dietary phytochemicals may alter cancer risk by modifications of epigenetic processes in the cells. The present study was designed to investigate whether tea catechins, particularly (-)-epigallocatechin-3-gallate (EGCG), would modify epigenetic events to regulate DNA methylation-silenced tumor suppressor genes in skin cancer cells. DNA methylation, histone modifications and tumor suppressor gene expressions were studied in detail using human epidermoid carcinoma A431 cells as an in vitro model after EGCG treatment using cytostaining, western blotting, dot blot analysis, real-time polymerase chain reaction and enzymatic activity assays. Our study shows that EGCG treatment decreased global DNA methylation levels in A431 cells in a dose-dependent manner. EGCG decreased the levels of 5-methylcytosine, DNA methyltransferase (DNMT) activity, messenger RNA (mRNA) and protein levels of DNMT1, DNMT3a and DNMT3b. EGCG decreased histone deacetylase activity and increased levels of acetylated lysine 9 and 14 on histone H3 (H3-Lys 9 and 14) and acetylated lysine 5, 12 and 16 on histone H4 but decreased levels of methylated H3-Lys 9. Additionally, EGCG treatment resulted in re-expression of the mRNA and proteins of silenced tumor suppressor genes, p16INK4a and Cip1/p21. Together, our study provides new insight into the epigenetic mechanism of action of EGCG that may contribute to the chemoprevention of skin cancer and may have important implications for epigenetic therapy.

Ohta, N., et al. (2015). "Human umbilical cord matrix mesenchymal stem cells suppress the growth of breast cancer by expression of tumor suppressor genes." PLoS One **10**(5): e0123756.

Human and rat umbilical cord matrix mesenchymal stem cells (UCMSC) possess the ability to control the growth of breast carcinoma cells. Comparative analyses of two types of UCMSC suggest that rat UCMSC-dependent growth regulation is significantly stronger than that of human UCMSC. Their different tumoricidal abilities were clarified by analyzing gene expression profiles in the two types of UCMSC. Microarray analysis revealed differential gene expression between untreated naive UCMSC and those co-cultured with species-matched breast carcinoma cells. The analyses screened 17 differentially expressed genes that are commonly detected in both human and rat UCMSC. The comparison between the two sets of gene expression profiles identified two tumor suppressor genes, adipose-differentiation related protein (ADRP) and follistatin (FST), that were specifically up-regulated in rat UCMSC, but down-regulated in human UCMSC when they were co-cultured with the corresponding species' breast carcinoma cells. Over-expression of FST, but not ADRP, in human UCMSC enhanced their ability to suppress the growth of MDA-231 cells. The growth of MDA-231 cells was also significantly lower when they were cultured in medium conditioned with FST, but not ADRP over-expressing human UCMSC. In the breast carcinoma lung metastasis model generated with MDA-231 cells, systemic treatment with FST-over-expressing human UCMSC significantly attenuated the tumor burden. These results suggest that FST may play an important role in exhibiting stronger tumoricidal ability in rat UCMSC than human UCMSC and also implies that human UCMSC can be transformed into stronger tumoricidal cells by enhancing tumor suppressor gene expression.

Okano, T., et al. (2006). "Alterations in novel candidate tumor suppressor genes, ING1 and ING2 in human lung cancer." Oncol Rep **15**(3): 545-549.

The ING1 gene is involved in the regulation of the cell cycle, senescence, and apoptosis and is a novel candidate tumor suppressor gene. ING2, another gene in the ING family, was identified and cloned. The functions of ING1 and ING2 largely depend on the activity of p53. To determine whether an alteration in these genes plays a role in carcinogenesis and tumor progression in lung cancer, we screened 30 human lung cancer cell lines and 31 primary lung cancer tumors for mutations in these genes using polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) and direct sequencing. Our findings failed to uncover any mutations in these genes. We also examined the expression of ING1 and ING2 in lung cancer cell lines that either had or lacked a p53 mutation, and in a control bronchial epithelium cell line, using quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR). ING1 expression was up-regulated in all 7 lung cancer cell lines that had a p53 mutation, while the expression of ING2 was down-regulated in 6 of 7 lung cancer cell lines that had a p53 mutation. These results suggest that the ING1 and ING2 genes have different roles in lung carcinogenesis and progression, and the ING2 gene may be an independent tumor suppressor candidate on p53.

Oliveira, A. M., et al. (2005). "Tumor suppressor genes in breast cancer: the gatekeepers and the caretakers." Am J Clin Pathol **124 Suppl**: S16-28.

Tumor suppressor genes encode for proteins whose normal function is to inhibit cell transformation and whose inactivation is advantageous for tumor cell growth and survival. A variety of mechanisms result in the inactivation of tumor suppressor genes, including intragenic mutations, chromosomal deletions, and loss of expression by methylation-mediated transcriptional silencing or increased proteolysis. Tumor suppressor genes participate in a variety of critical and highly conserved cell functions, including regulation of the cell cycle and apoptosis, differentiation, surveillance of genomic integrity and repair of DNA errors, signal transduction, and cell adhesion. Tumor suppressor functions can be separated into 2 major categories: gatekeepers and caretakers. Gatekeepers directly inhibit tumor growth or promote tumor death. Inactivation of these genes contributes directly to cancer formation and progression. Among them, the p53 gene is the most well known. Located on chromosome band 17p13, p53 encodes a 53-kd multifunctional transcription factor that regulates the expression of genes involved in cell cycle control, apoptosis, DNA repair, and angiogenesis. In breast cancer, most studies have shown that p53 mutation or down-regulation is associated with adverse prognosis. Other tumor suppressor genes of interest in breast cancer include the retinoblastoma gene (pRb), PTEN, p16, nm23, and maspin.

Olsen, J. (2006). "[Cancer-protecting genes (tumor-suppressor genes)]." Ugeskr Laeger **168**(24): 2329-2331.

Tumor-suppressor genes protect against the development of cancer. The prototypical tumor-suppressor gene is one that controls G1 to S progression in the cell cycle. However, genes that are involved in DNA-repair or induction of apoptosis also protect against the development of cancer. More generally speaking, tumor-suppressor genes either prevent the introduction of somatic mutations or the amplification of the population of somatic cells carrying mutations. Well-known tumor-suppressor genes that also give rise to inherited cancer disorders are described in the review.

Osborne, C., et al. (2004). "Oncogenes and tumor suppressor genes in breast cancer: potential diagnostic and therapeutic applications." Oncologist **9**(4): 361-377.

Carcinogenesis is a multistep process characterized by genetic alterations that influence key cellular pathways involved in growth and development. Oncogenes refer to those genes whose alterations cause gain-of-function effects, while tumor suppressor genes cause loss-of-function effects that contribute to the malignant phenotype. The effects of these alterations are complex due to the high number of changes in a typical case of breast cancer and the interactions of the biological pathways involved. This review focuses on the more common abnormalities in oncogenes and tumor suppressor genes in human breast cancer and their known associations with clinical outcome in terms of tumor classification, prognosis, and response to specific therapies. A better understanding of these relationships has led to new therapeutic applications. Agents that target oncogenes and their associated pathways are now in clinical use, with many more undergoing preclinical and clinical testing. The availability of antibodies, small synthetic molecules, cytokines, gene therapy techniques, and even natural compounds that are screened for specific biological properties has greatly increased the number of candidate drugs. Nevertheless, clinical successes have been limited because of the redundancy of many cancer-related pathways as well as the high degree of variability in genotype and phenotype among individual tumors. Likewise, strategies to replace tumor suppressor gene functions face numerous

technical hurdles. This review summarizes the current achievements and future prospects for the therapeutic targeting of oncogenes and tumor suppressor genes and new technology to better classify tumors and accurately predict responses to standard and novel agents.

Ostrakhovitch, E. A., et al. (2016). "Basal and copper-induced expression of metallothionein isoform 1,2 and 3 genes in epithelial cancer cells: The role of tumor suppressor p53." J Trace Elem Med Biol **35**: 18-29.

Metallothioneins (MTs) are a ubiquitous low-molecular weight, cysteine rich proteins with a high affinity for metal ions. The expression and induction of MTs have been associated with protection against DNA damage, oxidative stress, and apoptosis. Our past research had shown that p53 is an important factor in metal regulation of MTs. The present study was undertaken to explore further the interrelationship between p53 and MTs. We investigated whether silencing of p53 could affect expression pattern of basal and copper induced metallothioneins. The silencing of wild-type p53 (wt-p53) in epithelial breast cancer MCF7 cells affected the basal level of MT-2A RNA, whereas the levels of MT-1A and MT-1X RNA remained largely unchanged. The expression of MT-3 was undetectable in MCF7 with either functional or silenced p53. MCF7 cells with silenced wt-p53 failed to upregulate MT-2A in response to copper and showed a reduced sensitivity toward copper induced cell apoptotic death. Similarly in MCF7-E6 and MDA-MB-231 cells, the presence of inactive/mutated p53 halted MT-1A and MT-2A gene expression in response to copper. Constitutive expression of MT-3 RNA was detectable in the presence of mutated p53 (mtp53). Transient transfection of MDA-MB-231 cells with wt-p53 enabled copper induced upregulation of both MT-1A and MT-2A but not basal level of MT-2A, MT-1E, MT-1X and MT-3. Inactivation of p53 in HepG2 cells amplified the basal expression of studied MT isoforms, including MT-3, as well as copper-induced mRNA expression of MTs except MT-1H and MT-3. Presented data demonstrate a direct relation between p53 and MT-1A and MT-2A and they also indicate that wt-p53 might be a negative regulator of MT-3 in epithelial cancer cells.

Otani, K., et al. (2013). "Epigenetic-mediated tumor suppressor genes as diagnostic or prognostic biomarkers in gastric cancer." Expert Rev Mol Diagn **13**(5): 445-455.

Gastric cancer is believed to result in part from the accumulation of multiple genetic and epigenetic alterations leading to oncogene overexpression and tumor suppressor loss. Tumor suppressor genes are inactivated more frequently by promoter methylation than by mutation in gastric cancer. Identification of genes inactivated by promoter methylation is a powerful approach to discover novel tumor suppressor genes. We have previously identified tumor suppressor genes in gastric cancer by genome-wide methylation screening. The biological functions of these genes are related to cell adhesion, ubiquitination, transcription, p53 regulation and diverse signaling pathways. Some of the tumor suppressor genes are of particular clinical importance as they can be used as predictive biomarkers for early diagnosis or ongoing prognosis of gastric cancer.

Ozdemir, F., et al. (2012). "Methylation of tumor suppressor genes in ovarian cancer." Exp Ther Med **4**(6): 1092-1096.

Aberrant methylation of gene promoter regions is one of the mechanisms for inactivation of tumor suppressor genes in human malignancies. In this study, the methylation pattern of 24 tumor suppressor genes was analyzed in 75 samples of ovarian cancer using the methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) assay. Of the 24 tumor suppressor genes examined, aberrant methylation was observed in 17. The three most frequently methylated genes were CDKN2B, CDH13 and RASSF1, followed by ESR1 and MLH1. Methylation frequencies ranged from 1.3% for CDKN2A, RARbeta, CASP8, VHL and TP73 to 24% for CDKN2B. The corresponding normal DNA from each patient was also investigated. Methylation was detected in tumors, although not in normal tissues, with the exception of two samples, indicating aberrant methylation in tumors. Clear cell carcinoma samples exhibited a higher frequency of CDKN2B promoter hypermethylation compared to those of other histological types (P=0.05). Our data indicate that methylation of the CDKN2B gene is a frequent event in ovarian carcinogenesis and that analysis of only three genes is sufficient to detect the presence of methylation in 35% of ovarian cancer cases. However, more studies using a much larger sample size are needed to define the potential role of DNA methylation as a marker for ovarian cancer.

Pan, F. P., et al. (2016). "Emodin enhances the demethylation by 5-Aza-CdR of pancreatic cancer cell tumor-suppressor genes P16, RASSF1A and ppENK." Oncol Rep **35**(4): 1941-1949.

5-Aza-2'-deoxycytidine (5-Aza-CdR) is currently acknowledged as a demethylation drug, and causes a certain degree of demethylation in a variety of cancer cells, including pancreatic cancer cells. Emodin, a traditional Chinese medicine (TCM), is an effective monomer extracted from rhubarb and has been

reported to exhibit antitumor activity in different manners in pancreatic cancer. In the present study, we examined whether emodin caused demethylation and increased the demethylation of three tumor-suppressor genes P16, RASSF1A and ppENK with a high degree of methylation in pancreatic cancer when combined with 5-Aza-CdR. Our research showed that emodin inhibited the growth of pancreatic cancer Panc-1 cells in a dose- and time-dependent manner. Dot-blot results showed that emodin combined with 5-Aza-CdR significantly suppressed the expression of genome 5mC in PANC-1 cells. In order to verify the effect of methylation, methylation-specific PCR (MSP) and bisulfite genomic sequencing PCR (BSP) combined with TA were selected for the cloning and sequencing. Results of MSP and BSP confirmed that emodin caused faint demethylation, and 5-Aza-CdR had a certain degree of demethylation. When emodin was combined with 5-Aza-CdR, the demethylation was more significant. At the same time, fluorescent quantitative PCR and western blot analysis results confirmed that when emodin was combined with 5-Aza-CdR, the expression levels of P16, RASSF1A and ppENK were increased more significantly compared to either treatment alone. In contrast, the expression levels of DNA methyltransferase 1 (DNMT1) and DNMT3a were more significantly reduced with the combination treatment than the control or either agent alone, further proving that emodin in combination with 5-Aza-CdR enhanced the demethylation effect of 5-Aza-CdR by reducing the expression of methyltransferases. In conclusion, the present study confirmed that emodin in combination with 5-Aza-CdR enhanced the demethylation by 5-Aza-CdR of tumor-suppressor genes p16, RASSF1A and ppENK by reducing the expression of methyltransferases DNMT1 and DNMT3a.

Paredes-Zaglul, A., et al. (1998). "Analysis of colorectal cancer by comparative genomic hybridization: evidence for induction of the metastatic phenotype by loss of tumor suppressor genes." Clin Cancer Res **4**(4): 879-886.

Current models suggest that colon cancer initiation and progression are secondary to both the activation of oncogenes and the deletion of tumor suppressor genes. The role of each, however, is still poorly understood, particularly with regard to the induction of metastasis. We hypothesized that genetic differences exist between tumors that metastasize distantly and those that do not, and that oncogenes and tumor suppressor genes participate equally in this process. To address this hypothesis, human tumor specimens from localized [tumor-node-metastasis (TNM) stage I-III] and primary colon cancers (n = 10) were directly compared with metastatic (TNM stage IV) lesions (n = 10) using comparative genomic hybridization analysis. Although several alterations were shared equally between primary tumors and metastases (+7q, +19q, and +20q), two patterns of distinguishing alterations were observed: (a) alterations that were more extensive in liver metastases than in primary tumors (+8q, +13q, -4p, -8p, -15q, -17p, -18q, -21q, and -22q); and (b) alterations that were unique to metastatic lesions (-9q, -11q, and -17q). Overall, genetic losses were more common than gains, and, more importantly, the number of losses/tumor was significantly higher for metastases than for primary tumors (9.3 + 1.3 versus 4.1 + 0.7; P = 0.00062, Wilcoxon's rank-sum test). The distinct predominance of genetic losses in the metastatic lesions when compared with the primary localized tumors provides evidence that the metastatic phenotype is induced by the deletion of tumor suppressor genes and permits the construction of physical maps targeting regions where novel tumor suppressor genes are likely to exist.

Pastuszak-Lewandoska, D., et al. (2016). "Expression level and methylation status of three tumor suppressor genes, DLEC1, ITGA9 and MLH1, in non-small cell lung cancer." Med Oncol **33**(7): 75.

Despite therapeutic advances, lung cancer remains one of the most common causes of cancer-related death in the world. There is a need to develop biomarkers of diagnostic and/or prognostic value and to translate findings in basic science research to clinical application. Tumor suppressor genes (TSGs) represent potential useful markers for disease detection, progression and treatment target. We tried to elucidate the role of three 3p21.3 TSGs: DLEC1, ITGA9 and MLH1, in non-small cell lung cancer (NSCLC). We assessed their expression pattern by qPCR in 59 NSCLC tissues and in the matched macroscopically unchanged lung tissues. Additionally, we analyzed gene promoter methylation status by methylation-specific PCR in NSCLC samples. We did not find significant correlations between gene expression and methylation. In case of DLEC1 and ITGA9, expression levels were decreased in 71-78 % of tumor samples and significantly different between tumor and normal tissues (P = 0.0001). It could point to their diagnostic value. ITGA9 could also be regarded as a diagnostic marker differentiating NSCLC subtypes, as its expression level was significantly lower in squamous cell carcinoma (P = 0.001). The simultaneous down-regulation of DLEC1 and ITGA9 was observed in 52.5 % of NSCLCs. MSPs revealed high frequencies of gene promoter methylation in NSCLCs: 84 % for DLEC1 and MLH1 and 57 % for ITGA9. Methylation indexes reflected moderate gene methylation levels: 34 % for ITGA9, 27 % for MLH1

and 26 % for DLEC1. However, frequent simultaneous methylation of the studied genes in more than 50 % of NSCLCs suggests the possibility of consider them as a panel of epigenetic markers.

Pavan, J., et al. (1994). "[Tumor suppressor genes. New perspectives for clinical investigations in cancer]." Medicina (B Aires) **54**(2): 163-168.

Tumor origin is viewed as comprising a series of specific genetic events in target cells and their clonal descendants. The development of molecular biology during the last decade has led to the recognition that these events fall into two distinct categories: the activation of protooncogenes and the inactivation of tumor suppressor genes. The latter are genes the inactivation of which is required for the malignant transformation of a cell. Loss of tumor suppressor genes plays an important role in the development of human tumors. Studies with somatic cell hybrids have shown that tumor suppression occurs in neoplastic cells and can be corrected by cell fusion with normal human chromosome. These experiments proved that tumorigenicity is a recessive phenotype controlled by specific chromosomes. Certain tumor suppressor genes, e.g. p53 and RB1, may be involved in a variety of malignancies whereas others, e.g. the DCC gene, may be restricted to a single type of cancer. The detection of germline mutations in tumor suppressor genes should allow the identification of subjects at high risk of developing cancer.

Paz, M. F., et al. (2003). "Genetic unmasking of epigenetically silenced tumor suppressor genes in colon cancer cells deficient in DNA methyltransferases." Hum Mol Genet **12**(17): 2209-2219.

Hypermethylation associated silencing of the CpG islands of tumor suppressor genes is a common hallmark of human cancer. Here we report a functional search for hypermethylated CpG islands using the colorectal cancer cell line HCT-116, in which two major DNA methyltransferases, DNMT1 and DNMT3b, have been genetically disrupted (DKO cells). Using two molecular screenings for differentially methylated loci [differential methylation hybridization (DMH) and amplification of inter-methylated sites (AIMS)], we found that DKO cells, but not the single DNMT1 or DNMT3b knockouts, have a massive loss of hypermethylated CpG islands that induces the re-activation of the contiguous genes. We have characterized a substantial number of these CpG island associated genes with potentially important roles in tumorigenesis, such as the cadherin member FAT, or the homeobox genes LMX-1 and DUX-4. For other genes whose role in transformation has not been characterized, such as the calcium channel alpha1I or the thromboxane A2 receptor, their re-introduction in DKO cells inhibited colony formation. Thus, our results demonstrate the role of DNMT1 and DNMT3b in CpG island methylation associated silencing and the usefulness of genetic disruption strategies in searching for new hypermethylated loci.

Peng, H. Q., et al. (1995). "Loss of heterozygosity of tumor suppressor genes in testis cancer." Cancer Res **55**(13): 2871-2875.

Little is known of the molecular changes that occur in germ cell tumors (GCT) of the testis. We studied three GCT cell lines and 44 tumors for loss of heterozygosity (LOH) of the tumor suppressor genes APC, MCC, DCC, RB, TP53, and WT-1. We observed that LOH occurred in 55% (21 of 38) of informative cases at DCC, in 28% (10 of 36) of informative cases at APC, in 23% (6 of 26) at MCC, in 30% (13 of 43) at RB, and in 27% (6 of 22) at WT-1. The LOH level in these tumors using anonymous primers mapping to the short and long arms of chromosome 19, which is cytogenetically normal in GCT, revealed LOH of 11 and 5%, respectively. We also observed a LOH of 22% in the TP53 gene, despite the fact that mutations in TP53 do not occur in testis cancer. Since a high frequency of LOH at DCC (18q21.3) occurs equally at all histological subsets in GCT, we conclude that the loss of the function of this gene is an early event in testicular GCTs. However, the observed LOH levels at APC/MCC (5q21), RB (13q14), and WT-1 (11p13) could represent a functional loss of the corresponding tumor suppressor gene in some GCTs or reflect the loss of sequences in the same general chromosome region but involving a different tumor suppressor locus. Therefore, detailed mapping of these chromosomes is required to define the precise locations of maximal LOH in testis cancer.

Perinchery, G., et al. (1999). "High frequency of deletion on chromosome 9p21 may harbor several tumor-suppressor genes in human prostate cancer." Int J Cancer **83**(5): 610-614.

Chromosome 9p has been reported to be a critical region of loss in various cancers. Our present study was designed to determine the frequency of deletions at different loci of chromosome 9p in microdissected samples of normal prostatic epithelium and carcinoma from the same patients. For this purpose, DNA was extracted from the microdissected sections of normal and tumor cells of 40 prostate specimens, amplified by PCR and analyzed for loss of heterozygosity (LOH) on chromosome 9p using 15 microsatellite markers. Only 6 of 15 microsatellite markers exhibited LOH in prostate cancer specimens (D9S162, D9S1748, D9S171, D9S270, D9S273 and D9S153). LOH on

chromosome 9p was identified in 29 of 40 cases (72.5%) with at least 1 marker. The main deletion was found on 9p21, at loci D9S1748 (50%), D9S171 (51.4%) and D9S270 (21.8%). There was also a deletion on 9p22 at locus D9S162 (8.3%), on 9p13 at locus D9S273 (13.8%) and on 9p11 at locus D9S153 (7.7%). LOH data were correlated with stage of prostate cancer and revealed a high frequency of LOH at 3 or more loci in samples with stage T (3)N (0)M (0) (46%) compared with stage T (2)N (0)M (0) (15%), which suggests a higher incidence of LOH in the advanced stage of prostate cancer. One of the candidate target tumor-suppressor genes, p16 (MTS-1/CDKN2), has been identified within the 9p21 deleted region in tumor cell lines. Expression of P16 protein was either absent or very low in prostate cancer samples, suggesting that loss of the p16 gene may be involved in prostatic carcinogenesis.

Pietrusinski, M., et al. (2017). "Detection of bladder cancer in urine sediments by a hypermethylation panel of selected tumor suppressor genes." Cancer Biomark **18**(1): 47-59.

BACKGROUND: Promoter hypermethylation can be a useful biomarker for early detection and prognosis of bladder cancer, monitoring response to treatment and complement classical diagnostic procedures. OBJECTIVE: The molecular test was performed on DNA from bladder cancer cells in voided urine samples, tumor tissue DNA and normal control DNAs. We aimed to assess the diagnostic potential of epigenetic changes in urine DNA from bladder cancer cases at various clinico-pathological stages of the disease. METHODS: The methylation status of 5 genes (p14ARF, p16INK4A, RASSF1A, DAPK, APC) in 113 tumor samples paired with voided urine specimens was analyzed by MSP. We compared the results of methylation analysis with UroVysion test. RESULTS: The methylation profile in tumor/urine DNA was significantly correlated (p </= 0,05) with tumor grade in p14ARF, RASSF1a, APC/p14ARF, APC genes, respectively and with stage in p14ARF, RASSF1a/p14ARF genes, respectively. The results of UroVysion test were in correlation with hypermethylation both in tumor and urine DNA in p14ARF, RASSF1a and APC genes (p = 0,008; 0,02 and 0,04, respectively). CONCLUSIONS: Promoter hypermethylation of tumor suppressor genes is a frequent mechanism in bladder cancer. We found promoter hypermethylation in all grades and stages of all cases examined. Methylation profile of selected suppressor genes may be a potential useful biomarker and enhance early detection of bladder cancer using a noninvasive urine test.

Pluciennik, E., et al. (2014). "Alternating expression levels of WWOX tumor suppressor and cancer-related genes in patients with bladder cancer." Oncol Lett **8**(5): 2291-2297.

The aim of the present study was to determine the roles of the WWOX tumor suppressor and cancer-related genes in bladder tumor carcinogenesis. Reverse transcription-quantitative polymerase chain reaction was used to analyze the status of WWOX promoter methylation (using MethylScreen technology) and loss of heterozygosity (LOH) in papillary urothelial cancer tissues. The associations between the expression levels of the following tumorigenesis-related genes were also assessed: The WWOX tumor suppressor gene, the MKI67 proliferation gene, the BAX, BCL2 and BIRC5 apoptotic genes, the EGFR signal transduction gene, the VEGF vascular endothelial growth factor gene, and the CCND1 and CCNE1 cell cycle genes. The results reveal a high frequency of LOH in intron 1 in the WWOX gene, as well as an association between reduced WWOX expression levels and increased promoter methylation. In addition, the present study demonstrates that in bladder tumors, apoptosis is inhibited by increased expression levels of the BCL2 gene. A correlation between the proliferation indices of the MKI67 and the BIRC5 genes was also revealed. Furthermore, the expression levels of VEGF were identified to be positively associated with those of the EGFR gene.

Powell, J. A., et al. (2002). "Sequencing, transcript identification, and quantitative gene expression profiling in the breast cancer loss of heterozygosity region 16q24.3 reveal three potential tumor-suppressor genes." Genomics **80**(3): 303-310.

Loss of heterozygosity (LOH) of chromosome 16q24.3 is a common genetic alteration observed in invasive ductal and lobular breast carcinomas. We constructed a physical map and generated genomic DNA sequence data spanning 2.4 Mb in this region. Detailed in silico and in vitro analyses of the genomic sequence data enabled the identification of 104 genes. It was hypothesized that tumor-suppressor genes would exhibit marked mRNA expression variability in a panel of breast cancer cell lines as a result of downregulation due to mutation or hypermethylation. We examined the mRNA expression profiles of the genes identified at 16q24.3 in normal breast, a normal breast epithelial cell line, and several breast cancer cell lines exhibiting 16q24.3 LOH. Three of the genes, CYBA, Hs.7970, and CBFA2T3, exhibited variability ten times higher than the baseline. The possible role of these genes as tumor suppressors is discussed.

Qi, L. and Y. Ding (2017). "Screening of Tumor Suppressor Genes in Metastatic Colorectal Cancer." Biomed Res Int **2017**: 2769140.

Most tumor suppressor genes are commonly inactivated in the development of colorectal cancer (CRC). The activation of tumor suppressor genes may be beneficial to suppress the development and metastasis of CRC. This study analyzed genes expression and methylation levels in different stages of CRC. Genes with downregulated mRNA expression and upregulated methylation level in advanced CRC were screened as the potential tumor suppressor genes. After comparing the methylation level of screened genes, we found that MBD1 gene had downregulated mRNA expression and upregulated methylation levels in advanced CRC and continuously upregulated methylation level in the progression of CRC. Enrichment analysis revealed that genes expression in accordance with the elevated expression of MBD1 mainly located on chromosomes 17p13 and 17p12 and 8 tumor suppressor genes located on chromosome 17p13. Further enrichment analysis of transcription factor binding site identified that SP1 binding site had higher enrichment and could bind with MBD1. In conclusion, MBD1 may be a tumor suppressor gene in advanced CRC and affect the development and metastasis of CRC by regulating 8 tumor suppressor genes through binding with SP1.

Radpour, R., et al. (2011). "Hypermethylation of tumor suppressor genes involved in critical regulatory pathways for developing a blood-based test in breast cancer." PLoS One **6**(1): e16080.

BACKGROUND: Aberrant DNA methylation patterns might be used as a biomarker for diagnosis and management of cancer patients. METHODS AND FINDINGS: To achieve a gene panel for developing a breast cancer blood-based test we quantitatively assessed the DNA methylation proportion of 248 CpG sites per sample (total of 31,248 sites in all analyzed samples) on 10 candidate genes (APC, BIN1, BMP6, BRCA1, CST6, ESR-b, GSTP1, P16, P21 and TIMP3). The number of 126 samples consisting of two different cohorts was used (first cohort: plasma samples from breast cancer patients and normal controls; second cohort: triple matched samples including cancerous tissue, matched normal tissue and serum samples). In the first cohort, circulating cell free methylated DNA of the 8 tumor suppressor genes (TSGs) was significantly higher in patients with breast cancer compared to normal controls ($P<0.01$). In the second cohort containing triple matched samples, seven genes showed concordant hypermethylated profile in tumor tissue and serum samples compared to normal tissue ($P<0.05$). Using eight genes as a panel to develop a blood-based test for breast cancer, a sensitivity and specificity of more than 90% could be achieved in distinguishing between tumor and normal samples. CONCLUSIONS: Our study suggests that the selected TSG panel combined with the high-throughput technology might be a useful tool to develop epigenetic based predictive and prognostic biomarker for breast cancer relying on pathologic methylation changes in tumor tissue, as well as in circulation.

Rodriguez-Nieto, S. and M. Sanchez-Cespedes (2009). "BRG1 and LKB1: tales of two tumor suppressor genes on chromosome 19p and lung cancer." Carcinogenesis **30**(4): 547-554.

Losses of heterozygosity (LOH) of the short arm of chromosome 19 are frequent in lung cancer, suggesting that one or more tumor suppressor genes are present in this region. The LKB1 gene, also called STK11, is somatically inactivated through point mutations and large deletions in lung tumors, demonstrating that LKB1 is a target of the LOH of this chromosomal arm. Data from several independent groups have provided information about the profiles of lung tumors with LKB1 inactivation and it is generally agreed that this alteration strongly predominates in non-small cell lung cancer, in particular adenocarcinomas, in smokers. The LKB1 protein has serine-threonine kinase activity and is involved in the regulation of the cell energetic checkpoint through the phosphorylation and activation of adenosine monophosphate-dependent kinase (AMPK). LKB1 is also involved in other processes such as cell polarization, probably through substrates other than AMPK. Interestingly, another gene on chromosome 19p, BRG1, encoding a component of the SWI/SNF chromatin-remodeling complex, has emerged as a tumor suppressor gene that is altered in lung tumors. Similar to LKB1, BRG1 is somatically inactivated by point mutations or large deletions in lung tumors featuring LOH of chromosome 19p. These observations suggest an important role for BRG1 in lung cancer and highlight the need to further our understanding of the function of Brahma/SWI2-related gene 1 (BRG1) in cancer. Finally, simultaneous mutations at LKB1 and BRG1 are common in lung cancer cells, which exemplifies how a single event, LOH of chromosome 19p in this instance, targets two different tumor suppressors.

Ronsch, K., et al. (2011). "Class I and III HDACs and loss of active chromatin features contribute to epigenetic silencing of CDX1 and EPHB tumor suppressor genes in colorectal cancer." Epigenetics **6**(5): 610-622.

Aberrant Wnt/beta-catenin signaling is a driving force during initiation and progression of colorectal cancer. Yet, the Wnt/beta-catenin targets CDX1,

EPHB2, EPHB3 and EPHB4 (EPHB2-4) act as tumor suppressors in intestinal epithelial cells and frequently appear to be transcriptionally silenced in carcinomas. The molecular mechanisms which underlie the apparent loss of expression of a subset of Wnt/beta-catenin targets in a background of persistent pathway activity are largely unknown. To gain insight into this, we quantified expression of CDX1 and EPHB2-4 in human tissue specimens of case-matched colorectal normal mucosa, adenoma and invasive carcinoma. In particular EPHB2-4 display biphasic, albeit not strictly coincident, expression profiles with elevated levels in adenomas and decreased transcription in approximately 30% of the corresponding carcinomas. Consistent with their divergent and variable expression we observed considerable heterogeneity among the epigenetic landscapes at CDX1 and EPHB2-4 in a model of colorectal carcinoma cell lines. Unlike the inactive CDX1 locus, EPHB2-4 maintain DNA hypomethylation of their promoter regions in the silent state. A strong reduction of active histone modifications consistently parallels reduced expression of CDX1 and EPHB3 and to some extent of EPHB2. Accordingly, treatment with inhibitors for DNA methyltransferases (DNMTs) and histone deacetylases (HDACs) restored CDX1 and EPHB2-4 expression depending upon epigenetic features at their promoters but also upon cellular background. Overall our findings show that downregulation of CDX1 and EphB receptor genes occurs independently and that different branches of epigenetic control systems including class I and III HDACs contribute to epigenetic silencing of Wnt/beta-catenin targets during colorectal tumorigenesis.

Sarkar, S., et al. (2013). "Demethylation and re-expression of epigenetically silenced tumor suppressor genes: sensitization of cancer cells by combination therapy." Epigenomics **5**(1): 87-94.

Epigenetic regulation in eukaryotic and mammalian systems is a complex and emerging field of study. While histone modifications create an open chromatin conformation allowing for gene transcription, CpG methylation adds a further dimension to the expression of specific genes in developmental pathways and carcinogenesis. In this review, we will highlight DNA methylation as one of the distinct mechanisms for gene silencing and try to provide insight into the role of epigenetics in cancer progenitor cell formation and carcinogenesis. We will also introduce the concept of a dynamic methylation-demethylation system and the potential for the existence of a demethylating enzyme in this process. Finally, we will explain how re-expression of epigenetically silenced tumor suppressor genes could be exploited to develop effective drug therapies. In particular, we will consider how a combination therapy that includes epigenetic drugs could possibly kill cancer progenitor cells and reduce the chance of relapse following chemotherapy.

Sasaki, H., et al. (2010). "Hypermethylation of the large tumor suppressor genes in Japanese lung cancer." Oncol Lett **1**(2): 303-307.

Large tumor suppressor (LATS) 1 and 2 are tumor suppressor genes implicated in the regulation of the cell cycle. The methylation statuses of the promoter regions of these genes were studied in Japanese lung cancers. The methylation statuses of the promoter regions of LATS1 and LATS2 were investigated by methylation-specific PCR. The findings were compared to clinicopathological features of lung cancer. Methylation-specific PCR showed that the LATS1 promoter region was hypermethylated in 95 out of 119 (79.8%) lung cancers. The methylation status of LATS1 was significantly associated with squamous histology (p=0.0267) and smoking status (never smoker vs. smoker; p=0.0399). LATS1-ummethylated patients harbored more EGFR mutations (p=0.0143). The LATS2 promoter region was hypermethylated in 160 out of 203 (78.8%) lung cancers. However, the methylation status had no association with the clinicopathological characteristics of the lung cancers cases. Both the LATS1 and LATS2 methylation statuses did not correlate with survival of lung cancer patients. Thus, the EGFR methylation status of the LATS genes has limited value in Japanese lung cancers.

Sasaki, M., et al. (2003). "Alterations of tumor suppressor genes (Rb, p16, p27 and p53) and an increased FDG uptake in lung cancer." Ann Nucl Med **17**(3): 189-196.

OBJECTIVE: The FDG uptake in lung cancer is considered to reflect the degree of malignancy, while alterations of some tumor suppressor genes are considered to be related to the malignant biological behavior of tumors. The aim of this study is to examine the relationship between FDG-PET and alterations in the tumor suppression genes of lung cancer. METHODS: We examined 28 patients with primary lung cancer who underwent FDG-PET before surgery consisting of 17 patients with adenocarcinoma, 10 with squamous cell carcinoma and 1 with large cell carcinoma. The FDG-PET findings were evaluated based on the standardized uptake value (SUV). Alterations in the tumor suppressor genes, Rb, p16, p27 and p53, were evaluated immunohistochemically. RESULTS: The FDG uptake in lung cancer with alteration in each tumor suppressor gene tended to be higher than in those genes without alterations, although the differences were not significant. In 15

tumors with alterations in either tumor suppressor genes, the FDG uptake was 6.83 +/- 3.21. On the other hand, the mean FDG uptake was 1.95 in 2 tumors without alterations in any genes. The difference in the FDG uptake between the 2 groups was statistically significant ($p < 0.001$). CONCLUSIONS: In conclusion, the presence of abnormalities in the tumor suppressor genes, which results in an accelerated cell proliferation, is thus considered to increase the FDG uptake in lung cancer.

Sasaki, M. S., et al. (1991). "Somatic and germinal mutations of tumor-suppressor genes in the development of cancer." J Radiat Res **32 Suppl 2**: 266-276.

It is generally thought that the germinal mutation of tumor-suppressor genes predisposes the affected children to the development of certain types of hereditary tumors while the somatic mutation of the same genes links to the development of non-hereditary tumors. Retinoblastoma susceptibility gene (RB gene) is a prototype of such genes. We studied the parental origin of new mutation of the RB gene in the sporadic hereditary and non-hereditary retinoblastoma and osteosarcoma. The results showed a preferential involvement of parental genome in the new germinal as well as initial somatic mutations. The male-directed mutagenesis even in the somatic cells has been implicated as a reflection of germinal origin of mutation, even for non-hereditary tumors as a manifestation of mutational mosaicism associated with delayed mutation. The importance of the new mutations occurring as mosaics should be emphasized in the evaluation of cancer risks from parental exposures to radiation and chemicals.

Schulte, K. M., et al. (1998). "Rare loss of heterozygosity of the MTS1 and MTS2 tumor suppressor genes in differentiated human thyroid cancer." Horm Metab Res **30**(9): 549-554.

Loss of heterozygosity (LOH) of the MTS1 (p16) tumor suppressor gene has been reported to occur frequently in thyroid cancer cell lines. In order to determine the frequency of LOH for these multiple tumor suppressor genes, we used microsatellite markers IFNA and D9S171 to perform differential quantitative polymerase chain reaction. Tumor DNA was isolated from native sections of tumor tissue. Control DNA was isolated from blood. PCR products were separated on 6% polyacrylamide sequencing gels and quantified according to peak height and area. Analysis was informative in 70% of cases for both markers, and in 88% for at least one out of both. LOH was found in 3 out of 35 informative patients (8.6%) with papillary thyroid cancer, in 1 out of 7 patients with follicular thyroid cancer (14.2%), and in 0 out of 18 medullary cancers (0%). No LOH was found in 11 informative patients with multinodular goitre, 7 with follicular adenoma, 4 with Graves' disease, and 6 with other thyroid disease. 75% of LOH was found in T1 and T2 stages, it was not more frequent in patients with lymphonodular metastasis. The low frequency of LOH in these types of thyroid cancer argues against a role of loss of heterozygosity at the MTS 1 and 2 gene locus in the development of differentiated thyroid neoplasia.

Schwarzenbach, H., et al. (2012). "Loss of heterozygosity at tumor suppressor genes detectable on fractionated circulating cell-free tumor DNA as indicator of breast cancer progression." Clin Cancer Res **18**(20): 5719-5730.

PURPOSE: LOH on circulating DNA may provide tumor-specific information on breast cancer. As identification of LOH on cell-free DNA is impeded by the prevalence of wild type DNA in blood of cancer patients, we fractionated plasma DNA, and determined the diagnostic and prognostic value of both fractions. EXPERIMENTAL DESIGN: Our cohort of 388 patients with primary breast cancer before chemotherapy was selected from a multicenter study (SUCCESS). Postoperative plasma was fractionated in low- and high-molecular weight DNA by two different column systems. In both fractions, LOH was determined by a PCR-based microsatellite analysis using a panel of 8 polymorphic markers. Circulating tumor DNA in plasma from 30 patients after chemotherapy was additionally analyzed. The significance levels were adjusted for multiple comparisons. RESULTS: More patients (38%) had LOH at all markers in the fraction containing short DNA fragments than in the fraction containing the long DNA molecules (28%, $P = 0.0001$). In both fractions 32.85% of LOH were concordant. LOH at the markers D3S1605, D10S1765, D12S1725, D13S218, and D17S855 significantly correlated with tumor stage, tumor size, and lymph node metastasis, positive progesterone, and HER2 receptor status. Most importantly, LOH at D12S1725 mapping to cyclin D2 correlated with shorter overall survival ($P = 0.004$). CONCLUSIONS: The improved detection of LOH on cell-free DNA provides important information on DNA losses of tumor suppressor genes TIG1, PTEN, cyclin D2, RB1, and BRCA1 in breast cancer. In particular, loss of the cyclin D2 gene might become an important prognostic marker easily detectable in the peripheral blood.

Slattery, M. L., et al. (2017). "The co-regulatory networks of tumor suppressor genes, oncogenes, and miRNAs in colorectal cancer." Genes Chromosomes Cancer **56**(11): 769-787.

Tumor suppressor genes (TSGs) and oncogenes (OG) are involved in carcinogenesis. MiRNAs also contribute to cellular pathways leading to cancer. We use data from 217 colorectal cancer (CRC) cases to evaluate differences in TSGs and OGs expression between paired CRC and normal mucosa and evaluate how TSGs and OGs are associated with miRNAs. Gene expression data from RNA-Seq and miRNA expression data from Agilent Human miRNA Microarray V19.0 were used. We focus on genes most strongly associated with CRC (fold change (FC) of >/=1.5 or </=0.67) that were statistically significant after adjustment for multiple comparisons. Of the 74 TSGs evaluated, 22 were associated with carcinoma/normal mucosa differential expression. Ten TSGs were up-regulated (FAM123B, RB1, TP53, RUNX1, MSH2, BRCA1, BRCA2, SOX9, NPM1, and RNF43); six TSGs were down-regulated (PAX5, IZKF1, GATA3, PRDM1, TET2, and CYLD); four were associated with MSI tumors (MLH1, PTCH1, and CEBPA down-regulated and MSH6 up-regulated); and two were associated with MSS tumors (PHF6 and ASXL1 up-regulated). Thirteen of these TSGs were associated with 44 miRNAs. Twenty-seven of the 59 OGs evaluated were dysregulated: 14 down-regulated (KLF4, BCL2, SSETBP1, FGFR2, TSHR, MPL, KIT, PDGFRA, GNA11, GATA2, FGFR3, AR, CSF1R, and JAK3), seven up-regulated (DNMT1, EZH2, PTPN11, SKP2, CCND1, MET, and MYC); three down-regulated for MSI (FLT3, CARD11, and ALK); two up-regulated for MSI (IDH2 and HRAS); and one up-regulated with MSS tumors (CTNNB1). These findings suggest possible co-regulatory function between TSGs, OGs, and miRNAs, involving both direct and indirect associations that operate through feedback and feedforward loops.

Smith, C. G., et al. (2013). "Exome resequencing identifies potential tumor-suppressor genes that predispose to colorectal cancer." Hum Mutat **34**(7): 1026-1034.

Inherited factors account for around one third of all colorectal cancers (CRCs) and include rare high penetrance mutations in APC, MSH2, MSH6, and POLE. Here, we sought novel tumor-suppressor genes that predispose to CRC by exome resequencing 50 sporadic patients with advanced CRC (18 diagnosed </=35 years of age) at a mean coverage of 30x. To help identify potentially pathogenic alleles, we initially sought rare or novel germline truncating mutations in 1,138 genes that were likely to play a role in colorectal tumorigenesis. In total, 32 such mutations were identified and confirmed, and included an insertion in APC and a deletion in POLE, thereby validating our approach for identifying disease alleles. We sought somatic mutations in the corresponding genes in the CRCs of the patients harboring the germline lesions and found biallelic inactivation of FANCM, LAMB4, PTCHD3, LAMC3, and TREX2, potentially implicating these genes as tumor suppressors. We also identified a patient who carried a germline truncating mutation in NOTCH3, part of the Notch signaling cascade that maintains intestinal homeostasis. Our whole exome analyses provided further gene lists to facilitate the identification of potential predisposition alleles.

Smith, I. M., et al. (2010). "Inactivation of the tumor suppressor genes causing the hereditary syndromes predisposing to head and neck cancer via promoter hypermethylation in sporadic head and neck cancers." ORL J Otorhinolaryngol Relat Spec **72**(1): 44-50.

Fanconi anemia (FA) and dyskeratosis congenita (DC) are rare inherited syndromes that cause head and neck squamous cell cancer (HNSCC). Prior studies of inherited forms of cancer have been extremely important in elucidating tumor suppressor genes inactivated in sporadic tumors. Here, we studied whether sporadic tumors have epigenetic silencing of the genes causing the inherited forms of HNSCC. Using bisulfite sequencing, we investigated the incidence of promoter hypermethylation of the 17 Fanconi- and DC-associated genes in sporadic HNSCC. Genes that only showed methylation in the tumor patients were chosen for quantitative methylation-specific PCR (qMSP) in a set of 45 tumor and 16 normal patients. Three gene promoters showed differences in methylation: FancB (FAAP95, FA core complex), FancJ (BRIP1, DNA Helicase/ATPase), and DKC1 (dyskeratin). Bisulfite sequencing revealed that only FancB and DKC1 showed no methylation in normal patients, yet the presence of promoter hypermethylation in tumor patients. On qMSP, 1/16 (6.25%) of the normal mucosal samples from non-cancer patients and 14/45 (31.1%) of the tumor patients demonstrated hypermethylation of the FancB locus ($p < 0.05$). These results suggest that inactivation of FancB may play a role in the pathogenesis of sporadic HNSCC.

Song, H., et al. (2011). "[DNA methylation of tumor suppressor genes located on chromosome 3p in non-small cell lung cancer]." Zhongguo Fei Ai Za Zhi **14**(3): 233-238.

BACKGROUND AND OBJECTIVE: DNA methylation is one of the mechanisms of epigenetics. Allelic loss located on chromosome 3p happen frequently and early in non-small cell lung cancer (NSCLC). The aim of this study is to detect the promoter methylation status of tumor suppressor genes (TSGs) located on chromosome 3p in NSCLC and to

evaluate its correlation with clinicopathological features. METHODS: A total of 78 paired NSCLC specimens and their adjacent normal tissues were collected in the study. Promoter methylation status was determined by methylation-specific polymerase chain reaction (MSP). DLEC1 gene expression was determined by RT-PCR and immunohistochemistry. RESULTS: Aberrant methylation frequency of DLEC1, RASSF1A, hMLH1, RARbeta and FHIT genes detected in 78 NSCLC tissues were 41.03%, 39.74%, 30.77% and 16.67%, respectively, which were all significantly higher than that in adjacent normal tissues. However, FHIT gene was not detected methylation in both cancerous and non-cancerous tissues. DLEC1 hypermethylation was associated with advanced stage (P=0.011) and lymph metastasis (P=0.019), while RASSF1A, RARbeta, hMLH1 and mean methylation index (MI) were not correlated with any clinicopathological parameters. Moreover, DLEC1 gene downregulation was detected in 56.41% (44/78) NSCLC tissues and correlated with promoter hypermethylation. CONCLUSIONS: Frequent hypermethylation of TSGs located on chromosome 3p was a common event contributing to NSCLC pathogenesis and DLEC1 methylation was closely correlated with loss of expression.

Sourvinos, G., et al. (2001). "Genetic detection of bladder cancer by microsatellite analysis of p16, RB1 and p53 tumor suppressor genes." J Urol **165**(1): 249-252.

PURPOSE: We investigated the incidence of genetic alterations in urine specimens from patients with bladder cancer. MATERIALS AND METHODS: A total of 28 cytological urine specimens were assessed for microsatellite alternations, and 15 microsatellite markers were located on p53, RB1 and p16 regions. In 15 patients DNA from tumor specimens was also available. RESULTS: Loss of heterozygosity was detected in 26 of 28 patients (93%) in at least 1 microsatellite marker. Allelic losses were found in 18 patients (64%) for the p16 locus, in 8 (29%) for the RB1 locus and in 17 (61%) for the p53 region. In contrast, no microsatellite alterations were found in the normal group without evidence of bladder cancer. In 11 cases genetic alterations in the cytological urine specimens were not detectable in the corresponding tumor specimen, suggesting heterogeneity of bladder cancer. CONCLUSIONS: The detection of loss of heterozygosity in cytological urine specimens may be a prognostic indicator of early detection of bladder cancer. Our results suggest that microsatellite analysis of urine specimens represents a novel, potentially useful, noninvasive clinical tool to detect bladder cancer.

Suda, T., et al. (2012). "ER-activating ability of breast cancer stromal fibroblasts is regulated independently of alteration of TP53 and PTEN tumor suppressor genes." Biochem Biophys Res Commun **428**(2): 259-263.

Carcinoma-associated fibroblasts (CAFs) are associated with tumor progression and metastasis, and are able to activate estrogen receptor (ER) in breast cancer. We established a stable transformant of a human breast cancer cell line to detect CAF-specific ER-activating ability, and found that this CAF ability varied among tumors. Some studies have reported a high frequency of alterations among tumor suppressor genes in stromal cells, but do not generally agree as to the frequency. Moreover, the activation mechanism of CAF-induced estrogen signals, including the effects of these gene aberrations, is not fully understood. We investigated the relevance of tumor suppressor gene aberrations and ER-activating ability in CAFs derived from 20 breast cancer patients. Although CAF-specific ER-activating abilities varied among individual cases, all CAFs maintained wild-type alleles for TP53 and PTEN. Also, copy number aberrations in these genes were not observed in any CAFs. Our results suggest that the ER-activating ability of the CAFs is regulated independently of aberrations in these genes; and that other mechanisms of tumor-stromal interaction may affect activation of estrogen signals in breast cancer.

Sunamura, M., et al. (2002). "Gene therapy for pancreatic cancer targeting the genomic alterations of tumor suppressor genes using replication-selective oncolytic adenovirus." Hum Cell **15**(3): 138-150.

In order to develop an effective therapeutic intervention for patients with pancreatic cancer, we examined the genetic alternations of pancreatic cancer. Based on these results, we are developing a new gene therapy targeting the genetic character of pancreatic cancer using mutant adenoviruses selectively replication-competent in tumor cells. Loss of heterozygosity (LOH) of 30% or more were observed on chromosome arms 17p (47%), 9p (45%), 18q (43%), 12q (34%), and 6q (30%). LOH of 12q, 17p, and 18q showed the significant association with poor prognosis. These data strongly suggest that mutation of the putative suppressor genes, TP53 and SMAD4 play significant roles in the disease progression. Based on this rationale, we are developing a new gene therapy targeting tumors without normal TP53 function. E1B-55kDa-deleted adenovirus (AxE1AdB) can selectively replicate in TP53-deficient human tumor cells but not cells with functional TP53. We evaluated the therapeutic effect of this AxE1AdB on pancreatic cancer without normal TP53 function. The growth of human pancreatic tumor in SCID mice model was markedly inhibited by the consecutive

injection of AxE1AdB. Furthermore, AxE1AdB is not only the strong weapon but also useful carrier of genes possessing anti-tumor activities as a virus vector specific to tumors without normal TP53 function. It was reported that uracil phosphoribosyl transferase (UPRT) overcomes 5FU resistance. UPRT catalyzes the synthesis of 5-fluorouridine monophosphate (FUMP) from Uracil and phosphoribosylpyrophosphate (PRPP). The antitumor effect of 5FU is enhanced by augmenting 5-fluorodeoxyuridine monophosphate (FdUMP) converted from FUMP, which inhibits Thymidylate Synthetase (TS). The therapeutic advantage of restricted replication competent adenovirus that expresses UPRT (AxE1AdB-UPRT) was evaluatedin an intra-peritoneal disseminated tumor model. To study the anti-tumor effect of AxE1AdB-UPRT/5FU, mice with disseminated AsPC-1 tumors were administered the adenovirus, followed by the 5FU treatment. It was shown that the treatment with AxE1AdB-UPRT/5FU caused a dramatic reduction of the disseminated tumor burden without toxicity in normal tissues. These results revealed thatthe AxE1AdB-UPRT/5FU system is a promising tool for intraperitoneal disseminated pancreatic cancer.

Sunwoo, J. B., et al. (1996). "Evidence for multiple tumor suppressor genes on chromosome arm 8p in supraglottic laryngeal cancer." Genes Chromosomes Cancer **16**(3): 164-169.

Loss of heterozygosity studies of a variety of human tumors suggest that there are several tumor suppressor genes on chromosome arm 8p. To localize these genes more precisely, we utilized polymerase chain reaction amplification of microsatellite repeat polymorphisms and examined the allelic loss patterns of 17 marker loci on 8p in a population of 59 supraglottic laryngeal squamous cell carcinomas. Twenty-three of these tumors (39%) had an allelic loss at one or more of the markers examined. The allelic loss patterns of these tumors support the presence of at least three different tumor suppressor genes on 8p: one in 8p23, one in 8p22-23, and another in 8p21.

Tahara, E. and W. Yasui (1994). "The Third International Symposium of the Hiroshima Cancer Seminar: tumor-suppressor genes. Hiroshima, Japan, October 1993." J Cancer Res Clin Oncol **120**(10): 615-619.

Tahara, T., et al. (2010). "CpG island promoter methylation (CIHM) status of tumor suppressor genes correlates with morphological appearances of gastric cancer." Anticancer Res **30**(1): 239-244.

UNLABELLED: CpG island hypermethylation (CIHM) of tumor suppressor genes is one of the major events in the gastric carcinogenesis. We aimed to investigate the association between CIHM status of tumor suppressor genes and clinicopathological and morphological characteristics of gastric cancer. PATIENTS AND METHODS: CIHM of p14, p16, Death-associated protein kinase (DAPK) and E-cadherin (CDH1) genes were determined by methylation-specific-polymerase chain Reaction in 146 gastric cancer tissues. CIHM-high was defined as three or more methylated CpG islands. RESULTS: CIHM pf p14 was found in 70 (47.9%) cases, in 26 (17.8%) for p16, in 104 (71.2%) for CDH1 and in 127 (87.0%) for DAPK. CIHM-high was also found in 63 cases (43.2%). No association was found between between CIHM status and different staging, Lauren's subtypes, anatomic location, venous and lymphatic invasion, lymph node metastasis, distant metastasis, or peritoneal dissemination. However, among early gastric cancer cases, the depressed type with ulceration presented a significantly lower prevalence of CIHM of DAPK. In addition, Borrmann type IV cases presented significantly lower prevalence of CIHM-high among advanced gastric cancer. The Borrmann type IV cases also presented lower mean methylation number. CONCLUSION: The present results suggest that CIHM of DAPK and CIHM-high were associated with the morphological appearance of depressed type with ulceration in early gastric cancer, and Borrmann type IV advanced gastric cancer, respectively.

Tahara, T., et al. (2010). "Association between cyclin D1 polymorphism with CpG island promoter methylation status of tumor suppressor genes in gastric cancer." Dig Dis Sci **55**(12): 3449-3457.

BACKGROUND: CpG island hypermethylation of tumor suppressor genes is highly involved in gastric carcinogenesis, and enhanced cell proliferation could accelerate this process. Cyclin D1 regulates cell cycle function and may play a role in methylation-related carcinogenesis. AIMS: We investigated the association between Cyclin D1 gene G870A polymorphism and the methylation status of tumor suppressor genes in gastric cancer. METHODS: Polymorphisms at G870A in the Cyclin D1 gene were genotyped, and methylation status of the p14, p16, DAP-kinase, and CDH1 genes were determined by methylation-specific-polymerase chain reaction in 139 gastric cancer tissues. CIHM high was defined as three or more methylated CpG islands. RESULTS: Although no association was found between methylation status and different stages and Lauren's subtypes, patients with CIHM of DAP-kinase showed significantly worse survival than those without ($p = 0.017$). In addition, the number of methylated sites was also associated with survival curves ($p = 0.0397$). The 870G carrier a significantly lower prevalence of CIHM high

compared to the AA genotype in advanced-stage gastric cancer (adjusted OR = 0.32, p = 0.047). A weak correlation between the same genotypes and CIHM of p14 were found in the same subtype (adjusted OR = 0.32, p = 0.052). The mean methylation number was significantly lower in G carriers than in AA genotypes in advanced-stage gastric cancer (p = 0.017). CONCLUSIONS: Genetic polymorphism of CCND1 is associated with CIHM status in gastric cancer, especially in the advanced stage, but is independent of clinico-pathological features.

Tahara, T., et al. (2010). "Increased number of CpG island hypermethylation in tumor suppressor genes of non-neoplastic gastric mucosa correlates with higher risk of gastric cancer." Digestion **82**(1): 27-36.

BACKGROUND/AIM: We investigated the relationship of gastric cancer (GC) and CpG island hypermethylation (CIHM) in tumor suppressor genes of non-neoplastic gastric mucosa. METHODS: Gastric mucosa samples were obtained from 125 GC and 180 non-GC subjects. CIHM of p14, p16, DAP-kinase and CDH1 genes were determined by methylation-specific polymerase chain reaction. High CIHM was defined as three or all methylated CpG islands. RESULTS: Rates of CIHM of p14, CDH1, DAP-kinase, and high CIHM were significantly higher in all GC samples than non-GC samples (p14: 32.2 vs. 50.4%; OR = 1.70, 95% CI = 1.03-2.80, p = 0.04, CDH1: 36.1 vs. 84.0%; OR = 8.65, 95% CI = 14.74-15.77, p < 0.0001, DAP-kinase: 42.2 vs. 83.2%; OR = 5.98, 95% CI = 3.37-10.62, p < 0.0001, and high CIHM: 44.4 vs. 80.8%; OR = 4.40, 95% CI = 2.51-7.72, p < 0.0001). CIHM in CDH1 and DAP-kinase were associated with a greater risk of GC including all of its different subtypes. An increased number of CIHM was associated with an increased risk of all GC (1 CIHM; OR = 2.33, 95% CI = 0.82-6.64, p = 0.11, 2 CIHM; OR = 4.89, 95% CI = 1.79-13.37, p = 0.002, 3 CIHM; OR = 9.43, 95% CI = 3.20-27.78, p < 0.0001, and all CIHM OR = 24.71, 95% CI = 6.70-91.18, p < 0.0001). Three and all CIHM were closely associated with a higher risk of intestinal-type GC, Helicobacter pylori-positive infection status, male gender, and middle and older GC while 3 CIHM was closely associated with a higher risk of diffuse-type GC, H. pylori-negative infection status and younger GC. CONCLUSIONS: CIHM of CDH1 and DAP-kinase in non-neoplastic gastric mucosa corresponded to a risk of GC regardless of histological subtype, H. pylori infection status, gender and generation. An increased number of CIHM correlates with a higher GC risk including its various clinico-pathological subtypes.

Tajima, Y., et al. (2016). "[A Systematic Analysis of Oncogene and Tumor Suppressor Genes for Panitumumab-Resistant Rectal Cancer with Wild RAS Gene - A Case Report]." Gan To Kagaku Ryoho **43**(12): 2280-2282.

A 58-year-old man was admitted with the complaint of bloody stools. Colonoscopy and computed tomography revealed a rectal cancer with a liver metastasis and multiple lung metastases. After administering a regimen comprising 3 courses of XELOX plus bevacizumab chemotherapy, the sizes of the primary and metastatic lesions decreased remarkably. Abdominoperineal resection was performed for local control of the cancer; the specimen from the initial tumor was found to be KRAS wild type. After 14 courses of XELOX chemotherapy, brain metastases were detected. Although 3 courses of IRIS plus panitumumab were administered, the liver, lung, and brain metastases spread rapidly. A comprehensive genomic analysis focused on cancer-related genes with CancerPlex (R)found a mutation of the BRAF gene (I326V). BRAF is a downstream molecule of KRAS in the RAS-RAF-MAPK pathway. Therefore, this mutation of the BRAF gene has the possibility of causing resistance against panitumumab that was found in this case. Furthermore, we expect that the systematic analysis of oncogene and suppressor oncogenes will enable us to choose the optimal regimen of chemotherapy or molecular targeting therapy for each patient with colorectal cancer.

Takai, N., et al. (2005). "Discovery of epigenetically masked tumor suppressor genes in endometrial cancer." Mol Cancer Res **3**(5): 261-269.

Realization that many tumor suppressor genes are silenced by epigenetic mechanisms has stimulated the discovery of novel tumor suppressor genes. We used a variety of research tools to search for genes that are epigenetically silenced in human endometrial cancers. Changes in global gene expression of the endometrial cancer cell line Ishikawa was analyzed after treatment with the demethylating agent 5-aza-2'-deoxycytidine combined with the histone deacetylase inhibitor suberoylanilide bishydroxamide. By screening over 22,000 genes, candidate tumor suppressor genes were identified. Additional microarray analysis and real-time reverse transcription-PCR of normal and cancerous endometrial samples and search for CpG islands further refined the list. Tazarotene-induced gene-1 (Tig1) and CCAAT/enhancer binding protein-alpha (C/ebpalpha) were chosen for further study. Expression of both genes was low in endometrial cancer cell lines and clinical samples but high in normal endometrial tissues. Bisulfite sequencing, restriction analysis, and/or methylation-specific PCR revealed aberrant methylation of the CpG island in the

Tig1 gene of all 6 endometrial cancer cell lines examined and 4 of 18 clinical endometrial cancers, whereas the C/ebpalpha promoter remained unmethylated in endometrial cancers. Chromatin immunoprecipitation showed increased acetylated histone H3 bound to both Tig1 and C/ebpalpha genes after treatment with 5-aza-2'-deoxycytidine and/or suberoylanilide bishydroxamide. Forced expression of either TIG1 or C/EBPalpha led to significant growth reduction of Ishikawa cells. Our data suggest that C/ebpalpha and Tig1 function as tumor suppressor proteins in endometrial cancers and that their reexpression may be a therapeutic target.

Torabi, K., et al. (2015). "Patterns of somatic uniparental disomy identify novel tumor suppressor genes in colorectal cancer." Carcinogenesis **36**(10): 1103-1110.

Colorectal cancer (CRC) is characterized by specific patterns of copy number alterations (CNAs), which helped with the identification of driver oncogenes and tumor suppressor genes (TSGs). More recently, the usage of single nucleotide polymorphism arrays provided information of copy number neutral loss of heterozygosity, thus suggesting the occurrence of somatic uniparental disomy (UPD) and uniparental polysomy (UPP) events. The aim of this study is to establish an integrative profiling of recurrent UPDs/UPPs and CNAs in sporadic CRC. Our results indicate that regions showing high frequencies of UPD/UPP mostly coincide with regions typically involved in genomic losses. Among them, chromosome arms 3p, 5q, 9q, 10q, 14q, 17p, 17q, 20p, 21q and 22q preferentially showed UPDs/UPPs over genomic losses suggesting that tumor cells must maintain the disomic state of certain genes to favor cellular fitness. A meta-analysis using over 300 samples from The Cancer Genome Atlas confirmed our findings. Several regions affected by recurrent UPDs/UPPs contain well-known TSGs, as well as novel candidates such as ARID1A, DLC1, TCF7L2 and DMBT1. In addition, VCAN, FLT4, SFRP1 and GAS7 were also frequently involved in regions of UPD/UPP and displayed high levels of methylation. Finally, sequencing and fluorescence in situ hybridization analysis of the gene APC underlined that a somatic UPD event might represent the second hit to achieve biallelic inactivation of this TSG in colorectal tumors. In summary, our data define a profile of somatic UPDs/UPPs in sporadic CRC and highlights the importance of these events as a mechanism to achieve the inactivation of TSGs.

Turner, D. P. and D. K. Watson (2008). "ETS transcription factors: oncogenes and tumor suppressor genes as therapeutic targets for prostate cancer." Expert Rev Anticancer Ther **8**(1): 33-42.

ETS factors represent one of the largest families of transcriptional regulators and have known functional roles in many biological processes. Significantly, ETS factors have oncogenic and suppressive activity and their aberrant expression is associated with many of the processes that lead to prostate cancer progression. The targeting of transcription for therapeutic gain has met with some success. Therefore, better understanding the mechanisms that regulate ETS factor activity during both normal and aberrant transcription provides a novel means to identify processes that may be targeted in order to re-establish the normal ETS regulatory networks that are perturbed in cancer. Specific examples of altered ETS factor expression are highlighted, and therapeutic technologies that may be used to target ETS factors and their cofactors and downstream target genes in prostate cancer are discussed.

Zuberi, M., et al. (2016). "Utility of Serum miR-125b as a Diagnostic and Prognostic Indicator and Its Alliance with a Panel of Tumor Suppressor Genes in Epithelial Ovarian Cancer." PLoS One **11**(4): e0153902.

MicroRNAs (miRNAs) have been found to be dysregulated in epithelial ovarian cancer (EOC) and may function as either tumor suppressor genes (TSGs) or as oncogenes. Hypermethylation of miRNA silences the tumour suppressive function of a miRNA or hypermethylation of a TSG regulating that miRNA (or vice versa) leads to its loss of function. The present study aims to evaluate the impact of aberrant microRNA-125b (miR-125b) expression on various clinicopathological features in epithelial ovarian cancer and its association with anomalous methylation of several TSGs. We enrolled 70 newly diagnosed cases of epithelial ovarian cancer, recorded their clinical history and 70 healthy female volunteers. Serum miR-125b levels were determined by quantitative reverse transcription polymerase chain reaction (qRT-PCR) and the methylation status of various TSGs was investigated by methylation specific PCR. ROC curves were constructed to estimate the diagnostic and prognostic usefulness of miR-125b. The Kaplan-Meier method was applied to compare survival curves. Expression of miR-125b was found to be significantly upregulated ($p<0.0001$) in comparison with healthy controls. The expression level of miR-125b was found to be significantly associated with FIGO stage, lymph node and distant metastasis. ROC curve for diagnostic potential yielded significant AUC with an equitable sensitivity and specificity. ROC curves for prognosis yielded significant AUCs for

histological grade, distal metastasis, lymph node status and survival. The expression of miR-125b also correlated significantly with the hypermethylation of TSGs. Our results indicate that DNA hypermethylation may be involved in the inactivation of miR-125b and miR-125b may function as a potential independent biomarker for clinical outcome in EOC.

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