Cancer Suppressor-gene

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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 is literature collections for cancer suppressor-gene researches.
[Smith MH. **Cancer Suppressor-gene.** *Cancer Biology* 2011;1(2):272-296]. (ISSN: 2150-1041). http://www.cancerbio.net. 5

Keywords: cancer; biology; research; life; disease; suppressor-gene researches

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.

Adamovic, T., A. Hamta, et al. (2008). "Rearrangement and allelic imbalance on chromosome 5 leads to homozygous deletions in the CDKN2A/2B tumor suppressor gene region in rat endometrial cancer." Cancer Genet Cytogenet **184**(1): 9-21.

The inbred BDII rat is a valuable experimental model for the genetic analysis of hormone-dependent endometrial adenocarcinoma (EAC). One common aberration detected previously by comparative genomic hybridization in rat EAC is loss affecting mostly the middle part of rat chromosome 5 (RNO5). First, we applied an RNO5-specific painting probe and four region-specific gene probes onto tumor cell metaphases from 21 EACs, and found that rearrangements involving RNO5 were common. The copy numbers of loci situated on RNO5 were found to be reduced, particularly for the CDKN2A/2B locus. Second, polymerase chain reaction analysis was performed with 22 genes and markers and homozygous deletions of the CDKN2A exon 1beta and CDKN2B genes were detected in 13 EACs (62%) and of CDKN2A exon 1alpha in 12 EACs (57%) Third, the occurrence of allelic imbalance in RNO5 was analyzed using 39 microsatellite markers covering the entire chromosome and frequent loss of heterozygosity was detected. Even more intriguing was the repeated finding of allele switching in a narrow region of 7 Mb across the CDKN2A/2B locus. We conclude that genetic events affecting the middle part of RNO5 (including bands 5q31 approximately q33 and the CDKN2A locus) contribute to the development of EAC in rat, with the CDKN2A locus having a primary role.

Alvarez-Diaz, S., N. Valle, et al. (2009). "Cystatin D is a candidate tumor suppressor gene induced by vitamin D in human colon cancer cells." J Clin Invest **119**(8): 2343-58.

The active vitamin D metabolite 1alpha,25-dihydroxyvitamin D3 [1alpha,25(OH)2D3] has wide but not fully understood antitumor activity. A previous transcriptomic analysis of 1alpha,25(OH)2D3 action on human colon cancer cells revealed cystatin D (CST5), which encodes an inhibitor of several cysteine proteases of the cathepsin family, as a candidate target gene. Here we report that 1alpha,25(OH)2D3 induced vitamin D receptor (VDR) binding to, and activation of, the CST5 promoter and increased CST5 RNA and protein levels in human colon cancer cells. In cells lacking endogenous cystatin D, ectopic cystatin D expression inhibited both proliferation in vitro and xenograft tumor growth in vivo. Furthermore, cystatin D inhibited migration and anchorage-independent growth, antagonized the Wnt/beta-catenin signaling pathway, and repressed c-MYC expression. Cystatin D repressed expression of the epithelial-mesenchymal transition inducers SNAI1, SNAI2, ZEB1, and ZEB2 and, conversely, induced E-cadherin and other adhesion proteins. CST5 knockdown using shRNA abrogated the antiproliferative effect of

1alpha,25(OH)2D3, attenuated E-cadherin expression, and increased c-MYC expression. In human colorectal tumors, expression of cystatin D correlated with expression of VDR and E-cadherin, and loss of cystatin D correlated with poor tumor differentiation. Based on these data, we propose that CST5 has tumor suppressor activity that may contribute to the antitumoral action of 1alpha,25(OH)2D3 in colon cancer.

Anna, L., R. Holmila, et al. (2009). "Relationship between TP53 tumour suppressor gene mutations and smoking-related bulky DNA adducts in a lung cancer study population from Hungary." Mutagenesis **24**(6): 475-80.

Lung cancer rate in Hungary is one of the highest in the world among men and also very high among women, for reasons not clearly understood yet. The aim of the study was to explore characteristics of DNA damage and TP53 gene mutations in lung cancer from Hungary. Tissue samples from 104 lung resections for lung cancer patients, both men and women, operated on for non-small cell lung cancer, specifically, primary squamous cell carcinoma or adenocarcinoma were studied. Of the cases, 37% smoked up to the surgery, 24% stopped smoking within 1 year before the surgery, 26% stopped smoking more than a year before the surgery and 13% never smoked. TP53 mutations were detected by denaturant gradient gel electrophoresis, automated capillary electrophoresis single-strand conformation polymorphism and sequencing. Bulky DNA adduct levels were determined by (32)P-post-labelling in non-tumorous lung tissue. In total, 45% (47/104) of the cases carried TP53 mutation. The prevalence of TP53 mutations was statistically significantly associated with duration of smoking, tumour histology and gender. Smokers had approximately twice as high bulky adduct level as the combined group of former- and never-smokers (10.9 +/- 6.5 versus 5.5 +/- 3.4 adducts/10(8) nucleotides). The common base change G --> T transversion (8/43; 19%) was detected exclusively in smokers. For the first time, we demonstrate that most carriers of G --> T transversions had also a high level of bulky DNA adducts in their non-tumourous lung tissue. Our study provides evidence for a high burden of molecular alterations occurring concurrently in the lung of lung cancer patients.

Antonova, L. and C. R. Mueller (2008). "Hydrocortisone down-regulates the tumor suppressor gene BRCA1 in mammary cells: a possible molecular link between stress and breast cancer." Genes Chromosomes Cancer **47**(4): 341-52.

Psychological stress has been correlated with breast cancer development in numerous epidemiological studies. However, physiological and molecular models which may account for this association are not readily available. We have found that the stress hormone hydrocortisone (cortisol) down-regulates the expression of the breast cancer susceptibility gene BRCA1 in the nonmalignant mouse mammary cell line EPH4. This effect is concentration-dependent, is reliant on the continuous presence of hydrocortisone, and is not affected by the addition of lactogenic hormones, or growth conditions. Hydrocortisone was also found to negate a known positive effect of estrogen on BRCA1 expression and, therefore, may interfere with estrogen-related signaling in mammary epithelial cells. The repressive effect of hydrocortisone is diminished or lost in the mouse mammary lines HC-11 and SP1, respectively, suggesting regulation of the BRCA1 may differ between lines. We have uncovered two promoter regulatory sites, which are involved in BRCA1 regulation by hydrocortisone, namely the RIBS and UP regulatory elements. Binding of the transcription factor GABP to both sites is lost upon hydrocortisone addition, though the levels of these factors are not altered by hydrocortisone treatment. Because BRCA1 activity is important for a number of intracellular pathways involved in prevention of tumorigenesis, its observed down-regulation may represent a novel molecular mechanism for cortisol's involvement in breast cancer development.

Bandyopadhyay, S., Y. Wang, et al. (2006). "The tumor metastasis suppressor gene Drg-1 down-regulates the expression of activating transcription factor 3 in prostate cancer." Cancer Res **66**(24): 11983-90.

The tumor metastasis suppressor gene Drg-1 has been shown to suppress metastasis without affecting tumorigenicity in immunodeficient mouse models of prostate and colon cancer. Expression of Drg-1 has also been found to have a significant inverse correlation with metastasis or invasiveness in various types of human cancer. However, how Drg-1 exerts its metastasis suppressor function remains unknown. In the present study, to elucidate the mechanism of action of the Drg-1 gene, we did a microarray analysis and found that induction of Drg-1 significantly inhibited the expression of activating transcription factor (ATF) 3, a member of the ATF/cyclic AMP-responsive element binding protein family of transcription factors. We also showed that Drg-1 attenuated the endogenous level of ATF3 mRNA and protein in prostate cancer cells, whereas Drg-1 small interfering RNA up-regulated the ATF3 expression. Furthermore, Drg-1 suppressed the

promoter activity of the ATF3 gene, indicating that Drg-1 regulates ATF3 expression at the transcriptional level. Our immunohistochemical analysis on prostate cancer specimens revealed that nuclear expression of ATF3 was inversely correlated to Drg-1 expression and positively correlated to metastases. Consistently, we have found that ATF3 overexpression promoted invasiveness of prostate tumor cells in vitro, whereas Drg-1 suppressed the invasive ability of these cells. More importantly, overexpression of ATF3 in prostate cancer cells significantly enhanced spontaneous lung metastasis of these cells without affecting primary tumorigenicity in a severe combined immunodeficient mouse model. Taken together, our results strongly suggest that Drg-1 suppresses metastasis of prostate tumor cells, at least in part, by inhibiting the invasive ability of the cells via down-regulation of the expression of the ATF3 gene.

Benjamin, C. L., S. E. Ullrich, et al. (2008). "p53 tumor suppressor gene: a critical molecular target for UV induction and prevention of skin cancer." Photochem Photobiol **84**(1): 55-62.

The relationship between exposure to UV radiation and development of skin cancer has been well established. Several studies have shown that UVB induces unique mutations (C-->T and CC-->TT transitions) in the p53 tumor suppressor gene that are not commonly induced by other carcinogens. Our studies have demonstrated that UV-induced mouse skin cancers contain p53 mutations at a high frequency and that these mutations can be detected in UV-irradiated mouse skin well before the appearance of skin tumors. This observation suggested that it might be possible to use p53 mutations as a biologic endpoint for testing the efficacy of sunscreens in photoprotection studies. Indeed, application of SPF 15 sunscreens to mouse skin before each UVB irradiation resulted in reduction in the number of p53 mutations. Because p53 mutations represent an early essential step in photocarcinogenesis, these results imply that inhibition of this event may protect against skin cancer development. This hypothesis was confirmed by our finding that sunscreens used in p53 mutation inhibition experiments also protected mice against UVB-induced skin cancer.

Clarhaut, J., R. M. Gemmill, et al. (2009). "ZEB-1, a repressor of the semaphorin 3F tumor suppressor gene in lung cancer cells." Neoplasia **11**(2): 157-66.

SEMA3F is a secreted semaphorin with potent antitumor activity, which is frequently downregulated in lung cancer. In cancer cell lines, SEMA3F overexpression decreases hypoxia-induced factor 1alpha protein and vascular endothelial growth factor mRNA, and inhibits multiple signaling components. Therefore, understanding how SEMA3F expression is inhibited in cancer cells is important. We previously defined the promoter organization of SEMA3F and found that chromatin remodeling by a histone deacetylase inhibitor was sufficient to activate SEMA3F expression. In lung cancer, we have also shown that ZEB-1, an E-box transcription repressor, is predominantly responsible for loss of E-Cadherin associated with a poor prognosis and resistance to epidermal growth factor receptor inhibitors. In the present study, we demonstrated that ZEB-1 also inhibits SEMA3F in lung cancer cells. Levels of ZEB-1, but not ZEB-2, Snail or Slug, significantly correlate with SEMA3F inhibition, and overexpression or inhibition of ZEB-1 correspondingly affected SEMA3F expression. Four conserved E-box sites were identified in the SEMA3F gene. Direct ZEB-1 binding was confirmed by chromatin immunoprecipitation assays for two of these, and ZEB-1 binding was reduced when cells were treated with a histone deacetylase inhibitor. These results demonstrate that ZEB-1 directly inhibits SEMA3F expression in lung cancer cells. SEMA3F loss was associated with changes in cell signaling: increased phospho-AKT in normoxia and increase of hypoxia-induced factor 1alpha protein in hypoxia. Moreover, exogenous addition of SEMA3F could modulate ZEB-1-induced angiogenesis in a chorioallantoic membrane assay. Together, these data provide further support for the importance of SEMA3F and ZEB-1 in lung cancer progression.

Csontos, Z., E. Nadasi, et al. (2008). "Oncogene and tumor suppressor gene expression changes in the peripheral blood leukocytes of patients with colorectal cancer." Tumori **94**(1): 79-82.

AIMS AND BACKGROUND: The mortality of colorectal cancer continues to stagnate despite the development of new therapeutic approaches. Therefore, identifying high-risk population groups could contribute to the prevention of a considerable part of deaths caused by colorectal tumors. METHODS: Fifty patients with colon cancer and 50 patients with other, nonmalignant diseases were selected for the study. Expression of the c-myc, Ha-ras and p53 genes was determined in the peripheral leukocytes of the participants. RESULTS: Marked elevations of the expression of all three investigated genes were seen in the colon cancer patients when compared to the controls. CONCLUSIONS: Our investigations showed that increases in the expression of c-myc, Ha-ras and p53 genes can be demonstrated in the peripheral leukocytes of colon cancer patients. By applying our method to clinical investigations, individuals with a high risk of having developed colon

cancer may be identified and early diagnosis may be established.

Dong, X. Y., C. Rodriguez, et al. (2008). "SnoRNA U50 is a candidate tumor-suppressor gene at 6q14.3 with a mutation associated with clinically significant prostate cancer." Hum Mol Genet **17**(7): 1031-42.

Deletion of chromosome 6q14-q22 is common in multiple human cancers including prostate cancer, and chromosome 6 transferred into cancer cells induces senescence and reduces cell growth, tumorigenicity and metastasis, indicating the existence of one or more tumor-suppressor genes in 6q. To identify the 6q tumor-suppressor gene, we first narrowed the common region of deletion to a 2.5 Mb interval at 6q14-15. Of the 11 genes located in this minimal deletion region and expressed in normal prostates, only snoRNA U50 was mutated, demonstrated transcriptional downregulation and inhibited colony formation in prostate cancer cells. The mutation, a homozygous 2 bp (TT) deletion, was found in two of 30 prostate cancer cell lines/xenografts and nine of 89 localized prostate cancers (eleven of 119 or 9% cancers). Two of 89 (2%) patients with prostate cancer also showed the same mutation in their germline DNA, but none of 104 cancer-free control men did. The homozygous deletion abolished U50 function in a colony formation assay. Analysis of 1371 prostate cancer cases and 1371 matched control men from a case-control study nested in a prospective cohort showed that, although a germline heterozygous genotype of the deletion was detected in both patients and controls at similar frequencies, the homozygosity of the deletion was significantly associated with clinically significant prostate cancer (odds ratio 2.9; 95% confidence interval 1.17-7.21). These findings establish snoRNA U50 as a reasonable candidate for the 6q tumor-suppressor gene in prostate cancer and likely in other types of cancers.

Dova, L., V. Golfinopoulos, et al. (2008). "Systemic dissemination in cancer of unknown primary is independent of mutational inactivation of the KiSS-1 metastasis-suppressor gene." Pathol Oncol Res **14**(3): 239-41.

Cancer of unknown primary represents a heterogeneous group of malignancies characterised by early systemic dissemination and lack of primary site. KiSS1 is a member of the metastasis-suppressor gene family whose functional role is being investigated in human malignancies. We extracted DNA from 50 paraffin-embedded unknown primary tumors and screened KiSS1 exons III and IV for presence of mutations by means of Single Strand Conformational Polymorphism and direct sequencing. Only one tumor specimen harboured a cytosine to guanine point substitution in base 242 of exon IVa, resulting in a proline to arginine switch at codon 81 of the KiSS1 protein (P81R). The remaining 49 tumors harbored wild-type KiSS1 alleles, indistinguishable from those of peripheral blood lymphocytes of 50 healthy controls. Consequently, the propensity for systemic spread of unknown primary tumors may by due to mutations in genes other than KiSS1 or aberrant epigenetic regulation.

Duggan, D., S. L. Zheng, et al. (2007). "Two genome-wide association studies of aggressive prostate cancer implicate putative prostate tumor suppressor gene DAB2IP." J Natl Cancer Inst **99**(24): 1836-44.

BACKGROUND: The consistent finding of a genetic susceptibility to prostate cancer suggests that there are germline sequence variants predisposing individuals to this disease. These variants could be useful in screening and treatment. METHODS: We performed an exploratory genome-wide association scan in 498 men with aggressive prostate cancer and 494 control subjects selected from a population-based case-control study in Sweden. We combined the results of this scan with those for aggressive prostate cancer from the publicly available Cancer Genetic Markers of Susceptibility (CGEMS) Study. Single-nucleotide polymorphisms (SNPs) that showed statistically significant associations with the risk of aggressive prostate cancer based on two-sided allele tests were tested for their association with aggressive prostate cancer in two independent study populations composed of individuals of European or African American descent using one-sided tests and the genetic model (dominant or additive) associated with the lowest value in the exploratory study. RESULTS: Among the approximately 60,000 SNPs that were common to our study and CGEMS, we identified seven that had a similar (positive or negative) and statistically significant (P<.01) association with the risk of aggressive prostate cancer in both studies. Analysis of the distribution of these SNPs among 1032 prostate cancer patients and 571 control subjects of European descent indicated that one, rs1571801, located in the DAB2IP gene, which encodes a novel Ras GTPase-activating protein and putative prostate tumor suppressor, was associated with aggressive prostate cancer (one-sided P value = .004). The association was also statistically significant in an African American study population that included 210 prostate cancer patients and 346 control subjects (one-sided P value = .02). CONCLUSION: A genetic variant in DAB2IP may be associated with the risk of aggressive prostate cancer and should be evaluated further.

Ecke, T. H., H. H. Schlechte, et al. (2008). "Body mass index (BMI) and mutations of tumor suppressor gene p53 (TP53) in patients with urinary bladder cancer." Urol Oncol **26**(5): 470-3.

OBJECTIVE: Obesity is estimated to account for up to 20% of all cancer deaths. Mutations of TP53 are frequently correlated with tumor development and progression. We evaluated the effect of body mass index (BMI) and mutation status of tumor suppressor gene p53 (TP53) on patients with urinary bladder cancer. MATERIALS AND METHODS: Clinical samples were used from 75 patients with tumors of the urinary bladder. Mutation status in TP53 exons 5, 6, 7, and 8 was analyzed by temperature gradient gel electrophoresis of exon-specific PCR products and by sequence analysis. Statistical analysis included Pearson's correlation. RESULTS: For noninvasive bladder cancer, the mutation frequency in TP53 was 44.6%, while for invasive bladder cancer the mutation frequency in TP53 was 84.2%. Normal weight, overweight, and patients with obesity had a TP53 mutation frequency of 68.4%, 44.8%, and 25%, respectively ($P < 0.05$). CONCLUSIONS: TP53 mutation frequently occurs in higher stages of bladder tumors. Body mass index is not associated with a higher TP53 mutation frequency in our study, but BMI should be included for collecting data of bladder cancer risk profile.

Elangovan, S., T. C. Hsieh, et al. (2008). "Growth inhibition of human MDA-mB-231 breast cancer cells by delta-tocotrienol is associated with loss of cyclin D1/CDK4 expression and accompanying changes in the state of phosphorylation of the retinoblastoma tumor suppressor gene product." Anticancer Res **28**(5A): 2641-7.

Tocotrienols, a subgroup within the vitamin E family of compounds, have shown antiproliferative and anticancer properties, however, the molecular basis of these effects remains to be elucidated. In this study, the effect of 3-tocotrienol on cell cycle arrest was assessed by studying the retinoblastoma protein (Rb) levels and phosphorylation status, levels of E2F (a transcription factor critically involved in the G1/S-phase transition of the mammalian cell cycle; originally identified as a DNA-binding protein essential for early region 1A-dependent activation of the adenovirus promoter designated E2), and other cell cycle controlling proteins in estrogen receptor-negative MDA-MB-231 breast cancer cells. The cell growth assay demonstrated that exposure of the MDA-MB-231 cells to 6-tocotrienol (1-20 microM) resulted in a dose- and time-dependent inhibition of cell growth as compared with vehicle treated cells and the magnitude of growth inhibition was higher at 10 and 20 microM treatment for 48 and 72 h. The phosphorylation status of Rb plays a central role in the control of the cell cycle at the G0/G1-phase. delta-Tocotrienol treatment reduced the total Rb and its phosphorylation at the Ser780, Ser795, Ser 807/811 and Thr826 positions in a dose- and time-dependent fashion. The site-specific inhibition of the phosphorylation of Rb by delta-tocotrienol was tightly associated with a marked reduction in the expression of cyclin D1 and its regulatory partner cyclin-dependant kinase 4 (CDK4), which is responsible for the phosphorylation of Rb at Ser780, Ser795, Ser 807/811 and Thr826. In addition, delta-tocotrienol also reduced the expression of E2F that occurred simultaneously with the loss of Rb phosphorylation and inhibition of cell cycle progression. Interestingly, delta-tocotrienol also caused a marked reduction in the expression of G2/M regulatory proteins including cyclin B1 and CDK1. To the best of our knowledge, this study was the first to reveal that the target of cell proliferative inhibitory action of delta-tocotrienol in a model estrogen receptor-negative human breast cancer cell line MDA-MB-231 is mediated by the loss of cyclin D1 and associated suppression of site-specific Rb phosphorylation, suggesting its future development and use as an anticancer agent.

Feng, W., Z. Lu, et al. (2007). "Multiple histone deacetylases repress tumor suppressor gene ARHI in breast cancer." Int J Cancer **120**(8): 1664-8.

ARHI is a maternally imprinted tumor suppressor gene that is expressed in normal breast and ovarian epithelial cells but not in most breast and ovarian cancers. Our earlier studies showed that histone deacetylases (HDACs) in complexes with transcription factors E2F1 and E2F4 play an important role in downregulating ARHI expression in breast cancer cells. To determine which HDAC or HDACs are responsible for repressing ARHI, we cotransfected vectors expressing HDACs 1-11 with an ARHI/luciferase reporter into SKBr3 and MCF-7 breast cancer cells. Expression of multiple HDACs consistently reduced ARHI promoter activity in a dose-dependent manner. We also found that the expression level of HDACs 1-3 was higher in breast cancer cell lines than in normal breast epithelial cells. In agreement with their repressive function, depletion of HDACs 1, 3 and 11 not only significantly increased the ARHI promoter activity of the transfected reporter but also activated the transcription of the endogenous ARHI gene. Furthermore, depletion or inhibition of HDACs by small interfering RNA of HDAC11 or by trichostatin A, respectively, increased E2F acetylation. Chromatin immunoprecipitation assays revealed that HDACs 1 and 3 are bound to the ARHI promoter. Taken together, our results suggest that the activity of multiple HDACs contributes to the repression of the

ARHI tumor suppressor gene in breast cancer cells. Since HDAC inhibitors are now being used to treat breast cancer, the reactivation of ARHI in these cancer cells may serve as a new biomarker with which to monitor the treatment effects.

Frank, B., J. L. Bermejo, et al. (2007). "Copy number variant in the candidate tumor suppressor gene MTUS1 and familial breast cancer risk." Carcinogenesis **28**(7): 1442-5.

Copy number variants (CNVs), insertions, deletions and duplications, contribute considerably to human genetic variation and disease development. A recent study has characterized 100 CNVs including a deletion in the mitochondrial tumor suppressor gene 1 (MTUS1) lacking the coding exon 4. MTUS1 maps to chromosome 8p, a region frequently deleted and associated with disease progression in human cancers, including breast cancer (BC). To investigate the effect of the MTUS1 CNV on familial BC risk, we analyzed 593 BC patients and 732 control individuals using a case-control study design. We found a significant association of the deletion variant with a decreased risk for both familial and high-risk familial BC (odds ratio (OR) = 0.58, 95% confidence interval (CI) = 0.37-0.90, P = 0.01 and OR = 0.41, 95% CI = 0.23-0.74, P = 0.003), supporting its role in human cancer. To our knowledge, the present study is the first to determine the impact of a CNV in a tumor suppressor gene on cancer risk.

Fujii, T., G. Yokoyama, et al. (2008). "Preclinical and clinical studies of novel breast cancer drugs targeting molecules involved in protein kinase C signaling, the putative metastasis-suppressor gene Cap43 and the Y-box binding protein-1." Curr Med Chem **15**(6): 528-37.

Breast cancer is a common cause of tumors in women. The development of effective adjuvant therapies using drugs such as anthracyclines, taxanes, and aromatase inhibitors has improved the survival of breast cancer patients. Molecular cancer therapeutics are also attracting attention, and targeted molecular therapies, such as trastuzumab, have already contributed to effective new treatments for breast cancer. Other candidate targeted molecular therapies for breast cancer, including erlotinib, gefitinib, lapatinib, bevacizumab, and celecoxib, are currently undergoing clinical evaluation, and promising results are expected. The current review provides an up-to-date summary of the preclinical and clinical development of these drugs for breast cancer. In particular, we focus on therapies targeting protein kinase C (PKC) signaling, the putative metastasis-suppressor gene Cap43/N-myc downstream-regulated gene 1 (NDRG1)/differentiation-related gene-1 (Drg-1), and the Y-box binding protein-1 (YB-1). The PKC signaling pathway is widely considered to be a promising target for the development of novel therapeutics. Cap43 expression is significantly modulated by estrogen and/or anti-estrogens in breast cancer cells that are positive for estrogen receptor-alpha (ER-alpha). Cap43 is therefore of particular interest as a molecular indicator of the therapeutic efficacy of anti-estrogenic agents in breast cancer. The nuclear expression of YB-1 plays an essential role in the acquisition of malignant characteristics by breast cancer cells, through epidermal growth factor receptor 2 (HER2)-Akt-dependent pathways. Basic research investigating the key selective molecular changes that sustain breast cancer growth and progression, as demonstrated for PKC, Cap43, and YB-1, is allowing the development of specific targeted molecular diagnostics and therapeutics.

Gery, S., N. Komatsu, et al. (2007). "Epigenetic silencing of the candidate tumor suppressor gene Per1 in non-small cell lung cancer." Clin Cancer Res **13**(5): 1399-404.

PURPOSE: Epigenetic events are a critical factor contributing to cancer development. The purpose of this study was to identify tumor suppressor genes silenced by DNA methylation and histone deacetylation in non-small cell lung cancer (NSCLC). EXPERIMENTAL DESIGN: We used microarray analysis to screen for tumor suppressor genes. RESULTS: We identified Per1, a core circadian gene, as a candidate tumor suppressor in lung cancer. Although Per1 levels were high in normal lung, its expression was low in a large panel of NSCLC patient samples and cell lines. Forced expression of Per1 in NSCLC cell lines led to significant growth reduction and loss of clonogenic survival. Recent studies showed that epigenetic regulation, particularly histone H3 acetylation, is essential for circadian function. Using bisulfite sequencing and chromatin immunoprecipitation, we found that DNA hypermethylation and histone H3 acetylation are potential mechanisms for silencing Per1 expression NSCLC. CONCLUSIONS: These results support the hypothesis that disruption of circadian rhythms plays an important role in lung tumorigenesis. Moreover, our findings suggest a novel link between circadian epigenetic regulation and cancer development.

Grunt, T. W., K. Tomek, et al. (2007). "The DNA-binding epidermal growth factor-receptor inhibitor PD153035 and other DNA-intercalating cytotoxic drugs reactivate the expression of the retinoic acid receptor-beta tumor-suppressor gene in breast cancer cells." Differentiation **75**(9): 883-90.

We have previously shown that the epidermal growth factor-receptor (EGFR) tyrosine kinase inhibitor PD153035 induces retinoic acid receptor-beta (RAR-beta) expression in malignant cells by mechanisms that are independent of its blocking activity on EGFR (ErbB1) or on any other ErbB receptor (ErbB2, ErbB3, ErbB4). RAR-beta2, one of three human RAR-beta isoforms (RAR-beta1, RAR-beta2, RAR-beta4), is silenced in many tumors and acts as a tumor suppressor. Forced expression of RAR-beta2 reverts the malignant phenotype of RAR-beta2-negative breast cancer cells and reconstitutes retinoid sensitivity in these cells. Here, we demonstrate that the EGFR inhibitor PD153035 specifically induces RAR-beta2, but not the other two isoforms (RAR-beta1, RAR-beta4) in MDA-MB-468 and MDA-MB-453 human breast cancer cells. Induction was seen at the mRNA (reverse transcription-polymerase chain reaction) and protein level (Western analysis). PD153035-mediated induction of RAR-beta2 was associated with synergistic growth inhibition in cells co-treated with PD153035 and all-trans retinoic acid (tRA). Most importantly, PD153035 restored retinoic acid sensitivity in retinoic acid-resistant cells. Our previous work also revealed that PD153035 directly intercalates into the DNA suggesting that changes in the chromatin structure contribute to the RAR-beta2-inducing effect of PD153035. This prompted us to examine the effect of DNA intercalating chemotherapeutic drugs such as doxorubicin, amsacrine, and mitoxantrone on the expression of RAR-beta. Vincristine was used for comparative reasons, because this drug does not target DNA. All four compounds caused dose-dependent growth inhibition in MDA-MB-468 and MDA-MB-453 cells. Interestingly, compounds that directly interact with the DNA (doxorubicin, amsacrine, mitoxantrone) caused a time-dependent up-regulation of the RAR-beta expression in all cell lines examined, whereas the negative control drug vincristine, which causes disruption of microtubule structures, did not stimulate RAR-beta expression. These data further support the notion that induction of the RAR-beta tumor-suppressor gene in cancer cells by PD153035 is mediated at least in part by its DNA intercalating activity.

Guan, M., V. Tripathi, et al. (2008). "Adenovirus-mediated restoration of expression of the tumor suppressor gene DLC1 inhibits the proliferation and tumorigenicity of aggressive, androgen-independent human prostate cancer cell lines: prospects for gene therapy." Cancer Gene Ther **15**(6): 371-81.

Our recent study showing highly recurrent loss of function of DLC1 (deleted in liver cancer 1), a tumor suppressor gene in primary prostate carcinoma (PCA), implicates this gene in the pathogenesis of this disease. To evaluate the response of PCA to oncosuppressive activity of DLC1, we examined now the effects of adenoviral vector for human DLC1 transduction into the DLC1-deficient, androgen-independent (AI) and aggressive human PCA cell lines PC-3 and C4-2-B2. Adenovirus-mediated restoration of DLC1 expression inhibited the proliferation, invasiveness and anchorage-independent growth of PC-3 and C4-2-B2 cells in vitro as well as the tumorigenicity of PC-3 cells in nude mice. It also induced cell-cycle arrest, inhibited the activation of RhoA and the formation of actin stress fibers. DLC1 induced apoptosis in C4-2-B2 cells, whereas it did not elicit such an effect in PC-3 cells. The abundance of the antiapoptotic protein Bcl-2 was greater in PC-3 cells than in C4-2-B2 cells, and PC-3 cells were rendered sensitive to DLC1-induced apoptosis by treatment with the Bcl-2 inhibitor HA14-1. These results suggest that adenovirus-mediated DLC1 transfer, alone or together with other agents, such as inhibitors of Bcl-2 or histone deacetylase, might prove effective in the treatment of aggressive, AI-PCA.

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

INTRODUCTION: There is growing evidence that the Wilms' tumor 1 suppressor gene (WT1) behaves as an oncogene in some forms of breast cancer. Previous studies have demonstrated that the N-terminal domain of WT1 can act as a dominant negative through self-association. In the studies presented here we have explored the potential for the zinc finger domain (ZF) of WT1 to also have dominant-negative effects, and thus further our understanding of this protein. METHODS: Using full-length and ZF-only forms of WT1 we assessed their effect on the WT1 and c-myc promoter using luciferase and chromatin immunoprecipitation assays. The gene expression levels were determined by quantitative real-time RT-PCR, northern blot and western blot. We also assessed the effect of the ZF-only form on the growth of breast cancer cell lines in culture. RESULTS: Transfection with WT1-ZF plasmids resulted in a stronger inhibition of WT1 promoter than full-length WT1 in breast cancer cells. The WT1-ZF form lacking the lysine-threonine-serine (KTS) insert (ZF - KTS) can bind to the majority of WT1 consensus sites throughout the WT1 promoter region, while the ZF containing the insert (ZF + KTS) form only binds to sites in the proximal promoter. The abundances of endogenous WT1 mRNA and protein

were markedly decreased following the stable expression of ZF - KTS in breast cancer cells. The expressions of WT1 target genes, including c-myc, Bcl-2, amphiregulin and TERT, were similarly suppressed by ZF - KTS. Moreover, WT1-ZF - KTS abrogated the transcriptional activation of c-myc mediated by all four predominant isoforms of WT1 (including or lacking alternatively spliced exons 5 and 9). Finally, WT1-ZF - KTS inhibited colony formation and cell division, but induced apoptosis in MCF-7 cells. CONCLUSION: Our observations strongly argue that the WT1-ZF plasmid behaves as a dominant-negative regulator of the endogenous WT1 in breast cancer cells. The inhibition on proliferation of breast cancer cells by WT1-ZF - KTS provides a potential candidate of gene therapy for breast cancer.

Han, Y., L. Yang, et al. (2008). "Wilms' tumor 1 suppressor gene mediates antiestrogen resistance via down-regulation of estrogen receptor-alpha expression in breast cancer cells." Mol Cancer Res **6**(8): 1347-55.

The antiestrogen tamoxifen has been used in the treatment of hormone-responsive breast cancer for over a decade. The loss of estrogen receptor (ER) expression is the most common mechanism for de novo antiestrogen resistance. Wilms' tumor 1 suppressor gene (WT1) is a clinically useful marker that is associated with poor prognosis in breast cancer patients; its high level expression is frequently observed in cases of breast cancer that are estrogen and progesterone receptor negative. The lack of expression of these receptors is characteristic of tumor cells that are not responsive to hormonal manipulation. To determine whether there is a linkage between WT1 expression and antiestrogen resistance in breast cancer cells, we studied the effect of WT1 on tamoxifen responsiveness in ERalpha-positive MCF-7 cells. We found that overexpression of WT1 in MCF-7 markedly abrogated tamoxifen-induced cell apoptosis and 17beta-estradiol (E(2))-mediated cell proliferation. The expressions of ERalpha and its downstream target genes were significantly repressed following overexpression of WT1, whereas the down-regulation of WT1 by WT1 shRNA could enhance ERalpha expression and the sensitivity to tamoxifen treatment in ERalpha-negative MDA468 and HCC1954 cells that express high levels of WT1. Furthermore, we have confirmed that the WT1 protein can bind to endogenous WT1 consensus sites in the proximal promoter of ERalpha and thus inhibit the transcriptional activity of the ERalpha promoter in a WT1 site sequence-specific manner. Our study clearly implicates WT1 as a mediator of antiestrogen resistance in breast cancer through down-regulation of ERalpha expression and supports the development of WT1 inhibitors as a potential means of restoring antiestrogen responsiveness in breast cancer therapy.

Helmbold, P., C. Lahtz, et al. (2009). "Frequent hypermethylation of RASSF1A tumour suppressor gene promoter and presence of Merkel cell polyomavirus in small cell lung cancer." Eur J Cancer **45**(12): 2207-11.

In small cell lung cancer (SCLC), hypermethylation of the tumour suppressor Ras association domain family 1A (RASSF1A) is frequent. It is associated with SV40 polyomaviral infection in other tumours. Merkel cell polyomavirus (MCPyV) infection has been reported in Merkel cell carcinoma (MCC), a neuroendocrine carcinoma with biological similarity to SCLC. In our study, we investigated polyomavirus infection (SV40 and MCPyV) and promoter hypermethylation of the tumour suppressors RASSF1A and p16 in 18 SCLCs (14 primaries and 4 regional lymph node metastases) and 18 blood control samples. MCPyV was found in 39% (7 of 18) of the tumour tissues but not observed in controls. SV40 was not observed in the tumour tissue. RASSF1A promoter hypermethylation (94%; 17 of 18) was more frequent compared to p16 methylation (56%, 10 of 18). We found no significant correlation between RASSF1A or p16 promoter hypermethylation and infection with the investigated polyoma viruses. Our results show a high frequency of hypermethylation of the RASSF1A promoter and occurrence of MCPyV infection in the tumour tissue of SCLC. These events may contribute to the pathogenesis of SCLC.

Helmig, S. and J. Schneider (2007). "Oncogene and tumor-suppressor gene products as serum biomarkers in occupational-derived lung cancer." Expert Rev Mol Diagn **7**(5): 555-68.

Since lung cancer is the most frequent occupational cancer and one of the leading causes of cancer mortality in the world, it is one of the biggest challenges for research. In the literature, there are inconsistent results regarding the utility of the serum biomarkers p53, anti-p53 antibodies, EGF receptor or Ras. Based on the published results, routine use of these biomarkers for detection of occupationally derived lung carcinomas is not currently recommended. In this review, we summarize the literature and discuss the relevance of these oncogene and tumor-suppressor gene products as serum biomarkers in occupational-derived lung cancer.

Hsieh, Y. S., Y. L. Lee, et al. (2007). "Association of EcoRI polymorphism of the metastasis-suppressor gene NME1 with susceptibility to and severity of non-small cell lung cancer." Lung Cancer **58**(2): 191-5.

BACKGROUND: Human lung cancer cells with high metastatic potential show reduced expression of the metastasis-suppressor gene NME1. However, the biallelic EcoRI polymorphism of this gene has not been studied in lung cancer. With this allelic association study, we aimed to investigate the impact of polymorphisms of the NME1 gene on the susceptibility to and severity of non-small cell lung cancer (NSCLC). METHODS: Through a case-control study design, genomic DNA samples of 255 NSCLC patients and 303 controls, who were age and sex-matched and recruited from the health check-up unit, were subjected to polymorphism analysis with polymerase chain reaction-restriction fragment length polymorphism technique. The validity of this technique was proven by direct sequencing of polymerase chain reaction products. Statistical analyses were conducted to explore the contribution of polymorphism of the metastasis-suppressor gene NME1 in the susceptibility to and severity of NSCLC. RESULTS: Overall, the genotype frequencies of NME1 gene were significantly different between lung cancer patients and controls ($p < 0.0001$), and also different between patients with lung cancers of various stages ($p < 0.0001$). Logistic regression analysis revealed that higher odds ratios (ORs) for lung cancer were seen in patients homozygous (+/+) for variant allele (an OR of 4.02, 95% CI 2.39-6.76; $p < 0.0001$). Patients carrying a variant polymorphic homozygote (+/+) also had a tendency to advanced disease ($p = 0.001$). CONCLUSION: A significant association between the polymorphisms of NME1 gene and the susceptibility to and severity of lung cancer was demonstrated.

Hu, L., Y. T. Zhu, et al. (2009). "Identification of Smyd4 as a potential tumor suppressor gene involved in breast cancer development." Cancer Res **69**(9): 4067-72.

To identify genes involved in breast tumorigenesis, we applied the retroviral LoxP-Cre system to a nontumorigenic mouse mammary epithelial cell line NOG8 to create random chromosome deletion/translocation. We found that the disruption of one allele of Smyd4 (SET and MYND domain containing 4) gene through chromosome translocation led to tumorigenesis. The expression of Smyd4 was markedly decreased in tumor cells. Re-expression of Smyd4 resulted in growth suppression of tumor cells and inhibition of tumor formation in nude mice. Furthermore, the RNA interference-mediated suppression of Smyd4 expression in human MCF10A mammary epithelial cells caused their growth in soft agar. Microarray studies revealed that platelet-derived growth factor receptor alpha polypeptide (Pdgfr-alpha) was highly expressed in tumor cells compared with NOG8 cells. Re-expression of Smyd4 significantly reduced the expression of Pdgfr-alpha in tumor cells. In human breast cancers, reverse transcription-PCR results revealed that Smyd4 expression was totally silenced in 2 of 10 specimens. These findings indicate that Smyd4, as a potential tumor suppressor, plays a critical role in breast carcinogenesis at least partly through inhibiting the expression of Pdgfr-alpha, and could be a novel target for improving treatment of breast cancer.

Ishida, M., M. Sunamura, et al. (2008). "The PMAIP1 gene on chromosome 18 is a candidate tumor suppressor gene in human pancreatic cancer." Dig Dis Sci **53**(9): 2576-82.

Frequent loss of heterozygosity on the long arm of chromosome 18 is observed in pancreatic cancer. Previous studies suggested the existence of one or more tumor-suppressor genes other than SMAD4 on chromosome 18. To identify the candidate tumor-suppressor gene(s), we compared gene expression by cDNA microarray analyses using a pancreatic cancer cell line Panc-1 and its hybrid cell lines showing suppressed cell growth after introduction of one normal copy of chromosome 18. The microarray analyses identified 38 genes on chromosome 18 that showed differential expressional levels. Among these genes, phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1/APR/NOXA) was identified as one of the candidates for tumor suppressor. Expression vector-mediated introduction of PMAIP1 suppressed cell proliferation, and RNAi-mediated knockdown of PMAIP1 induced recovery of cell growth. These results suggest that PMAIP1 may play an important role in the progression of pancreatic cancer.

Jagadeesh, S., S. Sinha, et al. (2007). "Mahanine reverses an epigenetically silenced tumor suppressor gene RASSF1A in human prostate cancer cells." Biochem Biophys Res Commun **362**(1): 212-7.

It is becoming clear that frequent epigenetic silencing of tumor suppressor genes could be responsible for the development of cancer in various organs. Several recent reports suggest that suppression of RASSF1A is associated with the advanced grade and stage of prostate cancer and many other cancers. In this investigation, we demonstrated that, mahanine, a plant derived carbazole alkaloid, induced RASSF1A expression in both androgen-responsive (LNCaP) and androgen-negative (PC3) prostate cancer cells by inhibiting DNA methyltransferase (DNMT) activity. Mahanine-induced expression of RASSF1A in turn significantly reduced cyclin D1 but not other cyclins. To understand the inverse relationship between

RASSF1A and cyclin D1, we observed that mahanine treatment down-regulates cyclin D1 and addition of RASSF1A siRNA prevented this inhibition. This study show for the first time that mahanine can reverse an epigenetically silenced gene, RASSF1A in prostate cancer cells by inhibiting DNMT activity that in turn down-regulates a key cell cycle regulator, cyclin D1. Mahanine therefore, promises an encouraging therapeutic choice for advanced prostatic cancer.

Jenal, M., E. Trinh, et al. (2009). "The tumor suppressor gene hypermethylated in cancer 1 is transcriptionally regulated by E2F1." Mol Cancer Res **7**(6): 916-22.

The Hypermethylated in Cancer 1 (HIC1) gene encodes a zinc finger transcriptional repressor that cooperates with p53 to suppress cancer development. We and others recently showed that HIC1 is a transcriptional target of p53. To identify additional transcriptional regulators of HIC1, we screened a set of transcription factors for regulation of a human HIC1 promoter reporter. We found that E2F1 strongly activates the full-length HIC1 promoter reporter. Promoter deletions and mutations identified two E2F responsive elements in the HIC1 core promoter region. Moreover, in vivo binding of E2F1 to the HIC1 promoter was shown by chromatin immunoprecipitation assays in human TIG3 fibroblasts expressing tamoxifen-activated E2F1. In agreement, activation of E2F1 in TIG3-E2F1 cells markedly increased HIC1 expression. Interestingly, expression of E2F1 in the p53(-/-) hepatocellular carcinoma cell line Hep3B led to an increase of endogenous HIC1 mRNA, although bisulfite genomic sequencing of the HIC1 promoter revealed that the region bearing the two E2F1 binding sites is hypermethylated. In addition, endogenous E2F1 induced by etoposide treatment bound to the HIC1 promoter. Moreover, inhibition of E2F1 strongly reduced the expression of etoposide-induced HIC1. In conclusion, we identified HIC1 as novel E2F1 transcriptional target in DNA damage responses.

Kikuchi, R., H. Tsuda, et al. (2007). "Promoter hypermethylation contributes to frequent inactivation of a putative conditional tumor suppressor gene connective tissue growth factor in ovarian cancer." Cancer Res **67**(15): 7095-105.

Connective tissue growth factor (CTGF) is a secreted protein belonging to the CCN family, members of which are implicated in various biological processes. We identified a homozygous loss of CTGF (6q23.2) in the course of screening a panel of ovarian cancer cell lines for genomic copy number aberrations using in-house array-based comparative genomic hybridization. CTGF mRNA expression was observed in normal ovarian tissue and immortalized ovarian epithelial cells but was reduced in many ovarian cancer cell lines without its homozygous deletion (12 of 23 lines) and restored after treatment with 5-aza 2'-deoxycytidine. The methylation status around the CTGF CpG island correlated inversely with the expression, and a putative target region for methylation showed promoter activity. CTGF methylation was frequently observed in primary ovarian cancer tissues (39 of 66, 59%) and inversely correlated with CTGF mRNA expression. In an immunohistochemical analysis of primary ovarian cancers, CTGF protein expression was frequently reduced (84 of 103 cases, 82%). Ovarian cancer tended to lack CTGF expression more frequently in the earlier stages (stages I and II) than the advanced stages (stages III and IV). CTGF protein was also differentially expressed among histologic subtypes. Exogenous restoration of CTGF expression or treatment with recombinant CTGF inhibited the growth of ovarian cancer cells lacking its expression, whereas knockdown of endogenous CTGF accelerated growth of ovarian cancer cells with expression of this gene. These results suggest that epigenetic silencing by hypermethylation of the CTGF promoter leads to a loss of CTGF function, which may be a factor in the carcinogenesis of ovarian cancer in a stage-dependent and/or histologic subtype-dependent manner.

Kim, M., J. H. Kim, et al. (2008). "LRRC3B, encoding a leucine-rich repeat-containing protein, is a putative tumor suppressor gene in gastric cancer." Cancer Res **68**(17): 7147-55.

Leucine-rich repeat-containing 3B (LRRC3B) is an evolutionarily highly conserved leucine-rich repeat-containing protein, but its biological significance is unknown. Using restriction landmark genomic scanning and pyrosequencing, we found that the promoter region of LRRC3B was aberrantly methylated in gastric cancer. Gastric cancer cell lines displayed epigenetic silencing of LRRC3B, but treatment with the DNA methylation inhibitor 5-aza-2'-deoxycytidine and/or the histone deacetylase inhibitor trichostatin A increased LRRC3B expression in gastric cancer cell lines. Real-time reverse transcription-PCR analysis of 96 paired primary gastric tumors and normal adjacent tissues showed that LRRC3B expression was reduced in 88.5% of gastric tumors compared with normal adjacent tissues. Pyrosequencing analysis of the promoter region revealed that LRRC3B was significantly hypermethylated in gastric tumors. Stable transfection of LRRC3B in SNU-601 cells, a gastric cancer cell line, inhibited anchorage-dependent and anchorage-independent colony formation, and LRRC3B

expression suppressed tumorigenesis in nude mice. Microarray analysis of LRRC3B-expressing xenograft tumors showed induction of immune response-related genes and IFN signaling genes. H&E-stained sections of LRRC3B-expressing xenograft tumors showed lymphocyte infiltration in the region. We suggest that LRRC3B is a putative tumor suppressor gene that is silenced in gastric cancers by epigenetic mechanisms and that LRRC3B silencing in cancer may play an important role in tumor escape from immune surveillance.

Kostadima, L., G. Pentheroudakis, et al. (2007). "The missing kiss of life: transcriptional activity of the metastasis suppressor gene KiSS1 in early breast cancer." Anticancer Res **27**(4B): 2499-504.

BACKGROUND: KiSS-1 is a metastasis suppressor gene encoding a neuropeptide with potent antimetastatic activities in tumour cell lines. The transcriptional activity of the gene and its associations in resected breast cancer were analysed. MATERIALS AND METHODS: Tumour messenger RNA (mRNA) of the KiSS1 exon I/II boundary was extracted from paraffin-embedded stage II or III node-positive breast adenocarcinomas of 272 women. KiSS1 mRNA was examined for associations with outcome, disease and molecular characteristics. RESULTS: Only 8 out of 272 tumours (3%) yielded detectable KiSS1 mRNA levels. There was no evidence of correlation of KiSS1 transcription with the number of involved axillary nodes, grade, hormone receptor status or tumour size. Of women with increased KiSS1 mRNA tumour levels, 87.5% were postmenopausal, whereas only 48% were postmenopausal among patients without detectable KiSS1 mRNA ($p = 0.03$). No association of KiSS1 transcription was found with transcription of the cell cycle-regulators HER2, VEGF, p53, BCL2, PAEP, or BIRC5. At a median follow-up of 62 months, there was no statistically significant difference between women harbouring KiSS1 mRNA-negative versus-positive tumours in terms of disease-free and overall survival (log-rank test $p = 0.54$ and $p = 0.55$, respectively). CONCLUSION: The metastasis suppressor gene KiSS1 is silenced in the vast majority of resected node-positive breast adenocarcinomas. These findings support the antimetastatic role of the gene and warrant its study as a prognostic marker and a therapeutic target.

Kuiper, R. P., L. Vreede, et al. (2009). "The tumor suppressor gene FBXW7 is disrupted by a constitutional t(3;4)(q21;q31) in a patient with renal cell cancer." Cancer Genet Cytogenet **195**(2): 105-11.

FBXW7 (alias CDC4) is a p53-dependent tumor suppressor gene that exhibits mutations or deletions in a variety of human tumors. Mutation or deletion of the FBXW7 gene has been associated with an increase in chromosomal instability and cell cycle progression. In addition, the FBXW7 protein has been found to act as a component of the ubiquitin proteasome system and to degrade several oncogenic proteins that function in cellular growth regulatory pathways. By using a rapid breakpoint cloning procedure in a case of renal cell cancer (RCC), we found that the FBXW7 gene was disrupted by a constitutional t(3;4)(q21;q31). Subsequent analysis of the tumor tissue revealed the presence of several anomalies, including loss of the derivative chromosome 3. Upon screening of a cohort of 29 independent primary RCCs, we identified one novel pathogenic mutation, suggesting that the FBXW7 gene may also play a role in the development of sporadic RCCs. In addition, we screened a cohort of 48 unrelated familial RCC cases with unknown etiology. Except for several known or benign sequence variants such as single nucleotide polymorphisms (SNPs), no additional pathogenic variants were found. Previous mouse models have suggested that the FBXW7 gene may play a role in the predisposition to tumor development. Here we report that disruption of this gene may predispose to the development of human RCC.

Larson, P. S., B. L. Schlechter, et al. (2008). "CDKN1C/p57kip2 is a candidate tumor suppressor gene in human breast cancer." BMC Cancer **8**: 68.

BACKGROUND: CDKN1C (also known as p57KIP2) is a cyclin-dependent kinase inhibitor previously implicated in several types of human cancer. Its family members (CDKN1A/p21CIP1 and B/p27KIP1) have been implicated in breast cancer, but information about CDKN1C's role is limited. We hypothesized that decreased CDKN1C may be involved in human breast carcinogenesis in vivo. METHODS: We determined rates of allele imbalance or loss of heterozygosity (AI/LOH) in CDKN1C, using an intronic polymorphism, and in the surrounding 11p15.5 region in 82 breast cancers. We examined the CDKN1C mRNA level in 10 cancers using quantitative real-time PCR (qPCR), and the CDKN1C protein level in 20 cancers using immunohistochemistry (IHC). All samples were obtained using laser microdissection. Data were analyzed using standard statistical tests. RESULTS: AI/LOH at 11p15.5 occurred in 28/73 (38%) informative cancers, but CDKN1C itself underwent AI/LOH in only 3/16 (19%) cancers (p = ns). In contrast, CDKN1C mRNA levels were reduced in 9/10 (90%) cancers ($p < 0.0001$), ranging from 2-60% of paired normal epithelium. Similarly, CDKN1C protein staining was seen in 19/20 (95%) cases'

normal epithelium but in only 7/14 (50%) cases' CIS ($p < 0.004$) and 5/18 (28%) cases' IC ($p < 0.00003$). The reduction appears primarily due to loss of CDKN1C expression from myoepithelial layer cells, which stained intensely in 17/20 (85%) normal lobules, but in 0/14 (0%) CIS ($p < 0.00001$). In contrast, luminal cells displayed less intense, focal staining fairly consistently across histologies. Decreased CDKN1C was not clearly associated with tumor grade, histology, ER, PR or HER2 status. CONCLUSION: CDKN1C is expressed in normal epithelium of most breast cancer cases, mainly in the myothepithelial layer. This expression decreases, at both the mRNA and protein level, in the large majority of breast cancers, and does not appear to be mediated by AI/LOH at the gene. Thus, CDKN1C may be a breast cancer tumor suppressor.

Lemamy, G. J., M. E. Sahla, et al. (2008). "Is the mannose-6-phosphate/insulin-like growth factor 2 receptor coded by a breast cancer suppressor gene?" Adv Exp Med Biol **617**: 305-10.

The multifunctional growth factor mannose-6-phosphate/insulin-like growth factor 2 receptor (M6P/IGF2-R) binds proteins sharing M6P signals, including cathepsins and IGF2. It is involved in targeting newly synthesized mannose-6-phosphorylated lysosomal enzymes, activating transforming growth factor beta (TGFbeta), and neutralising the mitogen IGF2 by transporting it to lysosomes. The M6P/IGF2-R was proposed as being coded by a tumor suppressor gene. We measured gene expression at the protein level by quantitative immunohistochemistry, using chicken high affinity IgY antibodies directed against human M6P/IGF2-R. Chicken immunization was performed with human purified M6P/IGF2-R, and IgY antibodies were extracted from egg yolk by polyethylene glycol precipitation method. The biosensor analysis showed that IgY antibodies bind M6P/IGF2-R with high affinity (Kd = 7.5 nM). Quantitative immunohistochemical studies in sections from invasive breast carcinoma and ductal carcinoma in situ (DCIS) indicated various levels (from 5 to 400 units) of the M6P/IGF2-R protein, which did not correlate with tumor size, histological grade, estrogen and progesterone receptors. Moreover, the M6P/IGF2-R level was increased in DCIS relative to adjacent normal tissue ($p < 0.005$) and then decreased in invasive carcinoma compared with DCIS ($p < 0.02$). The hypothesis of tumor suppressor gene is not supported by these studies. However, it is not excluded for a small proportion of the tumors. Its assay might help to complement the cathepsin D assay to predict breast cancer prognosis and physiopathology.

Li, L. W., X. Y. Yu, et al. (2009). "Expression of esophageal cancer related gene 4 (ECRG4), a novel tumor suppressor gene, in esophageal cancer and its inhibitory effect on the tumor growth in vitro and in vivo." Int J Cancer **125**(7): 1505-13.

The ECRG4 gene was initially identified and cloned in our laboratory from human normal esophageal epithelium (GenBank accession no. AF325503). We revealed the expression of ECRG4 protein was downregulated in 68.5% (89/130) ESCC samples using tissue microarray. The low ECRG4 protein expression was significantly associated with regional lymph node metastasis, primary tumor size, and tumor stage in ESCC ($p < 0.05$). ECRG4 mRNA expression was downregulated in ESCC due to the hypermethylation in the gene promoter. The treatment with 5-aza-2'-deoxycytidine, which is a DNA methyltransferase inhibitor restored ECRG4 mRNA expression in ESCC cells. The result indicated that promoter hypermethylation may be 1 main mechanism leading to the silencing of ECRG4. The high expression of ECRG4 in patients with ESCC was associated with longer survival compared with those with low ECRG4 expression by Kaplan-Meier survival analysis ($p < 0.05$). ECRG4 protein was an independent prognostic factor for ESCC by multivariable Cox proportional hazards regression analysis ($p < 0.05$). The restoration of ECRG4 expression in ESCC cells inhibited cell proliferation, colony formation, anchorage-independent growth, cell cycle progression and tumor growth in vivo ($p < 0.05$). The transfection of ECRG4 gene in ESCC cells inhibited the expression of NF-kappaB and nuclear translocation, in addition to the expression of COX-2, a NF-kappaB target gene, was attenuated. Taken together, ECRG4 is a novel candidate tumor suppressor gene in ESCC, and ECRG4 protein is a candidate prognostic marker for ESCC.

Lin, J., T. Sun, et al. (2007). "Oncogenic activation of c-Abl in non-small cell lung cancer cells lacking FUS1 expression: inhibition of c-Abl by the tumor suppressor gene product Fus1." Oncogene **26**(49): 6989-96.

In lung cancer, frequent loss of one allele of chromosome 3p is seen in both small cell lung cancer and non-small cell lung cancer (NSCLC), providing evidence of tumor suppressor genes (TSGs) in this chromosomal region. The mechanism of Fus1 tumor suppressor activity is unknown. We have found that a Fus1 peptide inhibits the Abl tyrosine kinase in vitro (IC(50) 35 microM). The inhibitory Fus1 sequence was derived from a region that was deleted in a mutant FUS1 gene (FUS1 (1-80)) detected in some lung cancer cell lines. Importantly, a stearic acid-

modified form of this peptide was required for the inhibition, but stearic acid alone was not inhibitory. Two NSCLC cell lines, which lack expression of wild-type Fus1, contain activated c-Abl. Forced expression of an inducible FUS1 cDNA in H1299 NSCLC cells decreased levels of activated c-Abl and inhibited its tyrosine kinase activity. Similarly, treatment of c-Abl immune complexes with the inhibitory Fus1 peptide also reduced the level of c-Abl in these immune complexes. The size and number of colonies of the NSCLC cell line, H1,299, in soft agar was strongly inhibited by the Abl kinase inhibitor imatinib mesylate. Co-expression of FUS1 and c-ABL in COS1 cells blocked activation of c-Abl tyrosine kinase. In contrast, co-expression of mutant FUS1 (1-80) with c-ABL had little inhibitory activity against c-Abl. These findings provide strong evidence that c-Abl is a possible target in NSCLC patients that have reduced expression of Fus1 in their tumor cells.

Liu, X. M., X. F. Xiong, et al. (2009). "Possible roles of a tumor suppressor gene PIG11 in hepatocarcinogenesis and As2O3-induced apoptosis in liver cancer cells." J Gastroenterol **44**(5): 460-9.

BACKGROUND: Our previous studies demonstrated that p53-induced gene 11 (PIG11) was involved in arsenic trioxide (As(2)O(3))-induced apoptosis in human gastric cancer MGC-803 cells. Here, we studied further PIG11 expression in human hepatocellular carcinoma (HCC) tissues and cell lines and compared the sensitivity to As(2)O(3)-induced cell apoptosis in HepG2 and L-02 cells. METHODS: PIG11 expression in human normal liver tissues, HCC tissues, and cell lines was determined by immunohistochemistry and immunocytochemistry methods, using an anti-human PIG11 antibody. Cell viability was estimated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diplenyltetrazolium bromide (MTT) assay. Cell apoptosis was determined by flow cytometry. Reverse-transcriptase polymerase chain reaction (RT-PCR) and Western blotting were performed to analyze PIG11 mRNA and protein expression in cells. Protein intensity was calculated by comparison with the intensity of beta-actin, using densitometry. PIG11 was knocked down using small interfering RNA (siRNA). RESULTS: We found that PIG11 expression was significantly downregulated in HCC tissue and the cell lines (Bel-7402, SMMC-7721, HepG2 cells). Further, HepG2 cells were more sensitive to As(2)O(3)-induced apoptosis than L-02 cells. The expression of PIG11 mRNA and protein was upregulated to a greater extent in HepG2 than in L-02 cells. In the presence of actinomycin D or cycloheximide, the amount of PIG11 protein expression did not increase. Likewise, the inhibition of PIG11 by siRNA decreased As(2)O(3)-induced PIG11 protein expression by more than 85% and partially prevented As(2)O(3)-induced apoptosis in both HepG2 and L-02 cells. CONCLUSION: The above results demonstrated that the PIG11 gene may be involved in As(2)O(3)-induced apoptosis in HepG2 cells and suggested that the adaptive response of PIG11 expression is one of the important factors in enhancing cell sensitivity to As(2)O(3)-induced apoptosis.

Lu, Z., R. Z. Luo, et al. (2008). "The tumor suppressor gene ARHI regulates autophagy and tumor dormancy in human ovarian cancer cells." J Clin Invest **118**(12): 3917-29.

The role of autophagy in oncogenesis remains ambiguous, and mechanisms that induce autophagy and regulate its outcome in human cancers are poorly understood. The maternally imprinted Ras-related tumor suppressor gene aplasia Ras homolog member I (ARHI; also known as DIRAS3) is downregulated in more than 60% of ovarian cancers, and here we show that re-expression of ARHI in multiple human ovarian cancer cell lines induces autophagy by blocking PI3K signaling and inhibiting mammalian target of rapamycin (mTOR), upregulating ATG4, and colocalizing with cleaved microtubule-associated protein light chain 3 (LC3) in autophagosomes. Furthermore, ARHI is required for spontaneous and rapamycin-induced autophagy in normal and malignant cells. Although ARHI re-expression led to autophagic cell death when SKOv3 ovarian cancer cells were grown in culture, it enabled the cells to remain dormant when they were grown in mice as xenografts. When ARHI levels were reduced in dormant cells, xenografts grew rapidly. However, inhibition of ARHI-induced autophagy with chloroquine dramatically reduced regrowth of xenografted tumors upon reduction of ARHI levels, suggesting that autophagy contributed to the survival of dormant cells. Further analysis revealed that autophagic cell death was reduced when cultured human ovarian cancer cells in which ARHI had been re-expressed were treated with growth factors (IGF-1, M-CSF), angiogenic factors (VEGF, IL-8), and matrix proteins found in xenografts. Thus, ARHI can induce autophagic cell death, but can also promote tumor dormancy in the presence of factors that promote survival in the cancer microenvironment.

Ma, Y., B. Lu, et al. (2008). "Tumor suppressor gene insulin-like growth factor binding protein-related protein 1 (IGFBP-rP1) induces senescence-like growth arrest in colorectal cancer cells." Exp Mol Pathol **85**(2): 141-5.

Insulin-like growth factor binding protein-related protein 1 (IGFBP-rP1) is a potential tumor

suppressor gene. This study attempted to explore a potential senescence-like role for IGFBP-rP1 in suppressing human colorectal cancer. Recombinant IGFBP-rP1 inhibited cell proliferation and induced G1 cell cycle arrest in RKO and CW2 cells. It induced a senescence-like phenotype by showing 2-fold higher beta-galactosidase activity in IGFBP-rP1-transfectants over that in control cells. Western blot confirmed down-regulation of phosphorylated retinoblastoma protein (pRB) and up-regulation of p53 in IGFBP-rP1-transfectants as compared with control cells. Thus, IGFBP-rP1 might be a key molecule in the cellular senescence pathway. Our results uncovered a novel molecular mechanism involving the altered expression of pRB and p53 for tumor suppressor gene IGFBP-rP1 in colorectal cancer. Restoration of IGFBP-rP1 function might have potential therapeutic significance in colorectal cancer.

Majid, S., A. A. Dar, et al. (2009). "BTG3 tumor suppressor gene promoter demethylation, histone modification and cell cycle arrest by genistein in renal cancer." Carcinogenesis **30**(4): 662-70.

BTG3/ANA/APRO4 has been reported to be a tumor suppressor gene in some malignancies. It constitutes important negative regulatory mechanism for Src-mediated signaling, a negative regulator of the cell cycle and inhibits transcription factor E2F1. We report that BTG3 is downregulated in renal cancer and that the mechanism of inactivation is through promoter hypermethylation. Quantitative real-time polymerase chain reaction (PCR) showed that BTG3 was downregulated in cancer tissues and cells. Genistein and 5-aza-2'-deoxycytidine (5Aza-C) induced BTG3 messenger RNA (mRNA) expression in A498, ACHN and HEK-293 renal cell carcinoma (RCC) cell lines. Bisulfite-modified PCR and DNA sequencing results showed complete methylation of BTG3 promoter in tumor samples and cancer cell lines. Genistein and 5Aza-C treatment significantly decreased promoter methylation, reactivating BTG3 expression. Chromatin immunoprecipitation assay revealed that genistein and 5Aza-C increased levels of acetylated histones 3, 4, 2H3K4, 3H3K4 and RNA polymerase II at the BTG3 promoter indicative of active histone modifications. Enzymatic assays showed genistein and 5Aza-C decreased DNA Methyltransferase, methyl-CpG-binding domain 2 activity and increased HAT activity. Cell cycle and 3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide cell proliferation assays showed that genistein has antiproliferative effect on cancer cell growth through induction of cell cycle arrest. This is the first report to show that BTG3 is epigenetically silenced in RCC and can be reactivated by genistein-induced promoter demethylation and active histone modification. Genistein had similar effects to that of 5Aza-C, which is a potent demethylating agent with high toxicity and instability. Genistein being a natural, non-toxic, dietary isoflavone is effective in retarding the growth of RCC cells, making it a promising candidate for epigenetic therapy in renal carcinoma.

Marsit, C. J., E. A. Houseman, et al. (2008). "Genetic and epigenetic tumor suppressor gene silencing are distinct molecular phenotypes driven by growth promoting mutations in nonsmall cell lung cancer." J Cancer Epidemiol **2008**: 215809.

Both genetic and epigenetic alterations characterize human nonsmall cell lung cancer (NSCLC), but the biological processes that create or select these alterations remain incompletely investigated. Our hypothesis posits that a roughly reciprocal relationship between the propensity for promoter hypermethylation and a propensity for genetic deletion leads to distinct molecular phenotypes of lung cancer. To test this hypothesis, we examined promoter hypermethylation of 17 tumor suppressor genes, as a marker of epigenetic alteration propensity, and deletion events at the 3p21 region, as a marker of genetic alteration. To model the complex biology between these somatic alterations, we utilized an item response theory model. We demonstrated that tumors exhibiting LOH at greater than 30% of informative alleles in the 3p21 region have a significantly reduced propensity for hypermethylation. At the same time, tumors with activating KRAS mutations showed a significantly increased propensity for hypermethylation of the loci examined, a result similar to what has been observed in colon cancer. These data suggest that NSCLCs have distinct epigenetic or genetic alteration phenotypes acting upon tumor suppressor genes and that mutation of oncogenic growth promoting genes, such as KRAS, is associated with the epigenetic phenotype.

Martin, V., H. F. Jorgensen, et al. (2008). "MBD2-mediated transcriptional repression of the p14ARF tumor suppressor gene in human colon cancer cells." Pathobiology **75**(5): 281-7.

OBJECTIVE: The p14(ARF) and p16(INK4A) tumor suppressor genes are commonly inactivated by aberrant methylation of their promoter regions in human colon cancer. The methyl-CpG-binding domain protein MBD2 is physically associated with the methylated promoters of the p14(ARF) and p16(INK4A) genes in specific tumor cell lines. Moreover, deficiency of MBD2 strongly inhibits intestinal tumorigenesis in the Min mouse, raising the possibility that the protein might be involved in transcriptional repression of methylated tumor suppressor genes. The aim of this study was to

evaluate the role of MBD2 in the silencing of p14(ARF) and p16(INK4A) in cancer. METHODS: The MBD2 protein was stably knocked down by RNA interference in RKO, a colon cancer cell line in which both p14(ARF) and p16(INK4A) are silenced by methylation. RESULTS: We demonstrate here that MBD2 associates with the methylated promoter of the p14(ARF) gene in the RKO colon cancer cell line. Depletion of MBD2 by RNAi leads to selective upregulation of the p14(ARF) but not the p16(INK4A) gene transcript. In addition, p14(ARF) repression can be restored by expressing mouse MBD2 protein in MBD2-deficient RKO cells. CONCLUSION: These findings implicate MBD2 in transcriptional repression of the methylated p14(ARF) tumor suppressor gene and suggest that repression by MBD2 selectively affects a subset of methylated promoters.

Melotte, V., M. H. Lentjes, et al. (2009). "N-Myc downstream-regulated gene 4 (NDRG4): a candidate tumor suppressor gene and potential biomarker for colorectal cancer." J Natl Cancer Inst **101**(13): 916-27.

BACKGROUND: Identification of hypermethylated tumor suppressor genes in body fluids is an appealing strategy for the noninvasive detection of colorectal cancer. Here we examined the role of N-Myc downstream-regulated gene 4 (NDRG4) as a novel tumor suppressor and biomarker in colorectal cancer. METHODS: NDRG4 promoter methylation was analyzed in human colorectal cancer cell lines, colorectal tissue, and noncancerous colon mucosa by using methylation-specific polymerase chain reaction (PCR) and bisulfite sequencing. NDRG4 mRNA and protein expression were studied using real-time-PCR and immunohistochemistry, respectively. Tumor suppressor functions of NDRG4 were examined by colony formation, cell proliferation, and migration and invasion assays in colorectal cancer cell lines that were stably transfected with an NDRG4 expression construct. Quantitative methylation-specific PCR was used to examine the utility of NDRG4 promoter methylation as a biomarker in fecal DNA from 75 colorectal cancer patients and 75 control subjects. All P values are two-sided. RESULTS: The prevalence of NDRG4 promoter methylation in two independent series of colorectal cancers was 86% (71/83) and 70% (128/184) compared with 4% (2/48) in noncancerous colon mucosa ($P < .001$). NDRG4 mRNA and protein expression were decreased in colorectal cancer tissue compared with noncancerous colon mucosa. NDRG4 overexpression in colorectal cancer cell lines suppressed colony formation ($P = .014$), cell proliferation ($P < .001$), and invasion ($P < .001$). NDRG4 promoter methylation analysis in fecal DNA from a training set of colorectal cancer patients and control subjects yielded a sensitivity of 61% (95% confidence interval [CI] = 43% to 79%) and a specificity of 93% (95% CI = 90% to 97%). An independent test set of colorectal cancer patients and control subjects yielded a sensitivity of 53% (95% CI = 39% to 67%) and a specificity of 100% (95% CI = 86% to 100%). CONCLUSIONS: NDRG4 is a candidate tumor suppressor gene in colorectal cancer whose expression is frequently inactivated by promoter methylation. NDRG4 promoter methylation is a potential biomarker for the noninvasive detection of colorectal cancer in stool samples.

Meng, C. F., X. J. Zhu, et al. (2007). "Re-expression of methylation-induced tumor suppressor gene silencing is associated with the state of histone modification in gastric cancer cell lines." World J Gastroenterol **13**(46): 6166-71.

AIM: To identify the relationship between DNA hyper-methylation and histone modification at a hyperme-thylated, silenced tumor suppressor gene promoter in human gastric cancer cell lines and to elucidate whether alteration of DNA methylation could affect histone modification. METHODS: We used chromatin immunoprecipitation (ChIP) assay to assess the status of histone acetylation and methylation in promoter regions of the p16 and mutL homolog 1 (MLH1) genes in 2 gastric cancer cell lines, SGC-7901 and MGC-803. We used methylation-specific PCR (MSP) to evaluate the effect of 5-Aza-2'-deoxycytidine (5-Aza-dC), trichostatin A (TSA) or their combination treatment on DNA methylation status. We used RT-PCR to determine whether alterations of histone modification status after 5-Aza-dC and TSA treatment are reflected in gene expression. RESULTS: For the p16 and MLH1 genes in two cell lines, silenced loci associated with DNA hypermethylation were characterized by histone H3-K9 hypoacetylation and hypermethylation and histone H3-K4 hypomethylation. Treatment with TSA resulted in moderately increased histone H3-K9 acetylation at the silenced loci with no effect on histone H3-K9 methylation and minimal effects on gene expression. In contrast, treatment with 5-Aza-dC rapidly reduced histone H3-K9 methylation at the silenced loci and resulted in reactivation of the two genes. Combined treatment with 5-Aza-dC and TSA was synergistic in reactivating gene expression at the loci showing DNA hypermethylation. Similarly, histone H3-K4 methylation was not affected after TSA treatment, and increased moderately at the silenced loci after 5-Aza-dC treatment. CONCLUSION: Hypermethylation of DNA in promoter CpG islands is related to transcriptional

silencing of tumor suppressor genes. Histone H3-K9 methylation in different regions of the promoters studied correlates with DNA methylation status of each gene in gastric cancer cells. However, histone H3-K9 acetylation and H3-K4 methylation inversely correlate with DNA methylation status of each gene in gastric cancer cells. Alteration of DNA methylation affects histone modification.

Meng, F., R. Henson, et al. (2007). "MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer." Gastroenterology **133**(2): 647-58.

BACKGROUND AND AIMS: microRNAs (miRNAs) are short noncoding RNAs that regulate gene expression negatively. Although a role for aberrant miRNA expression in cancer has been postulated, the pathophysiologic role and relevance of aberrantly expressed miRNA to tumor biology has not been established. METHODS: We evaluated the expression of miRNA in human hepatocellular cancer (HCC) by expression profiling, and defined a target gene and biologically functional effect of an up-regulated miRNA. RESULTS: miR-21 was noted to be highly overexpressed in HCC tumors and cell lines in expression profiling studies using a miRNA microarray. Inhibition of miR-21 in cultured HCC cells increased expression of the phosphatase and tensin homolog (PTEN) tumor suppressor, and decreased tumor cell proliferation, migration, and invasion. In contrast-enhanced miR-21 expression by transfection with precursor miR-21 increased tumor cell proliferation, migration, and invasion. Moreover, an increase in cell migration was observed in normal human hepatocytes transfected with precursor miR-21. PTEN was shown to be a direct target of miR-21, and to contribute to miR-21 effects on cell invasion. Modulation of miR-21 altered focal adhesion kinase phosphorylation and expression of matrix metalloproteases 2 and 9, both downstream mediators of PTEN involved in cell migration and invasion. CONCLUSIONS: Aberrant expression of miR-21 can contribute to HCC growth and spread by modulating PTEN expression and PTEN-dependent pathways involved in mediating phenotypic characteristics of cancer cells such as cell growth, migration, and invasion.

Nakaya, K., H. D. Yamagata, et al. (2007). "Identification of homozygous deletions of tumor suppressor gene FAT in oral cancer using CGH-array." Oncogene **26**(36): 5300-8.

Homozygous deletions (HD) provide an important resource for identifying the location of candidate tumor suppressor genes. To identify the tumor suppressor gene in oral cancer, we employed high-resolution comparative genomic hybridization (CGH)-array analysis. We identified a homozygous loss of FAT (4q35), a new member of the human cadherin superfamily, from genome-wide screening of copy number alterations in one primary oral cancer. This result was evaluated by genomic polymerase chain reaction in 13 oral cancer cell lines and 20 primary oral cancers and Southern blot in the cell lines. We found frequent exonic HD of FAT in the cell lines (3/13, 23%) and in primary oral cancers (16/20, 80%). FAT expression was absent in these cell lines. Homozygous deletion hot spots were observed in exon 1 (9/20, 45%) and exon 4 (7/20, 35%). Moreover, loss of gene expression was identified in other types of squamous cell carcinoma. The methylation status of the FAT CpG island in squamous cell carcinomas correlated negatively with its expression. Our results identify mutations in FAT as an important factor in the development of oral cancer and indicate the importance of FATs function in some squamous cell carcinomas.

Oh, J. J., B. N. Boctor, et al. (2008). "Promoter methylation study of the H37/RBM5 tumor suppressor gene from the 3p21.3 human lung cancer tumor suppressor locus." Hum Genet **123**(1): 55-64.

Loss of heterozygosity (LOH) at chromosome 3p21.3 is one of the most prevalent genetic disturbances occurring at the earliest stage of tumor development for a wide variety of human cancers, culminated in lung cancer. The 19 genes residing at 3p21.3 have been vigorously characterized for tumor suppressor activity and gene inactivation mechanism because of their potentially significant merits of clinical applications. Many of these 19 genes have been shown to manifest various growth inhibitory properties, however none of them are inactivated by coding mutations in their remaining allele as in the Knudson's two- hits hypothesis. Thus far the most prevailing, alternative gene inactivation mechanism known for the 3p21.3 TSGs is epigenetic silencing by promoter hypermethylation. Previously, we have focused our investigation on one of the 19 genes at 3p21.3, H37/RBM5, and demonstrated its tumor suppressor activity both in vitro and in vivo as well as its mRNA/protein expression loss from the remaining allele in a majority of the primary lung tumors examined. The current study tested our hypothesis that the H37 inactivation in primary lung tumors may, as seen in most of the other 3p21.3 TSGs, be due to hypermethylation in its promoter CpG islands. Contrary to this most plausible postulation, however, we found no evidence of epigenetic gene silencing for the H37 TSG. Here we suggest some of the possible, further- alternative means of the H37 gene expression loss in tumor,

including defects in transcription and post-transcriptional/translational modifications as well as mechanisms related to haploinsufficiency.

Pappas, J. J., A. Toulouse, et al. (2008). "Allelic methylation bias of the RARB2 tumor suppressor gene promoter in cancer." Genes Chromosomes Cancer **47**(11): 978-93.

Retinoic acid receptor B2 (RARB2) is frequently inactivated in cancer. Methylation in the 5'-untranslated region and first exon is known to play a role; however, few studies have analyzed the detailed methylation pattern of the promoter region. We show that hypo- and hypermethylated alleles coexist in 5/11 cell lines in which RARB2 is inactivated. We present evidence supporting the mitotic transmission of these divergent methylation patterns and find a correlation between methylation divergence and heterozygosity at the 3p24 loci, suggesting an allelic methylation bias in these lines. Using a newly devised strategy based on allelic identification via methylation-sensitive restriction enzyme digestion combined with the use of a single nucleotide polymorphism, rs755661, we demonstrate that such a bias exists in three cancer cell specimens heterozygous at rs755661 and therefore amenable to this study. This previously unreported phenomenon of allelic methylation bias suggests that a promoter methylation-independent mechanism may be responsible for inactivation at the hypomethylated allele and this inactivation is reminiscent of an aberrant form of de novo imprinting. Approaches to interpreting methylation data should incorporate the notion of allelic methylation bias.

Park, J. Y., J. F. Helm, et al. (2008). "Silencing of the candidate tumor suppressor gene solute carrier family 5 member 8 (SLC5A8) in human pancreatic cancer." Pancreas **36**(4): e32-9.

OBJECTIVES: Few genetic mutations have been identified in pancreatic adenocarcinoma, whereas epigenetic changes that lead to gene silencing are known in several genes. Because SLC5A8 is regarded as a potential tumor suppressor gene that is down-regulated by epigenetic changes in several other cancers, we sought to characterize promoter methylation status and its relationship to SLC5A8 expression in pancreatic cancer. METHODS: Promoter methylation and expression of SLC5A8 were evaluated in pancreatic cancer cell lines, tumor, and adjacent nontumor tissues from pancreatic cancer patients using methylation-specific polymerase chain reaction analysis, quantitative real-time and semiquantitative reverse transcriptase-polymerase chain reaction, and bisulfate-modified sequencing. RESULTS: Complete or partial loss of SLC5A8 expression was observed in all tumor tissues. Bisulfite sequencing analysis on pancreatic cancer cell lines that did not express SLC5A8 detected dense methylation of the promoter region. SLC5A8 expression was reactivated by treatment with aza-deoxycytidine or trichostatin A. Methylation-specific polymerase chain reaction detected methylation in 7 of 10 pancreatic tumor tissues, whereas in only 3 of 28 adjacent nontumor tissues ($P < 0.001$). CONCLUSIONS: Our findings indicate loss of SLC5A8 expression as a result of aberrant promoter methylation in pancreatic adenocarcinoma. We suggest that SLC5A8 may function as a tumor suppressor gene whose silencing by epigenetic changes may contribute to carcinogenesis and progression of pancreatic cancer.

Plevova, P., S. Walczyskova, et al. (2009). "Germline variants of the promyelocytic leukemia tumor suppressor gene in patients with familial cancer." Neoplasma **56**(6): 500-7.

The promyelocytic leukemia (PML) gene is an important tumor suppressor gene. We tested the hypothesis that germline disruption of the PML gene may be associated with a cancer predisposition syndrome. Mutation analysis of the PML gene was performed in 111 patients with familial adult cancer or young age-onset adult cancer. These were mostly breast and colon cancer, or colon polyposis patients in whom mutation analyses of the BRCA1, BRCA2, MLH1, MSH2, APC or TP53 genes did not detect a pathogenic germline mutation. Heteroduplex analysis and direct sequencing were used for mutation screening. Mutation-specific methods were designed for frequency determination of novel variants in the general population. No deleterious nonsense or frameshift germline mutations were detected. Several missense single-nucleotide substitutions were found, including two novel missense variants, c.83C>T (p.Thr28Ile) in exon 1 in a 42-year-old breast cancer patient and c.1558C>T (p.Pro520Ser) in exon 6 in a 32-year-old colon cancer patient, that were not detected in 100 and 214 non-cancer persons, respectively. Frequency of the c.2260G>C (p.Ala754Pro) variant in isoform IV of the PML gene was higher in patients with colon polyposis and cancer than in the control group ($P = 0.029$). In conclusion, germline disruption of the PML gene is probably not associated with a highly penetrant susceptibility to adult-onset breast and colon cancer. Pathogenicity of c.83C>T and c.1558C>T variants in the PML gene is uncertain. Carriers of the c.2260 G>C variant in PMLIV isoform may be at an increased risk of colon polyposis and cancer.

Qu, S., J. Long, et al. (2008). "Genetic polymorphisms of metastasis suppressor gene NME1 and breast cancer survival." Clin Cancer Res **14**(15): 4787-93.

PURPOSE: Ample evidence supports an important role of tumor metastasis suppressor genes in cancer metastatic processes. We evaluated the association of genetic polymorphisms of metastasis suppressor gene NME1 with breast cancer prognosis in a follow-up study of patients with primary breast cancer and further investigated the functions of these polymorphisms. EXPERIMENTAL DESIGN: NME1 genotypes were analyzed in a cohort of 1,134 breast cancer patients recruited as part of the Shanghai Breast Cancer Study who were followed for a median of 7.1 years. In vitro biochemical analyses were carried out to examine the function of NME1 gene polymorphisms. RESULTS: Single nucleotide polymorphisms (SNP) in the promoter region of the NME1 gene were found to be associated with breast cancer prognosis. Patients carrying the C allele in rs16949649 were associated with higher breast cancer-specific mortality [hazard ratio (HR), 1.4; 95% confidence interval (95% CI), 1.1-1.9] compared with those carrying the wild-type allele, and the association was more evident in patients with an early-stage cancer (HR, 1.7; 95% CI, 1.2-2.5). SNP rs2302254 was also associated with breast cancer prognosis, and the association was statistically significant for the risk of breast cancer relapse, metastasis, and death (HR, 1.3; 95% CI, 1.0-1.6). In vitro biochemical analyses showed that minor alleles in rs2302254 and rs3760468, which is in strong linkage disequilibrium with rs16949646, altered nuclear proteins binding capacity and reduced NME1 promoter activity, supporting the results from an association study of these SNPs with breast cancer survival. CONCLUSION: Promoter polymorphisms in the NME1 gene may alter its expression and influence breast cancer survival.

Reisman, D. and K. Boggs (2007). "Transcriptional regulation of the p53 tumor suppressor gene: a potential target for cancer therapeutics?" Recent Pat DNA Gene Seq **1**(3): 176-85.

The tightly regulated expression of p53 contributes to genomic stability and transcription of the p53 gene is induced prior to cells entering S-phase, possibly as a mechanism to insure a rapid p53 response in the event of DNA damage. We have previously described the cloning of an additional 1000bp of upstream p53 sequences that we have demonstrated play a role in the regulated expression of p53. As described in earlier reports we identified that a member of the C/EPB family of transcription factors may play a role in regulating p53. A particular C/EBPbeta isoform, C/EBPbeta-2, efficiently binds to the p53 promoter and induces its expression in a fashion that reflects the pattern of p53 expression seen as cells are induced to enter S-phase and is absent from cells that are defective in proper p53 regulation. We conclude from these findings that C/EBPbeta-2 plays a central role in regulating p53 transcription during the transition into S-phase. The recent development of novel compounds that restore wild-type p53 activity to tumor cells raises the possibility that understanding the means by which p53 gene expression is deregulated in tumors cells could likely lead to the development of novel therapeutic strategies designed to return p53 to its normal expression.

Revillion, F., C. Puech, et al. (2009). "Expression of the putative tumor suppressor gene PTPN13/PTPL1 is an independent prognostic marker for overall survival in breast cancer." Int J Cancer **124**(3): 638-43.

Although it is well established that some protein tyrosine kinases have a prognostic value in breast cancer, the involvement of protein tyrosine phosphatases (PTPs) is poorly substantiated for breast tumors. Three of these enzymes (PTP-gamma, LAR, and PTPL1) are already known to be regulated by estrogens or their antagonists in human breast cancer cells. We used a real-time reverse transcriptase polymerase chain reaction method to test the expression levels of PTP-gamma, LAR and its neuronal isoform, and PTPL1 in a training set of RNA from 59 breast tumors. We sought correlations between levels of these molecular markers, current tumor markers, and survival. We then quantified the expression level of the selected phosphatase in 232 additional samples, resulting in a testing set of 291 breast tumor RNAs from patients with a median follow-up of 6.4 years. The Spearman nonparametric test revealed correlations between PTPL1 expression and differentiation markers. Cox univariate analysis of the overall survival studies demonstrated that PTPL1 is a prognostic factor [risk ratio (RR)=0.45], together with the progesterone receptor (PR) (RR=0.52) and node involvement (RR=1.58). In multivariate analyses, PTPL1 and PR retained their prognostic value (RRs of 0.48 and 0.55, respectively). This study demonstrates for the first time that PTPL1 expression level is an independent prognostic indicator of favorable outcome for patients with breast cancer. In conjunction with our mechanistic studies, this finding identifies PTPL1 as an important regulatory element of human breast tumor aggressiveness and sensitivity to treatments such as antiestrogens and antiaromatase.

Sangodkar, J., J. Shi, et al. (2009). "Functional role of the KLF6 tumour suppressor gene in gastric cancer." Eur J Cancer **45**(4): 666-76.

Gastric cancer is the second most common cancer and a leading cause of cancer-related death worldwide. The Kruppel-like factor 6 (KLF6) tumour suppressor gene had been previously shown to be inactivated in a number of human cancers through loss of heterozygosity (LOH), somatic mutation, decreased expression and increased alternative splicing into a dominant negative oncogenic splice variant, KLF6-SV1. In the present study, 37 gastric cancer samples were analysed for the presence of loss of heterozygosity (LOH) of the KLF6 locus and somatic mutation. In total, 18 of 34 (53%) of the gastric cancer samples analysed demonstrated KLF6 locus specific loss. Four missense mutations, such as T179I, R198G, R71Q and S180L, were detected. Interestingly, two of these mutations R71Q and S180L have been identified independently by several groups in various malignancies including prostate, colorectal and gastric cancers. In addition, decreased wild-type KLF6 (wtKLF6) expression was associated with loss of the KLF6 locus and was present in 48% of primary gastric tumour samples analysed. Functional studies confirmed that wtKLF6 suppressed proliferation of gastric cancer cells via transcriptional regulation of the cyclin-dependent kinase inhibitor p21 and the oncogene c-myc. Functional characterisation of the common tumour-derived mutants demonstrated that the mutant proteins fail to suppress proliferation and function as dominant negative regulators of wtKLF6 function. Furthermore, stable overexpression of the R71Q and S180L tumour-derived mutants in the gastric cancer cell line, Hs746T, resulted in an increased tumourigenicity in vivo. Combined, these findings suggest an important role for the KLF6 tumour suppressor gene in gastric cancer development and progression and identify several highly cancer-relevant signalling pathways regulated by the KLF6 tumour suppressor gene.

Singh, L. S., M. Berk, et al. (2007). "Ovarian cancer G protein-coupled receptor 1, a new metastasis suppressor gene in prostate cancer." J Natl Cancer Inst **99**(17): 1313-27.

BACKGROUND: Metastasis is a process by which tumors spread from primary organs to other sites in the body and is the major cause of death for cancer patients. The ovarian cancer G protein-coupled receptor 1 (OGR1) gene has been shown to be expressed at lower levels in metastatic compared with primary prostate cancer tissues. METHODS: We used an orthotopic mouse metastasis model, in which we injected PC3 metastatic human prostate cancer cells stably transfected with empty vector (vector-PC3) or OGR1-expressing vector (OGR1-PC3) into the prostate lobes of athymic or NOD/SCID mice (n = 3-8 mice per group). Migration of PC3 cells transiently transfected with vector control or with OGR1- or GPR4 (a G protein-coupled receptor with the highest homology to OGR1)-expressing vectors was measured in vitro by Boyden chamber assays. G protein alpha-inhibitory subunit 1 (G alpha(i1)) expression after treatment with pertussis toxin (PTX) was measured using immunoblotting analysis. The inhibitory factor present in the conditioned medium was extracted using organic solvents and analyzed by mass spectrometry. RESULTS: In vivo, all 26 mice carrying tumors that were derived from vector-PC3 cells developed prostate cancer metastases (mean = 100%, 95% confidence interval [CI] = 83.97% to 100%) but few (4 of 32) mice carrying tumors derived from OGR1-expressing PC3 cells (mean = 12.50%, 95% CI = 4.08% to 29.93%) developed metastases. However, exogenous OGR1 overexpression had no effect on primary prostate tumor growth in vivo. In vitro, expression of OGR1, but not GPR4, inhibited cell migration (mean percentage of cells migrated, 30.2% versus 100%, difference = 69.8%, 95% CI = 63.0% to 75.9%; P<.001) via increased expression of G alpha(i1) and the secretion of a chloroform/methanol-extractable heat-insensitive factor into the conditioned medium through a PTX-sensitive pathway. CONCLUSION: OGR1 is a novel metastasis suppressor gene for prostate cancer. OGR1's constitutive activity via G alpha(i) contributes to its inhibitory effect on cell migration in vitro.

Singh, R. K., D. Indra, et al. (2007). "Deletions in chromosome 4 differentially associated with the development of cervical cancer: evidence of slit2 as a candidate tumor suppressor gene." Hum Genet **122**(1): 71-81.

The aim of this study was to locate the candidate tumor suppressor genes (TSGs) loci in the chromosomal 4p15-16, 4q22-23 and 4q34-35 regions associated with the development of uterine cervical carcinoma (CA-CX). Deletion mapping of the regions by microsatellite markers identified six discrete areas with high frequency of deletions, viz. 4p16.2 (D1: 40%), 4p15.31 (D2: 35-38%), 4p15.2 (D3: 37-40%), 4q22.2 (D4: 34%), 4q34.2-34.3 (D5: 37-59%) and 4q35.1 (D6: 40-50%). Significant correlation was noted among the deleted regions D1, D2 and D3. The deletions in D1, D2, D5 and D6 regions are suggested to be associated with the cervical intraepithelial neoplasia (CIN), and deletions in the D2, D3, D5 and D6 regions seems to be associated with progression of CA-CX. The deletions in the D2 and D6 regions showed significant prognostic implications (P = 0.001; 0.02). The expression of the candidate TSG SLIT2 mapped to D2 region gradually reduced from normal cervix uteri -->CIN --> CA-CX. SLIT2 promoter hypermethylation was seen in 28% CIN

samples and significantly increased with tumor progression (P = 0.04). Significant correlation was seen between SLIT2 deletion and its promoter methylation (P = 0.001), indicating that both these phenomena could occur simultaneously to inactivate this gene. Immunohistochemical analysis showed reduced expression of SLIT2 in cervical lesions and CA-CX cell lines. Although no mutation was detected in the SLIT2 promoter region (-432 to + 55 bp), CC and AA haplotypes were seen in -227 and -195 positions, respectively. Thus, it indicates that inactivation of SLIT2-ROBO1 signaling pathway may have an important role in CA-CX development.

Spillman, M. A., J. Lacy, et al. (2007). "Regulation of the metastasis suppressor gene MKK4 in ovarian cancer." Gynecol Oncol **105**(2): 312-20.

OBJECTIVES: MKK4 is a metastasis suppressor that is downregulated in some ovarian cancers. We sought to investigate whether promoter methylation, loss of heterozygosity, or changes in phosphorylation are involved in MKK4 dysregulation during ovarian carcinogenesis. METHODS: Bisulfite sequencing was used to determine MKK4 promoter methylation. PCR analysis of tumor/normal DNA was performed to determine LOH at the MKK4 locus. Normal human ovarian surface epithelium (HOSE) and SKOV-3 cells were serum starved and treated with EGF, TGFbeta, or wortmannin. Western blotting was performed using antibodies that detect total and phosphorylated MKK4. RESULTS: No MKK4 promoter hypermethylation was detected in 21 ovarian cancers. LOH was detected at the MKK4 intragenic marker D17S969 in 35% of cases and at D17S1303 in 20%. MKK4 protein was detected in 97% of ovarian tumors. The inactivated phosphoserine 80 (ser-80) form comprised 62% of phosphorylated MKK4 protein in ovarian tumors. Treatment of HOSE or SKOV-3 cells with EGF induced a 1.7- to 4.2-fold increase in phosphorylation of ser-80 MKK4 without altering total MKK4 protein. TGFbeta increased MKK4 ser-80 phosphorylation by 5.4-fold above baseline. The PI3K/Akt pathway inhibitor wortmannin decreased the amount of ser-80 MKK4 by 50%, and inhibited EGF stimulation of MKK4 ser-80 phosphorylation by 60%. CONCLUSIONS: LOH of MKK4 occurs in some ovarian cancers, but without loss of MKK4 protein. MKK4 expression does not appear to be downregulated by promoter methylation. Peptide growth factors induce MKK4 ser-80 phosphorylation, which downregulates its activity. PI3K/Akt pathway inhibitors can partially block ser-80 phosphorylation and this may have therapeutic implications.

Stec-Michalska, K., L. Peczek, et al. (2009). "Helicobacter pylori infection and family history of gastric cancer decrease expression of FHIT tumor suppressor gene in gastric mucosa of dyspeptic patients." Helicobacter **14**(5): 126-34.

BACKGROUND: The expression of a fragile histidine triad (FHIT) protein is lost in stomach tumors. The study aimed at determining whether FHIT expression is affected by Helicobacter pylori infection, strain virulence (vacA and cagA genes) and histopathological changes in the gastric mucosa of patients with functional dyspepsia having first-degree relatives with gastric cancer. MATERIALS AND METHODS: Eighty-eight never-smoking patients with functional dyspepsia were selected for the study, and 48 of them had first-degree relatives with gastric cancer. Bacterial DNA amplification was used to identify H. pylori colonization. The level of FHIT gene expression was determined by qRT-PCR (mRNA) and Western blot (FHIT protein) analyses. RESULTS: For patients having first-degree relatives with gastric cancer FHIT expression was lower (mRNA by ca. 40-45% and protein by 30%) compared with the control patients ($p < .05$). H. pylori infection decreased the FHIT mRNA level by 10-35% and the protein level by 10-20%. Bacterial strain vacA(+)cagA(+) lowered FHIT mRNA by ca. 30-35% in the antrum samples of both groups and in corpus samples of patients with first-degree relatives with gastric cancer ($p < .05$). The FHIT mRNA level was twice as high in control H. pylori-negative patients with intestinal metaplasia, compared with those with non-atrophic gastritis. CONCLUSIONS: The decreased FHIT gene expression associated with hereditary factors and with H. pylori infection, especially with vacA(+)cagA(+)-positive strains, may be related to gastric carcinoma development.

Tokumaru, Y., K. Yamashita, et al. (2008). "The role of PGP9.5 as a tumor suppressor gene in human cancer." Int J Cancer **123**(4): 753-9.

PGP9.5 is a controversial molecule from an oncologic point of view. We recently identified frequent methylation of PGP9.5 gene exclusively in primary head and neck squamous cell carcinoma (HNSCC), suggesting that it could be a tumor suppressor gene. On the other hand, PGP9.5 was reported to be overexpressed in a subset of human cancers presumably due to intrinsic oncogenic properties or as a result of transformation. To demonstrate that PGP9.5 possesses tumor suppressive activity, we examined forced expression by stable transfection of PGP9.5 in 4 HNSCC cell lines. Although all 4 cell lines demonstrated reduced log growth rates in culture after transfection, only 2 cell lines with wild type p53 (011, 022) demonstrated

decreased growth in soft agar. In 2 cell lines with mutant p53 (013, 019), we observed no altered growth in soft agar and increased sensitivity to UV irradiation. We then tested for and found a high frequency of promoter methylation in a larger panel of primary tumors including HNSCC, esophageal SCC, gastric, lung, prostate and hepatocellular carcinoma. Our data support the notion that PGP9.5 is a tumor suppressor gene that is inactivated by promoter methylation or gene deletion in several types of human cancers.

Turley, R. S., E. C. Finger, et al. (2007). "The type III transforming growth factor-beta receptor as a novel tumor suppressor gene in prostate cancer." Cancer Res **67**(3): 1090-8.

The transforming growth factor-beta (TGF-beta) signaling pathway has an important role in regulating normal prostate epithelium, inhibiting proliferation, differentiation, and both androgen deprivation-induced and androgen-independent apoptosis. During prostate cancer formation, most prostate cancer cells become resistant to these homeostatic effects of TGF-beta. Although the loss of expression of either the type I (TbetaRI) or type II (TbetaRII) TGF-beta receptor has been documented in approximately 30% of prostate cancers, most prostate cancers become TGF-beta resistant without mutation or deletion of TbetaRI, TbetaRII, or Smads2, 3, and 4, and thus, the mechanism of resistance remains to be defined. Here, we show that type III TGF-beta receptor (TbetaRIII or betaglycan) expression is decreased or lost in the majority of human prostate cancers as compared with benign prostate tissue at both the mRNA and protein level. Loss of TbetaRIII expression correlates with advancing tumor stage and a higher probability of prostate-specific antigen (PSA) recurrence, suggesting a role in prostate cancer progression. Taken together, these studies define the loss of TbetaRIII expression as a common event in human prostate cancer and suggest that this loss is important for prostate cancer progression through effects on cell motility, invasiveness, and tumorigenicity.

Vallian, S., M. Sedaghat, et al. (2009). "Methylation status of p16 INK4A tumor suppressor gene in Iranian patients with sporadic breast cancer." J Cancer Res Clin Oncol **135**(8): 991-6.

INTRODUCTION: p16(INK4A) is a tumor suppressor encoding the Cdk inhibitor protein, which acts to repress Cdk4/6 and pRb phosphorylation. p16(INK4A) gene can be inactivated by a variety of events, including promoter hypermethylation. MATERIALS AND METHODS: To investigate the methylation status of the p16(INK4A) gene in Iranian patients with breast carcinoma, promoter methylation was studied by methylation-specific PCR (MSP) and restriction enzyme-related PCR (REP). In addition, p16(INK4A) promoter was analyzed by PCR-SSCP in order to detection of mutation and single nucleotide polymorphisms. RESULTS: Analysis of 70 patients by MPS and REP showed hypermethylation of p16(INK4A) promoter in 35.7% (25/70) and 40% (28/70) of samples, respectively. Comparison of the molecular data and pathological information of the samples suggested that p16(INK4A) gene might be inactivated at the early stages in breast cancer. CONCLUSION: Therefore, it could be suggested that hypermethylation of p16(INK4A) promoter is one of the epigenetic factors affecting the progress of sporadic breast carcinogenesis in Iranian patients.

Veena, M. S., G. Lee, et al. (2008). "Inactivation of the cystatin E/M tumor suppressor gene in cervical cancer." Genes Chromosomes Cancer **47**(9): 740-54.

We have previously localized a cervical cancer tumor suppressor gene to a 300 kb interval of 11q13. Analysis of candidate genes revealed loss of expression of cystatin E/M, a lysosomal cysteine protease inhibitor, in 6 cervical cancer cell lines and 9 of 11 primary cervical tumors. Examination of the three exons in four cervical cancer cell lines, 19 primary tumors, and 21 normal controls revealed homozygous deletion of exon 1 sequences in one tumor. Point mutations were observed in six other tumors. Two tumors contained mutations at the consensus binding sites for cathepsin L, a lysosomal protease overexpressed in cervical cancer. Introduction of these two point mutations using site directed mutagenesis resulted in reduced binding of mutated cystatin E/M to cathepsin L. Although mutations were not observed in any cell lines, four cell lines and 12 of 18 tumors contained promoter hypermethylation. Reexpression of cystatin E/M was observed after 5'aza 2-deoxycytidiene and/or Trichostatin A treatment of cervical cancer cell lines, HeLa and SiHa, confirming promoter hypermethylation. Ectopic expression of cystatin E/M in these two cell lines resulted in growth suppression. There was also suppression of soft agar colony formation by HeLa cells expressing the cystatin E/M gene. Reexpression of cystatin E/M resulted in decreased intracellular and extracellular expression of cathepsin L. Overexpression of cathepsin L resulted in increased cell growth which was inhibited by the reintroduction of cystatin E/M. We conclude, therefore, that cystatin E/M is a cervical cancer suppressor gene and that the gene is inactivated by somatic mutations and promoter hypermethylation.

Versmold, B., J. Felsberg, et al. (2007). "Epigenetic silencing of the candidate tumor suppressor gene PROX1 in sporadic breast cancer." Int J Cancer **121**(3): 547-54.

Extensive hypermethylation and consecutive transcriptional silencing of tumorsuppressor genes have been documented in multiple tumor entities including breast cancer. In a microarray based genome-wide methylation analysis of five sporadic breast carcinomas we identified a hypermethylated CpG island within the first intron of the prospero related homeobox gene 1 (PROX1). We, therefore, investigated CpG island methylation of PROX1 in a series of 33 pairs of primary breast cancer and corresponding normal tissue samples by bisulfite sequencing and COBRA analyses. Seventeen of these (52%) breast cancer samples revealed a significant accumulation of methylated CpG sites along with a significant reduction of PROX1 transcription compared to normal breast tissues of the same patients. Frequent methylation was also observed in brain metastases from primary breast cancer (21/37 = 57% of cases). Secondary, we analysed 38 brain metastases of primary breast carcinomas and detected a significantly reduced expression of PROX1 compared to normal breast tissue ($p < 0.001$) and primary breast carcinomas ($p < 0.05$), respectively. Additionally, treatment of breast cancer cell lines with demethylating agents could reactivate PROX1 transcription. In summary, we have identified PROX1 as a novel target gene that is hypermethylated and transcriptionally silenced in primary and metastatic breast cancer.

Wu, H., Y. Cao, et al. (2008). "Effect of tumor suppressor gene PTEN on the resistance to cisplatin in human ovarian cancer cell lines and related mechanisms." Cancer Lett **271**(2): 260-71.

The aim of this study was to explore role of PTEN gene in chemosensitivity to cisplatin in human ovarian cancer cells and related mechanisms. METHOD: A PTEN-targeted short hairpin RNA (shRNA) expression vector and a wild-type sense PTEN plasmid were constructed, human ovarian cisplatin-sensitive cancer cell line OV2008 and its resistant variant C13 * cells were transfected with PTEN shRNA or wild-type PTEN plasmid, respectively, and cells were then treated with cisplatin. Next, AKT activity was regulated with co-transfection of antisense or sense AKT plasmid in OV2008 /PTENshRNA cells or C13 */p-PTEN cells, respectively. Effects of transfection of above vectors on cell growth, apoptosis and expression of PTEN and AKT were evaluated. RESULTS: Expression of PTEN in OV2008 cells was significantly higher than that in C13 * cells. Transfection of PTEN shRNA into OV2008 cells remarkably down-regulated expression of PTEN and up-regulated expression of phospho-AKT protein, with transfected cells being resistant to cisplatin. Overexpression of PTEN by transfection with sense PTEN obviously enhanced cisplatin-induced apoptosis of C13 * cells. Furthermore, decreased AKT activity could increase cisplatin-induced apoptosis in OV2008/PTENshRNA cells; while, transfection of pcDNA3.1-AKT plasmid into C13 */p-PTEN cells resulted in increased activity of AKT, with cisplatin-induced apoptosis being inhibited significantly. PTEN might reverse chemoresistance to cisplatin in human ovarian cancer cells through inactivation of the PI3K/AKT cell survival pathway and may serve as a potential molecular target for the treatment of chemoresistant ovarian cancer.

Wu, L., Z. Li, et al. (2008). "Adenovirus-expressed human hyperplasia suppressor gene induces apoptosis in cancer cells." Mol Cancer Ther **7**(1): 222-32.

Hyperplasia suppressor gene (HSG), also called human mitofusin 2, is a novel gene that markedly suppresses the cell proliferation of hyperproliferative vascular smooth muscle cells from spontaneously hypertensive rat arteries. This gene encodes a mitochondrial membrane protein that participates in mitochondrial fusion and contributes to the maintenance and operation of the mitochondrial network. In this report, we showed that an adenovirus vector encoding human HSG (Ad5-hHSG) had an antitumor activity in a wide range of cancer cell lines. Confocal laser scanning microscopy and electron microscopy revealed that cells infected with Ad5-hHSG formed dose-dependent perinuclear clusters of fused mitochondria. Adenovirus-mediated hHSG overexpression induced apoptosis, cell cycle arrest, mitochondrial membrane potential (DeltaPsim) reduction and release of cytochrome c, caspase-3 activation, and cleavage of PARP in vitro. Overexpression of hHSG also significantly suppressed the growth of subcutaneous tumors in nude mice both ex vivo and in vivo. In addition, Ad5-hHSG increased the sensitivity of these cell lines to two chemotherapeutic agents, VP16 and CHX, and radiation. These results suggest that Ad5-hHSG may serve as an effective therapeutic drug against tumors.

Xiang, S., S. B. Coffelt, et al. (2008). "Period-2: a tumor suppressor gene in breast cancer." J Circadian Rhythms **6**: 4.

Previous reports have suggested that the ablation of the Period 2 gene (Per 2) leads to enhanced development of lymphoma and leukemia in mice. Employing immunoblot analyses, we have demonstrated that PER 2 is endogenously expressed in human breast epithelial cell lines but is not

expressed or is expressed at significantly reduced level in human breast cancer cell lines. Expression of PER 2 in MCF-7 breast cancer cells significantly inhibited the growth of MCF-7 human breast cancer cells, and, when PER 2 was co-expressed with the Crytochrome 2 (Cry 2) gene, an even greater growth-inhibitory effect was observed. The inhibitory effect of PER 2 on breast cancer cells was also demonstrated by its suppression of the anchorage-independent growth of MCF-7 cells as evidenced by the reduced number and size of colonies. A corresponding blockade of MCF-7 cells in the G1 phase of the cell cycle was also observed in response to the expression of PER 2 alone or in combination with CRY 2. Expression of PER 2 also induced apoptosis of MCF-7 breast cancer cells as demonstrated by an increase in PARP [poly (ADP-ribose) polymerase] cleavage. Finally, our studies demonstrate that PER 2 expression in MCF-7 breast cancer cells is associated with a significant decrease in the expression of cyclin D1 and an up-regulation of p53 levels.

Yeh, L. S., Y. Y. Hsieh, et al. (2007). "Mutation analysis of the tumor suppressor gene PPP2R1B in human cervical cancer." Int J Gynecol Cancer **17**(4): 868-71.

Protein phosphatase 2A (PP2A) holoenzyme plays a critical role in cell cycle control and growth factor signaling. The PPP2R1B gene encodes the beta isoforms of the subunit A of the PP2A. We aimed to evaluate the role of the PPP2R1B gene in the pathogenesis of cervical cancer. Twenty-four women with primary cervical cancer were included. All resected specimens were divided into two groups: (1) cervical cancers (n = 24), (2) nearby noncancerous tissues (n = 24). We performed nested reverse transcriptase-polymerase chain reaction analysis and complementary DNA sequencing on the genomic DNA samples of all specimens. The aberrant transcripts and gene mutation as well as the genotype and allele frequencies of codon 66 CTA/CTG of PPP2R1B genes in both groups were compared. The percentages of aberrant transcripts between both groups were nonsignificantly different (20.8% vs 33.3%). There was no mutation in all specimens. The genotype and allele frequencies between both groups were non-different. Proportions of CTA homozygote/heterozygote/CTG homozygote were (1) 66.7/8.3/25% and (2) 58.3/12.5/29.2%. Proportions of CTA/CTG alleles in both groups were (1) 70.8/29.2% and (2) 64.6/35.4%. We conclude that PPP2R1B genes may not play a role in the carcinogenesis of cervical cancer. Mutations of PPP2R1B gene are not frequent in cervical cancer.

Zhijun, X., Z. Shulan, et al. (2007). "Expression and significance of the protein and mRNA of metastasis suppressor gene ME491/CD63 and integrin alpha5 in ovarian cancer tissues." Eur J Gynaecol Oncol **28**(3): 179-83.

PURPOSE: To investigate the expression and significance of the proteins and mRNA of metastasis suppressor gene Me491/cd63 and integrin alpha5 in ovarian cancer tissues. METHODS: RT-PCR and in situ hybridization were used to detect the expression of the proteins and mRNA of ME491/CD63 and integrin alpha5 in normal ovarian tissues (Group I), ovarian benign tumor tissues (Group II), ovarian borderline tumor tissues (Group III) and ovarian cancer tissues (Group IV), and the correlation between the expression and the age of patient, degree of differentiation, lymphatic metastasis, stage and pathological type was analyzed. RESULTS: There was a significant change in gene expression between the well and moderately differentiated tumors and poorly differentiated tumors. Gene expression in Groups III and IV was significantly weak, and significantly different from that in Group II and the early-stages of Groups III and IV. There was a significant difference in gene expression between each group. CONCLUSION: There is low expression of the proteins and mRNA of ME491/CD63 and integrin alpha5 in ovarian cancer. The lower the pathological differentiation is, the more significant the loss of expression is and the more likely metastasis is.

Zuo, T., L. Wang, et al. (2007). "FOXP3 is an X-linked breast cancer suppressor gene and an important repressor of the HER-2/ErbB2 oncogene." Cell **129**(7): 1275-86.

The X-linked Foxp3 is a member of the forkhead/winged helix transcription factor family. Germline mutations cause lethal autoimmune diseases in males. Serendipitously, we observed that female mice heterozygous for the "scurfin" mutation of the Foxp3 gene (Foxp3(sf/+)) developed cancer at a high rate. The majority of the cancers were mammary carcinomas in which the wild-type Foxp3 allele was inactivated and HER-2/ErbB2 was overexpressed. Foxp3 bound and repressed the HER-2/ErbB2 promoter. Deletion, functionally significant somatic mutations, and downregulation of the FOXP3 gene were commonly found in human breast cancer samples and correlated significantly with HER-2/ErbB2 overexpression, regardless of the status of HER-2 amplification. Our data demonstrate that FOXP3 is an X-linked breast cancer suppressor gene and an important regulator of the HER-2/ErbB2 oncogene.

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1/23/2011