Cancer Type literatures

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Abstract: Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries. This is a literature collection on cancer types.
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1. Introduction

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

In the U.S., according to the National Cancer Institute in 2010, the most common cancers (excluding non-melanoma skin cancers) are listed below (Table 1).

Table 1. The most common cancers in USA

Cancer type	Estimated new cases	Estimated deaths
Bladder	70,530	14,680
Breast (female-male)	207,090-1,970	39,840-390
Colon and rectal (combined)	142,570	51,370
Endometrial	43,470	7,950
Kidney (renal cell)	53,581	11,997
Leukemia	43,050	21,840
Lung (including bronchus)	222,520	157,300
Melanoma	68,130	8,700
Non-Hodgkin lymphoma	65,540	20,210
Pancreatic	43,140	36,800
Prostate	217,730	32,050
Thyroid	44,670	1,690

The three most common cancers in men, women and children in the U.S. are as follows:

- **Men**: Prostate, lung, and colorectal
- **Women**: Breast, colorectal, and lung
- **Children**: Leukemia, brain tumors, and lymphoma

The incidence of cancer and cancer types are influenced by many factors such as age, sex, race, local environmental factors, diet, and genetics. Consequently, the incidence of cancer and cancer types vary depending on these variable factors. For example, the World Health Organization (WHO) provides the following general information about cancer worldwide:

- Cancer is a leading cause of death worldwide. It accounted for 7.4 million deaths (around 13% of all deaths) in 2004 (statistics published in 2009).
- Lung, stomach, liver, colon, and breast cancer cause the most cancer deaths each year.
- Deaths from cancer worldwide are projected to continue rising, with an estimated 12 million deaths in 2030.

Different areas of the world may have cancers that are either more or less predominant then those found in the U.S. One example is that stomach cancer is often found in Japan, while it is rarely found in the U.S.

The objective of this article is to introduce the reader to general aspects of cancers. It is designed to be an overview of cancer and cannot cover every cancer type. This article will also attempt to help guide the reader to more detailed sources about specific cancer types.

Literatures

Amado, R. G., M. Wolf, et al. (2008). "Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer." J Clin Oncol **26**(10): 1626-34.

PURPOSE: Panitumumab, a fully human antibody against the epidermal growth factor receptor (EGFR), has activity in a subset of patients with

metastatic colorectal cancer (mCRC). Although activating mutations in KRAS, a small G-protein downstream of EGFR, correlate with poor response to anti-EGFR antibodies in mCRC, their role as a selection marker has not been established in randomized trials. PATIENTS AND METHODS: KRAS mutations were detected using polymerase chain reaction on DNA from tumor sections collected in a phase III mCRC trial comparing panitumumab monotherapy to best supportive care (BSC). We tested whether the effect of panitumumab on progression-free survival (PFS) differed by KRAS status. RESULTS: KRAS status was ascertained in 427 (92%) of 463 patients (208 panitumumab, 219 BSC). KRAS mutations were found in 43% of patients. The treatment effect on PFS in the wild-type (WT) KRAS group (hazard ratio [HR], 0.45; 95% CI: 0.34 to 0.59) was significantly greater ($P < .0001$) than in the mutant group (HR, 0.99; 95% CI, 0.73 to 1.36). Median PFS in the WT KRAS group was 12.3 weeks for panitumumab and 7.3 weeks for BSC. Response rates to panitumumab were 17% and 0%, for the WT and mutant groups, respectively. WT KRAS patients had longer overall survival (HR, 0.67; 95% CI, 0.55 to 0.82; treatment arms combined). Consistent with longer exposure, more grade III treatment-related toxicities occurred in the WT KRAS group. No significant differences in toxicity were observed between the WT KRAS group and the overall population. CONCLUSION: Panitumumab monotherapy efficacy in mCRC is confined to patients with WT KRAS tumors. KRAS status should be considered in selecting patients with mCRC as candidates for panitumumab monotherapy.

Amantini, C., P. Ballarini, et al. (2009). "Triggering of transient receptor potential vanilloid type 1 (TRPV1) by capsaicin induces Fas/CD95-mediated apoptosis of urothelial cancer cells in an ATM-dependent manner." Carcinogenesis **30**(8): 1320-9.

Herein, we provide evidence on the expression of transient receptor potential vanilloid type 1 (TRPV1) on human urothelial cancer (UC) cells and its involvement in the apoptosis induced by the selective agonist capsaicin (CPS). We analyzed TRPV1 messenger RNA and protein expression on human UC cell lines demonstrating its progressive decrease in high-grade UC cells. Treatment of RT4 cells with CPS induced cell cycle arrest in G(0)/G(1) phase and apoptosis. These events were associated with rapid co-ordinated transcription of pro-apoptotic genes including Fas/CD95, Bcl-2 and caspase families and ataxia telangiectasia mutated (ATM)/CHK2/p53 DNA damage response pathway. CPS induced Fas/CD95 upregulation, but more importantly Fas/CD95 ligand independent, TRPV1-dependent death receptor clustering and triggering of both extrinsic and intrinsic mitochondrial-dependent pathways. Moreover, we observed that CPS activates ATM kinase that is involved in Ser15, Ser20 and Ser392 p53 phosphorylation as shown by the use of the specific inhibitor KU55933. Notably, ATM activation was also found to control upregulation of Fas/CD95 expression and its co-clustering with TRPV1 as well as RT4 cell growth and apoptosis. Altogether, we describe a novel connection between ATM DNA damage response pathway and Fas/CD95-mediated intrinsic and extrinsic apoptotic pathways triggered by TRPV1 stimulation on UC cells.

Awata, H., K. Hirose, et al. (2002). "Heterogeneity of apoptosis and proliferative activity within superficial spreading type early gastric cancer." Oncol Rep **9**(1): 153-8.

Using the in situ DNA nick end-labeling method and immunohistochemistry, the indices of apoptosis (AI) and Ki-67 labeling index (LI) among 3 different portions within the tumor (the central surface vs. the lateral margin or deepest portion) were determined and compared in 14 superficial spreading type early gastric cancers. The AI was the lowest and the LI remained high in the lateral margin compared with the central surface, whereas the AI remained high and the LI was the lowest in the deepest portion. This heterogeneity of apoptosis and proliferation within the tumor might be related to the predominantly horizontal growth of superficial spreading type early gastric cancer.

Bahn, D. K., P. Silverman, et al. (2004). "In treating localized prostate cancer the efficacy of cryoablation is independent of DNA ploidy type." Technol Cancer Res Treat **3**(3): 253-7.

While the prognostic value of DNA ploidy has been well established for radical prostatectomy, external beam radiation, brachytherapy and androgen deprivation therapy its role as a survival outcome predictor for prostate cancer patients treated with cryoablation has not yet been examined. Anecdotal evidence suggesting that cryoablation may be independent of DNA ploidy type led to the implementation of the current study. Retrospective analysis of data including flow digital cytometry was performed on 447 archival specimens taken from patients who had undergone cryosurgical ablation of primary prostate cancer. Five-year biochemical disease free survivals (bDFS) (defined as PSA thresholds of 0.5 and 1.0 ng/ml) were determined with Kaplan-Meier analysis. Patients were grouped according to DNA ploidy types then stratified by Gleason grade, risk group, pre-surgical PSA level, and disease stage. Mean and median age of the cohort was

65 and 64.6 years. Mean follow-up was 65.7 months. The DNA ploidy status of the population was found to be 59% diploid, 13% tetraploid, and 28% aneuploid. Using PSA < 1.0 ng/ml criterion, the bDFS rates for diploid, tetraploid, and aneuploid were 78%, 75%, and 79% respectively. The bDFS rates using a PSA < 0.5 ng/ml criterion were 67%, 59%, and 69% for diploid, tetraploid, and aneuploid groups. No significant outcome differences were found in stratified analysis. This investigation demonstrates that the efficacy of cryoablation is independent of DNA ploidy type.

Bartlett, J. M., A. Munro, et al. (2008). "Type 1 receptor tyrosine kinase profiles identify patients with enhanced benefit from anthracyclines in the BR9601 adjuvant breast cancer chemotherapy trial." J Clin Oncol **26**(31): 5027-35.

PURPOSE: Patients with early breast cancer who receive anthracycline-containing chemotherapy experience improved relapse-free (RFS) and overall survival (OS) compared with those who receive non-anthracycline-containing chemotherapy. Such benefit, however, may be restricted to women whose tumors have specific molecular characteristics. We tested the hypothesis that HER2, epidermal growth factor receptor (EGFr)/HER1, HER3, Ki67, and topoisomerase IIalpha expression are predictive of outcome after anthracycline-based chemotherapy. METHODS: Tissue microarrays from 322 of 374 women in the BR9601 trial, which compared cyclophosphamide, methotrexate, and fluorouracil (CMF) with epirubicin followed by CMF (epi-CMF), were analyzed for HER1, 2, 3, 4; Ki67; and topoisomerase IIalpha protein expression and for HER2/topoisomerase IIalpha gene amplification. Their relationships to RFS and OS were investigated, and multiple regression analysis was used to identify interactions. RESULTS: A significant interaction was seen between tumors with normal HER1, HER2 fluorescent in situ hybridization (FISH), or HER3 levels and the enhanced benefit from epi-CMF versus CMF for RFS (hazard ratio [HR], 0.36; HR for overexpressed HER1 or HER2 FISH or HER3, 0.92; P = .035) and for OS (HR, 0.30; HR for overexpressed HER1 or HER2 FISH or HER3), 0.98; P = .023). Neither Ki67 nor TIIalpha expressions or gene alterations showed clear predictive value for benefit from the addition of the anthracycline. CONCLUSION: Patients with HER2 amplified and those with HER1, HER2 FISH, or HER3-positive tumors did not benefit from the addition of epirubicin to CMF. Conversely, patients with HER2 nonamplified and HER1 through HER3-negative tumors showed significantly increased RFS and OS rates when treated with epi-CMF compared with CMF.

Bossi, G. and A. Sacchi (2007). "Restoration of wild-type p53 function in human cancer: relevance for tumor therapy." Head Neck **29**(3): 272-84.

BACKGROUND: In the majority of human cancers, the tumor suppressor activity of p53 is impaired because of mutational events or interactions with other proteins (ie, MDM2). The loss of p53 function is responsible for increased aggressiveness of cancers, while tumor chemoresistance and radioresistance are dependent upon the expression of mutant p53 proteins. METHODS: Review of the literature indicates that p53 acts primarily as a transcription factor whose function is subject to a complex and diverse array of covalent post-translational modifications that markedly influence the expression of p53 target genes responsible for cellular responses such as growth arrest, senescence, or apoptosis. The ability of p53 to induce apoptosis in cancer cells is believed essential for cancer therapy. RESULTS: Numerous data indicate that p53 dependent apoptosis is a relevant factor in determining the efficacy of anticancer treatments. Thus, the development of new strategies for restoration of p53 function in human tumors is considered an important issue. Two main approaches for restoration of p53 function have been pursued that impact anticancer treatments: (a) de novo expression of wild-type p53 (wt-p53) through gene therapy and (b) identification of small molecules reactivating wt-p53 function. CONCLUSIONS: The extensive body of knowledge acquired has identified manipulations of p53 signaling as a relevant issue for successful therapies. In this context, the recognition of p53 status in cancer cells is significant and would help considerably in the selection of an appropriate therapeutic approach. p53 manipulations for cancer therapy have revealed the need for specificity of p53 activation and ability to spare body tissues. Furthermore, the promising results obtained by using molecules competent to reactivate wt-p53 functions in cancer cells provide the basis for the design of new molecules with lower side effects and higher anti-tumor efficiency. The reexpression and reactivation of p53 protein in human cancer cells would increase tumor susceptibility to radiation or chemotherapy enhancing the efficacy of standard therapeutic protocols.

Bouvet, M., R. J. Bold, et al. (1998). "Adenovirus-mediated wild-type p53 tumor suppressor gene therapy induces apoptosis and suppresses growth of human pancreatic cancer [seecomments]." Ann Surg Oncol **5**(8): 681-8.

BACKGROUND: The p53 tumor suppressor gene is mutated in up to 70% of pancreatic

adenocarcinomas. We determined the effect of reintroduction of the wild-type p53 gene on proliferation and apoptosis in human pancreatic cancer cells using an adenoviral vector containing the wild-type p53 tumor suppressor gene. METHODS: Transduction efficiencies of six p53-mutant pancreatic cancer cell lines (AsPC-1, BxPC-3, Capan-1, CFPAC-1, MIA PaCa-2, and PANC-1) were determined using the reporter gene construct Ad5/CMV/beta-gal. Cell proliferation was monitored using a 3H-thymidine incorporation assay, Western blot analysis for p53 expression was performed, and DNA laddering and fluorescence-activated cell sorter analysis were used to assess apoptosis. p53 gene therapy was tested in vivo in a subcutaneous tumor model. RESULTS: The cell lines varied in transduction efficiency. The MIA PaCa-2 cells had the highest transduction efficiency, with 65% of pancreatic tumor cells staining positive for beta-galactosidase (beta-gal) at a multiplicity of infection (MOI) of 50. At the same MOI, only 15% of the CFPAC-1 cells expressed the beta-gal gene. Adenovirus-mediated p53 gene transfer suppressed growth of all human pancreatic cancer cell lines in a dose-dependent manner. Western blot analysis confirmed the presence of the p53 protein product at 48 hours after infection. DNA ladders demonstrated increased chromatin degradation, and fluorescence-activated cell sorter analysis demonstrated a four-fold increase in apoptotic cells at 48 and 72 hours following infection with Ad5/CMV/p53 in the MIA PaCa-2 and PANC-1 cells. Suppression of tumor growth mediated by induction of apoptosis was observed in vivo in an established nude mouse subcutaneous tumor model following intratumoral injections of Ad5/CMV/p53. CONCLUSIONS: Introduction of the wild-type p53 gene using an adenoviral vector in pancreatic cancer with p53 mutations induces apoptosis and inhibits cell growth. These data provide preliminary support for adenoviral mediated p53 tumor suppressor gene therapy of human pancreatic cancer.

Brouwer, E., M. J. Havenga, et al. (2007). "Human adenovirus type 35 vector for gene therapy of brain cancer: improved transduction and bypass of pre-existing anti-vector immunity in cancer patients." Cancer Gene Ther **14**(2): 211-9.

Clinical trials in malignant glioma have demonstrated excellent safety of recombinant adenovirus type 5 (Ad5) but lack of convincing efficacy. The overall low expression levels of the Coxsackie and Adenovirus receptor and the presence of high anti-Ad5-neutralizing antibody (NAb) titers in the human population are considered detrimental for consistency of clinical results. To identify an adenoviral vector better suited to infect primary glioma cells, we tested a library of fiber-chimeric Ad5-based adenoviral vectors on 12 fresh human glioma cell suspensions. Significantly improved marker gene expression was obtained with several Ad5-chimeric vectors, predominantly vectors carrying fiber molecules derived from B-group viruses (Ad11, Ad16, Ad35 and Ad50). We next tested Ad35 sero prevalence in sera derived from 90 Dutch cancer patients including 30 glioma patients and investigated the transduction efficiency of this vector in glioma cell suspensions. Our results demonstrate that the sero prevalence and the titers of NAb against Ad35 are significantly lower than against Ad5. Also, recombinant Ad35 has significantly increased ability to transfer a gene to primary glioma cells compared to Ad5. We thus conclude that Ad35 represents an interesting candidate vector for gene therapy of malignant glioma.

Burfeind, P., C. L. Chernicky, et al. (1996). "Antisense RNA to the type I insulin-like growth factor receptor suppresses tumor growth and prevents invasion by rat prostate cancer cells in vivo." Proc Natl Acad Sci U S A **93**(14): 7263-8.

Prostate carcinoma is the second leading cause of death from malignancy in men in the United States. Prostate cancer cells express type I insulin-like growth factor receptor (IGF-IR) and prostate cancer selectively metastazises to bone, which is an environment rich in insulin-like growth factors (IGFs), thereby supporting a paracrine action for cancer cell proliferation. We asked whether the IGF-IR is coupled to tumorigenicity and invasion of prostate cancer. When rat prostate adenocarcinoma cells (PA-III) were stably transfected with an antisense IGF-IR expression construct containing the ZnSO4-inducible metallothionein-1 transcriptional promoter, the transfectants expressed high levels of IGF-IR antisense RNA after induction with ZnSO4, which resulted in dramatically reduced levels of endogenous IGF-IR mRNA. A significant reduction in expression both of tissue-type plasminogen activator and of urokinase-type plasminogen activator occurred in PA-III cells accompanying inhibition of IGF-IR. Subcutaneous injection of either nontransfected PA-III or PA-III cells transfected with vector minus the IGF-IR insert into nude mice resulted in large tumors after 4 weeks. However, mice injected with IGF-IR antisense-transfected PA-III cells either developed tumors 90% smaller than controls or remained tumor-free after 60 days of observation. When control-transfected PA-III cells were inoculated over the abraded calvaria of nude mice, large tumors formed with invasion of tumor cells into the brain parenchyma. In contrast, IGF-IR antisense transfectants formed significantly smaller tumors with

no infiltration into brain. These results indicate an important role for the IGF/IGF-IR pathway in metastasis and provide a basis for targeting IGF-IR as a potential treatment for prostate cancer.

Cascallo, M., J. Calbo, et al. (2000). "Modulation of drug cytotoxicity by reintroduction of wild-type p53 gene (Ad5CMV-p53) in human pancreatic cancer." Cancer Gene Ther **7**(4): 545-56.

Chemotherapy does not significantly improve prognosis in pancreatic cancer. New therapeutical approaches involving p53 gene replacement appear to be very encouraging due to the key role of p53 in the cell response to DNA damage. Here, we have evaluated the effectiveness of combining wild-type p53 (wt-p53) gene reintroduction (Ad5CMV-p53) and exposure to two genotoxic drugs, gemcitabine and cisplatin, in several human pancreatic cell lines. The efficiency of the combinations was clearly dependent upon timing, as assessed by cell survival determinations. Although wt-p53 transduction before drug treatment induced chemoresistance, p53 transduction in cells treated previously with gemcitabine increased cytotoxicity. Cell cycle profiles showed significant decreases in the percentage of cells in the S phase as a consequence of arrests provoked by the expression of exogenous p53, reducing the number of cells susceptible to the drug. The sensitivity of cells to cisplatin, which has a lower degree of S-phase specificity, was not modified as much by p53 gene replacement. In contrast, the recognition of the previous drug-induced DNA damage by the newly expressed wt-p53 elicited increases in sub-G1 populations, consistent with the annexin determinations and bax/bcl-2 ratios observed. Experiments on subcutaneous pancreatic xenografts corroborated the effectiveness of this approach in vivo. Thus, the combination of p53 transduction and chemotherapy, under a correct schedule of administration, appears to be a very promising therapy for human pancreatic cancer.

Chang, J., C. Lee, et al. (2000). "Over-expression of ERT(ESX/ESE-1/ELF3), an ets-related transcription factor, induces endogenous TGF-beta type II receptor expression and restores the TGF-beta signaling pathway in Hs578t human breast cancer cells." Oncogene **19**(1): 151-4.

The epithelium-specific transcription factor, ERT/ESX/ESE-1/ELF3, binds to the TGF-beta RII promoter in a sequence specific manner and regulates its expression. In this study, we investigated whether ERT could regulate endogenous TGF-beta RII expression in Hs578t breast cancer cells. Analyses of the Hs578t parental cell line revealed low RII mRNA expression and resistance to the growth inhibitory effects of TGF-beta. Infection of this cell line with a retroviral construct expressing ERT induced higher levels of endogenous RII mRNA expression and protein expression relative to cells infected with chloramphenicol acetyltransferase (CATneo) as a control. Relative to control cells, the ERTneo-expressing Hs578t cells show approximately a 50% reduction in cell growth in the presence of exogenous TGF-beta1, as well as a fourfold higher induction of activation in transient transfection assays using the 3TP-luciferase reporter construct. When transplanted into athymic mice, ERT-expressing Hs578t cells showed decreased and delayed tumorigenicity compared with control cells. This data strongly suggests that ERT plays an important role as a transcriptional activator of TGF-beta RII expression, and that deregulated ERT expression may play a critical role in rendering Hs578t human breast cancer cells insensitive to TGF-beta's growth inhibitory effects.

Chang, J., K. Park, et al. (1997). "Expression of transforming growth factor beta type II receptor reduces tumorigenicity in human gastric cancer cells." Cancer Res **57**(14): 2856-9.

Expression of transforming growth factor beta (TGF-beta) receptor type II (RII) is required for the growth-inhibitory effects of TGF-beta on proliferating epithelial cells. TGF-beta RII mutations have been identified in a broad spectrum of human epithelial malignancies, including colon and gastric cancers, and are highly correlated with development of TGF-beta resistance in cell lines derived from these tumors. In this study, the role of TGF-beta RII in regulating the tumorigenic potential of the SNU-638 human gastric cancer cell line was investigated by infecting these cells with retroviral construct (MFG) expressing TGF-beta RII. The SNU-638 cell line displays the DNA replication error phenotype and encodes a truncated, inactive TGF-beta RII protein. Infection of these cells with retroviral constructs expressing wild-type TGF-beta RII led to significant increases in TGF-beta RII mRNA and protein expression. These cells responded to exogenous TGF-beta with reduced proliferation compared to that of control cells infected with retroviral vector expressing chloramphenicol acetyltranferase. Addition of TGF-beta-neutralizing antibodies led to increased proliferation of wild-type TGF-beta RII-expressing SNU-638 cells but had no effect on control cells. The latter finding suggests that TGF-beta acts in an autocrine fashion to inhibit cell proliferation in SNU-638 cells. When transplanted into athymic nude mice, wild-type TGF-beta RII-expressing SNU-638 cells showed decreased and delayed tumorigenicity compared with control cells. This study suggests a

strong association between the expression of wild-type TGF-beta RII and the degree of malignancy in human gastric cancer cells.

Czegledy, J., M. Evander, et al. (1994). "Human papillomavirus type 18 E6* mRNA in primary tumors and pelvic lymph nodes of Hungarian patients with squamous cervical cancer." Int J Cancer **56**(2): 182-6.

Seven biopsy specimens from squamous-cell carcinomas of the uterine cervix were examined by RT-PCR for human-papilloma-virus(HPV)-specific transcripts. With our HPV18-transcription-specific primer pair (5' nts 127-149; 3' nts 587-607), all 7 were shown to contain one strong viral mRNA signal from the early 6/early 7 open reading frames (E6/E7 ORFs). Sequence analysis of the cloned PCR product proved that the transcript was generated by splicing out an intron in E6 from nucleotides 233 to 416, thereby corresponding to the HPV18 E6* spliced mRNA. Nine out of 9 metastatic and 5 of 7 histologically negative lymph nodes from the same patients were also found to be positive for the same mRNA transcript. However, 4 HPV18 unrelated primary tumors and the connected regional pelvic lymph nodes (3 metastatic, 7 histologically negative) were negative for the HPV18 E6* mRNA. Cytokeratin signals indicating tumor cells of epithelial origin were detected in 7 out of the 9 transcript-positive lymph nodes with histological signs of metastasis and in 2 out of the 5 transcript-positive histologically negative lymph nodes. This suggests that the dispersion of the epithelial monoclonal tumor cells was lymphogenic in origin.

Das, G. C., D. Holiday, et al. (2001). "Taxol-induced cell cycle arrest and apoptosis: dose-response relationship in lung cancer cells of different wild-type p53 status and under isogenic condition." Cancer Lett **165**(2): 147-53.

The effective dose, schedule, molecular basis of the cytotoxicity of taxol and their dependence on the genetic background in tumor cells are still not well understood. Here, we examined how the dose-response relationship for taxol varies in lung cancer cells with different p53 status and under isogenic conditions. DNA content analyses in A 549 (p53, +/+) and H 1299 (p53, -/-) cells, showed that taxol progressively induced G2/M arrest in both cell lines in a concentration-dependent manner, which was accompanied by a parallel decrease in the G1 population. G2/M arrest, however, occurred at a lower concentration in A 549 cell lines than in H 1299 cells. The S-phase population in A 549 cells was not significantly changed up to 0.025 microM, but dropped by six-fold at 1.0 microM taxol, in contrast to that in H 1299 cells. A sub-G1 apoptotic population was present at 24 h, even at 0.002 microM taxol, when G2/M arrest was not appreciably detected. In both cell lines, the maximum apoptosis of about 28% was achieved at 0.025 microM taxol, implicating that wild-type p53 does not modulate the level of taxol-induced apoptosis. When we examined the role of the wild-type p53 in isogenic cell lines developed in a H 1299 background, the maximum level of apoptosis was in the range of 28-34% at a drug concentration around 0.03 microM, not significantly different from that observed in parental H 1299 cells. We conclude that taxol is effective in inducing apoptosis at very low doses (0.020-0.035 microM), and that the presence or absence of the wild-type p53 does not make a statistically significant difference in the level of apoptotic cell death in these lung cancer cell lines, but the maximum is attained at a lower drug concentration in the presence of p53.

Day, J. M., P. A. Foster, et al. (2008). "17beta-hydroxysteroid dehydrogenase Type 1, and not Type 12, is a target for endocrine therapy of hormone-dependent breast cancer." Int J Cancer **122**(9): 1931-40.

Oestradiol (E2) stimulates the growth of hormone-dependent breast cancer. 17beta-hydroxysteroid dehydrogenases (17beta-HSDs) catalyse the pre-receptor activation/inactivation of hormones and other substrates. 17beta-HSD1 converts oestrone (E1) to active E2, but it has recently been suggested that another 17beta-HSD, 17beta-HSD12, may be the major enzyme that catalyses this reaction in women. Here we demonstrate that it is 17beta-HSD1 which is important for E2 production and report the inhibition of E1-stimulated breast tumor growth by STX1040, a non-oestrogenic selective inhibitor of 17beta-HSD1, using a novel murine model. 17beta-HSD1 and 17beta-HSD12 mRNA and protein expression, and E2 production, were assayed in wild type breast cancer cell lines and in cells after siRNA and cDNA transfection. Although 17beta-HSD12 was highly expressed in breast cancer cell lines, only 17beta-HSD1 efficiently catalysed E2 formation. The effect of STX1040 on the proliferation of E1-stimulated T47D breast cancer cells was determined in vitro and in vivo. Cells inoculated into ovariectomised nude mice were stimulated using 0.05 or 0.1 microg E1 (s.c.) daily, and on day 35 the mice were dosed additionally with 20 mg/kg STX1040 s.c. daily for 28 days. STX1040 inhibited E1-stimulated proliferation of T47D cells in vitro and significantly decreased tumor volumes and plasma E2 levels in vivo. In conclusion, a model was developed to study the inhibition of the major oestrogenic 17beta-HSD, 17beta-HSD1, in breast cancer. Both E2 production and tumor growth were inhibited by STX1040,

suggesting that 17beta-HSD1 inhibitors such as STX1040 may provide a novel treatment for hormone-dependent breast cancer.

Dominguez, G., J. M. Silva, et al. (2001). "Wild type p73 overexpression and high-grade malignancy in breast cancer." Breast Cancer Res Treat **66**(3): 183-90.

The overexpression of wild type p73 is the most frequent alteration of p73 in malignancies. We investigated, in 70 breast carcinomas, p73 mRNA expression and its relationship to p53 mutations, determined by an immunohistochemical method, and loss heterozygosity (LOH) status of the 1p36 region, together with its possible implication in the pathogenesis of breast carcinomas. LOH, amplifying DNA by PCR using 5 markers, of 1p36 region (one intragenic to p73 gene) was found in 17% of cases but no significant correlation was observed with p73 overexpression. p53 positive immunostaining was present in 33% of breast carcinomas, and these exhibited a statistically significant relation with p73 overexpressed tumors. Overexpression of p73 mRNA was observed in 19 tumors (27%). The analysis of cases with p73 overexpression and cases with normal mRNA expression, in terms of age and pathologic characteristics of the tumors showed a significant association of p73 overexpression and tumors with lymph node metastases, vascular invasion and higher pathologic stage. These results suggest that p73 overexpression is a molecular alteration that could be implicated in the tumorigenesis of breast carcinomas and, eventually, in a poor clinical behavior.

Fechner, G., F. G. Perabo, et al. (2003). "Preclinical evaluation of a radiosensitizing effect of gemcitabine in p53 mutant and p53 wild type bladder cancer cells." Urology **61**(2): 468-73.

OBJECTIVES: Despite clinical use, the radiosensitizing effect of gemcitabine (2'2'-difluorodeoxycytidine) in human transitional cell carcinoma (TCC) has not been shown to date. We investigated gemcitabine as a radiosensitizer for human TCC cells. METHODS: Monolayer cultures of RT112 (G1, p53 wild type), RT4 (G1-G2, p53 wild type), T24 (G3, p53, mutant type), and SUP (G4, p53 mutant type) cells were incubated in medium with gemcitabine. Electron beam radiation was applied alone, simultaneous, or 3, 6, 12, and 24 hours after gemcitabine. Jurkat leukemia cells were used as controls for radiation toxicity. Cell survival was determined 6, 12, 24, 48, and 72 hours after radiation by microculture tetrazolium assay. DNA damage was evaluated by flow cytometric assessment of poly(ADP-ribose) polymerase, and apoptosis was determined by terminal-deoxynucleotidyltransferase-mediated dUTP nick-end labeling and flow cytometric assessment after annexin-V and propidium iodide labeling. RESULTS: In all TCC cell lines, radiation alone caused only little and insignificant growth inhibitory effects at 10 Gy. Gemcitabine alone had a dose-dependent cytotoxic and apoptosis inducing effect on all TCC cell lines independent of p53 status. Assays combining radiation with gemcitabine in different dose and time schedules demonstrated no radiosensitizing effect in TCC cells. CONCLUSIONS: Gemcitabine is effective in TCC cell lines independent of p53 status. A radiosensitizing effect could not be demonstrated. Again, p53 status was not predictive of the radioresponse in the bladder cancer cell lines. Clinical studies with gemcitabine and radiotherapy might nevertheless yield different results but should be performed with utmost caution.

Fernando, G. J., B. Murray, et al. (1999). "Expression, purification and immunological characterization of the transforming protein E7, from cervical cancer-associated human papillomavirus type 16." Clin Exp Immunol **115**(3): 397-403.

E7 is the major oncogenic protein produced in cervical cancer-associated human papillomavirus type 16 (HPV16). This protein was expressed in Escherichia coli as a glutathione-S-transferase (GST) fusion protein. E7-enriched inclusion bodies were collected from bacterial lysates, were solubilized in 10 M urea, and the protein was purified using anion exchange column chromatography. After removal of endotoxin with serial Triton X-114 extractions, material of high purity (about 90%) was obtained, which is suitable for use in a human clinical trial. This material was immunogenic, and when used as a vaccine, protected mice against challenge with an HPV16 E7 DNA transfected tumour cell line. Based on this observation, the E7GST fusion protein is currently being used in a human clinical trial of a vaccine against HPV16-induced cervical cancer. This fusion protein could be cleaved with thrombin to remove the GST fusion part and further purified by preparative SDS gel electrophoresis to obtain free E7 with > 98% purity.

Ferrandina, G., G. Almadori, et al. (1998). "Growth-inhibitory effect of tamoxifen and quercetin and presence of type II estrogen binding sites in human laryngeal cancer cell lines and primary laryngeal tumors." Int J Cancer **77**(5): 747-54.

Quercetin and tamoxifen, in a range of concentrations between 0.01 and 5 microM, exert a dose-dependent inhibition on the anchorage-dependent and anchorage-independent cell growth of Hep2 and CO-K3 laryngeal cancer cell lines. Cell cycle analysis revealed that the growth-inhibitory

effect was associated with a block of the cells at the G2/M checkpoint of the cell cycle followed by DNA fragmentation. This suggests that the failure of cells to proceed through the G2/M checkpoint can be a trigger for apoptosis. The induction of apoptosis by quercetin and tamoxifen was confirmed immunocytochemically by the in situ nick end labeling (TUNEL) reaction. These compounds also exerted a dose-dependent growth-inhibitory effect on primary tumor cells, as assessed by colony-forming assay and bromodeoxyuridine labeling. Laryngeal cancer cell lines and primary tumor cells expressed Type II estrogen binding sites (Type II EBS) with binding characteristics similar to those of Type II EBS in other tumor cells. Since the affinities of quercetin and tamoxifen for Type II EBS were correlated with their growth-inhibitory potential while ipriflavone neither interacted with these sites nor inhibited cell growth, the possibility exists that the action of these compounds is mediated, at least in part, by the interaction with Type II EBS. In conclusion, our data indicate that quercetin and tamoxifen could be potentially useful in laryngeal cancer treatment.

Fujii, T., Y. Matsushima, et al. (1995). "Serum antibody against unfused recombinant E7 protein of human papillomavirus type 16 in cervical cancer patients." Jpn J Cancer Res **86**(1): 28-34.

Sera were examined for the presence of antibody against E7 protein of human papillomavirus type 16 (HPV-16) by Western blot analysis using the bacterially derived unfused protein. The occurrence rates of anti-E7 antibody against HPV-16 were 14.1% (10/71) in cervical cancer patients, 0% (0/48) in cervical intraepithelial neoplasia patients, and 0% (0/41) in female non-malignant patients. Three patients (one with endometrial cancer, one with breast cancer, and one male patient with colon polyp) out of 115 patients with tumors in organs other than the cervix, had antibody against E7 protein of HPV-16. The serum antibody, once positive, could be detected for a long time after surgical removal of the cancers in all cases that could be followed up. HPV-16 DNA could be detected in 50% (13/26) of cervical cancer patients. Sixty-nine percent (9/13) of patients with HPV-16 DNA in cancers had the antibody and all the patients with stages II, III, and IV cervical cancer (8/8) harboring HPV-16 DNA showed the presence of the antibody against E7 protein of HPV-16. In contrast, only 20% (1/5) of cervical cancer patients with stage Ia or Ib harboring HPV-16 DNA showed positive for the anti-E7 antibody in sera. These findings suggest that the presence of anti-E7 antibody in serum depends on the staging of cervical cancer and extent of HPV infection.

Fujiwara, T., E. A. Grimm, et al. (1993). "A retroviral wild-type p53 expression vector penetrates human lung cancer spheroids and inhibits growth by inducing apoptosis." Cancer Res **53**(18): 4129-33.

Multicellular tumor spheroids approximate the three-dimensional configuration of primary and metastatic tumors. The effects of retrovirus-mediated transduction of wild-type p53 (wt-p53) were studied on multicellular tumor spheroids of human non-small cell lung cancer cell lines H322a, the p53 gene of which is homozygously mutated at codon 248, and WT226b, which has endogenous wt-p53. The growth of WT226b spheroids was not affected by exogenous wt-p53 transduction; the growth of H322a spheroids, however, was significantly inhibited by the addition of wt-p53 virus stocks. Transduction of cells by the wt-p53 retroviral vector and penetration of multiple cell layers in H322a spheroids was demonstrated by in situ polymerase chain reaction/hybridization with the neomycin-resistant neo probe. Apoptotic changes indicating programmed cell death were observed in H322a spheroids treated with the wt-p53 virus. These results suggest that retroviral vectors can penetrate into multiple cell layers of three-dimensional tumor masses and induce potentially therapeutic effects.

Gao, N., Y. D. Hu, et al. (2001). "The exogenous wild-type p14ARF gene induces growth arrest and promotes radiosensitivity in human lung cancer cell lines." J Cancer Res Clin Oncol **127**(6): 359-67.

The cyclin-dependent kinase inhibitor p16INK4a encoded by the INK4A/CDKN2A/MTS1 gene is a frequent target of 9p21 inactivation in human lung cancers. The p14ARF transcript, which is an alternative spliced form of this locus, is also altered or deleted in a proportion of human lung cancers and has been shown to inhibit cell cycle progression as an endogenous cellular regulator of the p53 protein, raising the possibility that it might constitute an additional lung tumor suppressor gene at the 9p21 locus. To test the candidacy of p14ARF as a lung cancer suppressor and assess the role it plays in radiosensitivity, we transfected the wild-type p14ARF gene into four cell lines which had various endogenous gene backgrounds of INK4A-/p53+/RB+ (A549 and H460), INK4A+/p53+/RB- (H446) as well as p14ARF+/p53-/RB+ (Calu-1). We found that transfection of p14ARF is related to an obvious growth inhibition in all wtp53 cell lines, regardless of INK4A/ARF and RB status. Although it has been shown that p53-induced G1 checkpoint in response to DNA damage by ionizing radiation is p14ARF-independent, we found the radiosensitivity of two p14ARF-deficient cell lines was increased after p14ARF gene transfer. The results indicated that cell cycle redistribution after acquiring the exogenous

gene might be the main explanation for the enhanced sensitization. An increased radiation-induced apoptotic proportion in one cell line also suggested a fortified p53 function that might be triggered by the restored p14ARF protein.

Gingras, S., R. Moriggl, et al. (1999). "Induction of 3beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerase type 1 gene transcription in human breast cancer cell lines and in normal mammary epithelial cells by interleukin-4 and interleukin-13." Mol Endocrinol **13**(1): 66-81.

Sex steroids play a crucial role in the development and differentiation of normal mammary gland as well as in the regulation of breast cancer growth. Local intracrine formation of sex steroids from inactive precursors secreted by the adrenals, namely, dehydroepiandrosterone and its sulfate, may regulate growth and function of peripheral target tissues, including the breast. Both endocrine and paracrine influences on the proliferation of human breast cancer cells are well recognized. Breast tumors harbor tumor-associated macrophages and tumor-infiltrating lymphocytes that secrete a wide spectrum of cytokines. These factors may also contribute to neoplastic cell activity. The present study was designed to investigate the action of cytokines on 3beta-hydroxysteroid dehydrogenase (3beta-HSD) activity, which is an essential step in the biosynthesis of active estrogens and androgens in human breast cancer cell lines and in normal human mammary epithelial cells in primary culture. 3Beta-HSD activity was undetectable in ZR-75-1 and T-47D estrogen receptor-positive (ER)+ cells under basal growth conditions. This activity was markedly induced after exposure to picomolar concentrations of interleukin (IL)-4 or IL-13. The potent stimulatory effect of these cytokines on 3beta-HSD activity was also observed in the ER- MDA-MB-231 human breast cancer cell line and in normal human mammary epithelial cells (HMECs) in primary culture. The stimulation of 3beta-HSD activity by IL-4 and IL-13 results from a rapid increase in 3beta-HSD type 1 mRNA levels as measured by RT-PCR and Northern blot analyses. Such an induction of the 3beta-HSD activity may modulate androgenic and estrogenic biological responses as demonstrated using ZR-75-1 cells transfected with androgen- or estrogen-sensitive reporter constructs and treated with the adrenal steroid 5-androstene-3beta,17beta-diol. The DNA-binding activity of Stat6, a member of the signal transducers and activators of transcription gene family, is activated 30 min after exposure to IL-4 and IL-13 in human breast cancer cell lines as well as in HMECs in primary culture. In these cells, Stat6 activated by IL-4 or IL-13 binds to two regions of the 3beta-HSD type 1 gene promoter, containing Stat6 consensus sequences. IL-4 induction of 3beta-HSD mRNA and activity is sensitive to staurosporine. This protein kinase inhibitor also inhibits IL-4-induced Stat6 DNA-binding activity. Our data demonstrate for the first time that IL-4 and IL-13 induce 3beta-HSD type 1 gene expression, thus suggesting their involvement in the fine control of sex steroid biosynthesis from adrenal steroid precursors in normal and tumoral human mammary cells. Furthermore, aromatase and/or 5alpha-reductase(s) are expressed in the mammary gland and in a large proportion of human breast tumors. An increase in the formation of their substrates, namely, 4-androstenedione and testosterone, may well have a significant impact on the synthesis of active estrogens and androgens in these tissues.

Gonzalez-Zuloeta Ladd, A. M., A. Arias Vasquez, et al. (2007). "Differential roles of Angiotensinogen and Angiotensin Receptor type 1 polymorphisms in breast cancer risk." Breast Cancer Res Treat **101**(3): 299-304.

While angiotensinogen (AGT) seems to have anti proliferative properties, angiotensin II (ATII) is a potent growth factor and it mediates its actions through the angiotensin type 1 receptor (AGTR1). In the AGT gene, the M235T polymorphism has been associated with the variation in angiotensinogen levels and in the AGTR1 gene; the C573T variant is associated with different pathologies. We aimed to evaluate the relationship of these two variants and the risk of breast cancer. These polymorphisms were genotyped in 3787 women participating the Rotterdam Study. We performed a logistic regression and a disease free survival analysis by genotype. The logistic regression yielded an odds ratio of 1.4 (95% CI: 1.1-1.9) for the MM genotype carriers versus the T allele carriers. The breast cancer free survival by AGT genotype was significantly reduced in MM genotype carriers compared to non-carriers (hazard ratio (HR) = 1.5; 95% CI: 1.1-2.2). We did not find any association of the AGTR1 polymorphism and breast cancer risk or disease free survival. Our results suggest that AGT plays a role in breast cancer risk in postmenopausal women, whereas the role of AGTR1 needs further studying.

Gorka, M., W. M. Daniewski, et al. (2005). "Autophagy is the dominant type of programmed cell death in breast cancer MCF-7 cells exposed to AGS 115 and EFDAC, new sesquiterpene analogs of paclitaxel." Anticancer Drugs **16**(7): 777-88.

The molecular mechanism of cell death induced by AGS 115 and EFDAC, sesquiterpene analogs of paclitaxel, was investigated in human

breast cancer MCF-7 cells. The study was carried out using laser scanning cytometry, homeostatic confocal microscopy, atomic force microscopy and electron microscopy. AGS 115 and EFDAC exhibited a microtubule-stabilizing effect as confirmed by a significant increase in alpha-tubulin aggregation. Both paclitaxel analogs also induced death in MCF-7 cells. Evaluation of biochemical and morphological features suggested that the major form of programmed cell death induced by AGS 115 and EFDAC was autophagy. This was confirmed by MAP I LC3 expression and the ultrastructural pattern revealed by electron microscopy. Surface images of cells undergoing autophagy showed that, unlike during apoptosis, the dimensions remained unchanged, but the surface of the cell was deformed. The occurrence of apoptosis was confirmed by the efflux of Smac/DIABLO from mitochondria, caspase-7 activation and DNA loss, and did not exceed 9.7%. Therefore, AGS 115 and EFDAC appear to be promising candidates for further investigation in anti-cancer therapy.

Graniela Sire, E. A., F. Vikhanskaya, et al. (1995). "Sensitivity and cellular response to different anticancer agents of a human ovarian cancer cell line expressing wild-type, mutated or no p53." Ann Oncol **6**(6): 589-93.

BACKGROUND: The cytotoxicity and gene expression induced by anticancer drugs with different mechanisms of action was tested in clones from a human ovarian cancer cell line expressing no p53, mutated p53 or wild type (wt) p53. MATERIALS AND METHODS: We used clones from SKOV3 cells transfected with a temperature-sensitive mutant p53 which expresses mutated p53 at 37 degrees C and a wild type-like p53 at 32 degrees C. Cytotoxicity and expression of p53-related genes (WAF1 and GADD45) were tested after 24 hours of treatment with different drugs. RESULTS: All of the drugs were equally active in the different systems, independently of the presence of p53, with the exception of doxorubicin which was less cytotoxic in cells expressing a wtp53. An increase in the transcription of WAF1 and GADD45 genes was found in cells expressing p53 and treated with the drugs. GADD45 and WAF1 expression was also found in cells not expressing p53 but treated with the drugs, suggesting that these genes can also be activated by DNA damage through a pathway independent of p53. A highly DNA-sequence-specific alkylator, tallimustine (FCE 24517), which causes a very small number of DNA lesions, does not increase the expression of these genes. Cyclin D1 gene expression was not changed after treatment with the drugs tested in cells both expressing and not expressing wtp53. CONCLUSIONS: Our data suggest that p53 expression does not play a role in increasing the susceptibility of cells not undergoing apoptosis after DNA damage, but that, at least in the case of doxorubicin, it can enhance the repair systems and reduce the cytotoxicity.

Han, B., M. Nakamura, et al. (2006). "Calcitonin inhibits invasion of breast cancer cells: involvement of urokinase-type plasminogen activator (uPA) and uPA receptor." Int J Oncol **28**(4): 807-14.

There is a growing body of evidence indicating that calcitonin (CT) and calcitonin receptor (CTR) are involved in the regulation of cell growth, differentiation, and survival and in tissue development. However, the precise functional role of CT/CTR in breast cancer is still unknown. It is well established that the urokinase plasminogen activator (uPA) system plays an important role in breast cancer invasion and metastasis. The goal of this study was to investigate the effects of CT on regulation of the uPA system and invasive capacity of breast cancer cells. In the highly invasive MDA-MB-231 cell line, 10(-8) M CT decreased both uPA and uPAR mRNA and protein expression which was associated with inhibition of the extracellular signal-regulated kinase (ERK) 1/2 pathway. Furthermore, two weeks of CT administration to nude mice inhibited the expression of uPA mRNA in primary tumors by 25% ($P<0.05$), as compared to control, untreated animals. CT also inhibited the invasiveness of MDA-MB-231 cells by 37% (10(-8) M CT, $P<0.05$), as determined by a Matrigel invasion assay. To the best of our knowledge, this is the first report describing a direct effect of CT on breast cancer cell invasion. Our data might suggest a close link between CT signaling, the uPA-mediated pathway, and breast cancer invasion.

Hasegawa, Y., N. Emi, et al. (1993). "Gene transfer of herpes simplex virus type I thymidine kinase gene as a drug sensitivity gene into human lung cancer cell lines using retroviral vectors." Am J Respir Cell Mol Biol **8**(6): 655-61.

One of the recent strategies for gene therapy as a cancer control is the targeted introduction of a drug-sensitivity gene into tumor cells. We investigated the gene transfer of herpes simplex virus type I thymidine kinase (HSV-TK) gene as a drug-sensitivity gene into human lung cancer cell lines. We used a recombinant retroviral vector derived from Moloney murine leukemia virus (MuLV) as one of potential vectors for gene therapy. The amphotropic retroviral vector consisted of the HSV-TK gene and the neomycin-resistant gene under Rous sarcoma virus (RSV) promoter control. The antiherpes drugs, acyclovir (ACV) and ganciclovir (GCV), were chosen

for testing the activity of HSV-TK that was transferred into human lung cancer cell lines. ACV and GCV are nucleoside analogs specifically converted by HSV-TK to a toxic form capable of inhibiting DNA synthesis. The cytotoxicity was determined by using a tetrazolium-based colorimetric assay (MTT assay). The results obtained from our experiments demonstrated that the retroviral vector-mediated HSV-TK gene transfer leads to ACV- and GCV-dependent cytotoxicity in human lung cancer cell lines, which were both small cell carcinoma and non-small cell carcinoma established from human specimens. These findings suggest that the gene transfer of HSV-TK gene into tumor cells would be one of the models for the use of gene therapy to control lung cancer.

Hu, Y., R. K. Le Leu, et al. (2008). "The potential of sphingomyelin as a chemopreventive agent in AOM-induced colon cancer model: wild-type and p53+/- mice." Mol Nutr Food Res **52**(5): 558-66.

A protective effect of sphingolipids on colorectal cancer (CRC) has been reported in certain mouse strains. It is unknown if sphingolipids are protective in a p53 deficiency mouse model of CRC. This study investigated the effect of sphingomyelin (SM) on intestinal sphingomyelinase (SMase) activity, colonic epithelial biology and azoxymethane (AOM)-induced CRC. Groups of wild-type (C57BL/6J) and p53+/- mice were fed 0.1% SM diet for 4 wk, administered a single AOM injection and then killed 6 h later to measure apoptosis and proliferation. Separately, both mouse types were fed 0.05% SM diet, administered three AOM injections and killed 33-38 wk later to measure tumour formation. SM significantly increased SMase activity and reduced proliferation ($p < 0.05$) in wild-type and p53+/- mice. SM did not regulate baseline apoptosis, apoptotic response to AOM or apoptosis in tumours, nor did it restore defective apoptosis in p53+/- mice. There was a nonsignificant trend to reduced tumour incidence with SM in wild-type ($p = 0.15$) and p53+/- ($p = 0.12$) mice. In conclusion, while increasing intestinal SMase activity and suppressing proliferation, SM did not promote any form of apoptosis and failed to achieve significant protection in these mice. Further investigation to understand the variable effect of SM in preventing CRC is warranted.

Husain, I., J. L. Mohler, et al. (1994). "Elevation of topoisomerase I messenger RNA, protein, and catalytic activity in human tumors: demonstration of tumor-type specificity and implications for cancer chemotherapy." Cancer Res **54**(2): 539-46.

Topoisomerase I has been identified as an intracellular target of camptothecin, a plant alkaloid with anticancer activity. Various lines of evidence suggest that the sensitivity of cells to this drug is directly related to the topoisomerase I content. In humans, the levels of topoisomerase I have been shown to be elevated in colorectal tumors, compared to normal colon mucosa. The aim of our study was to determine whether (a) topoisomerase I levels are elevated in other solid tumors, (b) the elevated enzyme is catalytically active in these tumors, and (c) the increase in topoisomerase I levels in colorectal tumors is a result of increased transcription or translation. Topoisomerase I levels were quantitated in crude extracts from colorectal, prostate, and kidney tumors and their matched normal counterparts by Western blotting and by direct determination of catalytic activity, and mRNA levels were determined by Northern blotting. By Western blotting, colorectal tumors showed 5-35-fold increases in topoisomerase I levels, compared to their normal colon mucosa. In the case of prostate tumors, the increase was 2-10-fold, compared with benign hyperplastic prostate tissue from the same patients. However, no difference was observed in topoisomerase I levels in kidney tumors, compared to their normal counterparts. The catalytic activity of topoisomerase I was determined by a quantitative 32P-transfer assay in crude homogenates, without isolating nuclei. Colorectal and prostate tumors exhibited 11-40- and 4-26-fold increases, respectively, in catalytic activity. However, kidney tumors did not show any alteration in catalytic activity, compared to their normal matched samples. Thus, for all three tumor types there was a good correlation between enzyme levels and catalytic activity. Finally, colorectal tumors were analyzed for steady state mRNA levels. A 2-33-fold increase in mRNA levels was found in colorectal tumors, compared to normal colon mucosa. These results suggest that alterations in topoisomerase I expression in humans are tumor type specific and that the increase in topoisomerase I levels results from either increased transcription of the topoisomerase I gene or increased mRNA stability.

Hwang, R. F., E. M. Gordon, et al. (1998). "Gene therapy for primary and metastatic pancreatic cancer with intraperitoneal retroviral vector bearing the wild-type p53 gene." Surgery **124**(2): 143-50; discussion 150-1.

BACKGROUND: Metastatic pancreatic cancer is uniformly fatal because no effective chemotherapy is available. Mutations in the p53 tumor suppressor gene are found in up to 70% of pancreatic adenocarcinomas. We examined the efficacy of a retroviral vector containing the wild-type p53 gene on metastatic pancreatic cancer in a nude mouse model. METHODS: Bxpc3 human pancreatic cancer cells

were transduced with either a retroviral p53 vector or an LXSN empty vector. Cells were examined for incorporation of tritiated thymidine to determine the effect of p53 retroviral transduction on DNA synthesis, and a TACS2 assay for apoptosis was performed. The functional activity of p53 in transduced cells was assessed by Western blot analysis with an antibody to WAF1/p21. In vivo effects of intraperitoneal injections of the p53 vector were examined in a nude mouse model of peritoneal carcinomatosis. RESULTS: Cells treated with the p53 vector exhibited a 59% to 85.5% reduction in cell number compared with the control cells (P < .05). p53-treated cells demonstrated decreased incorporation of tritiated thymidine (12.7% +/- 0.7% vs 17.5% +/- 1.4%; P = .002), increased staining for apoptosis, and increased expression of the WAF1/p21 protein. Treatment of nude mice with the retroviral p53 vector resulted in a significant inhibition of growth of the primary pancreatic tumor, as well as the peritoneal tumor deposits, compared with the LXSN control vector. CONCLUSIONS: Intraperitoneal delivery of a retroviral p53 vector may provide a novel treatment approach for peritoneal carcinomatosis from pancreatic cancer.

Ikegami, S., T. Tadakuma, et al. (2005). "Selective gene therapy for prostate cancer cells using liposomes conjugated with IgM type monoclonal antibody against prostate-specific membrane antigen." Hum Cell **18**(1): 17-23.

Prostate cancer cells express prostate-specific membrane antigen (PSMA). We developed an IgM type monoclonal antibody against PSMA. The antibody was coupled to poly-L-lysine and thereafter this conjugate was mixed with cationic liposomes containing plasmid DNA. The antibody-liposome complex was tested whether it could deliver the gene of interest selectively to the PSMA positive cells. As assessed by beta-galactosidase reporter gene, the transfection efficiency was 13.2% with anti-PSMA-liposome complex as compared to 4% with control IgM liposome complex. In contrast, no such differences were observed in PSMA negative PC-3, DU145 and T24 cells. Furthermore, in the suicide gene therapy in vitro with thymidine kinase gene plus ganciclovir system, anti-PSMA liposome complex demonstrated a selective growth inhibitory effect on PSMA positive LNCaP cells but not on PSMA negative cell lines.

Ip, C., S. P. Briggs, et al. (1996). "The efficacy of conjugated linoleic acid in mammary cancer prevention is independent of the level or type of fat in the diet." Carcinogenesis **17**(5): 1045-50.

The objective of the present study was to investigate whether the anticarcinogenic activity of conjugated linoleic acid (CLA) is affected by the amount and composition of dietary fat consumed by the host. Because the anticancer agent of interest is a fatty acid, this approach may provide some insight into its mechanism of action, depending on the outcome of these fat feeding experiments. For the fat level experiment, a custom formulated fat blend was used that simulates the fatty acid composition of the US diet. This fat blend was present at 10, 13.3, 16.7 or 20% by weight in the diet. For the fat type experiment, a 20% (w/w) fat diet containing either corn oil (exclusively) or lard (predominantly) was used. Mammary cancer prevention by CLA was evaluated using the rat dimethylbenz[a]anthracene model. The results indicated that the magnitude of tumor inhibition by 1% CLA was not influenced by the level or type of fat in the diet. It should be noted that these fat diets varied markedly in their content of linoleate. Fatty acid analysis showed that CLA was incorporated predominantly in mammary tissue neutral lipids, while the increase in CLA in mammary tissue phospholipids was minimal. Furthermore, there was no evidence that CLA supplementation perturbed the distribution of linoleate or other fatty acids in the phospholipid fraction. Collectively these carcinogenesis and biochemical data suggest that the cancer preventive activity of CLA is unlikely to be mediated by interference with the metabolic cascade involved in converting linoleic acid to eicosanoids. The hypothesis that CLA might act as an antioxidant was also examined. Treatment with CLA resulted in lower levels of mammary tissue malondialdehyde (an end product of lipid peroxidation), but failed to change the levels of 8-hydroxydeoxyguanosine (a marker of oxidatively damaged DNA). Thus while CLA may have some antioxidant function in vivo in suppressing lipid peroxidation, its anticarcinogenic activity cannot be accounted for by protecting the target cell DNA against oxidative damage. The finding that the inhibitory effect of CLA maximized at 1% (regardless of the availability. of linoleate in the diet) could conceivably point to a limiting step in the capacity to metabolize CLA to some active product(s) which is essential for cancer prevention.

Israyelyan, A. H., J. M. Melancon, et al. (2007). "Effective treatment of human breast tumor in a mouse xenograft model with herpes simplex virus type 1 specifying the NV1020 genomic deletion and the gBsyn3 syncytial mutation enabling high viral replication and spread in breast cancer cells." Hum Gene Ther **18**(5): 457-73.

A new oncolytic and fusogenic herpes simplex virus type 1 (HSV-1) was constructed on the

basis of the wildtype HSV-1(F) strain. To provide for safety and tumor selectivity, the virus carried a large deletion including one of the two alpha4, gamma(1)34.5, alpha0 genes and the latency-associated transcript region. The gamma(1)34.5 gene, a major neurovirulence factor, was replaced by a gene cassette constitutively expressing the red fluorescent protein gene. Homologous recombination was used to transfer the fusogenic gBsyn3 mutation to the viral genome to produce the OncSyn virus. OncSyn causes extensive virus-induced cell fusion (syncytia) and replicates to higher titers than the parental Onc and HSV-1(F) strains in breast cancer cells. Biochemical analysis revealed that the OncSyn virus retains a stable genome and expresses all major viral glycoproteins. A xenograft mouse model system using MDA-MB-435S-luc (MM4L) human breast cancer cells constitutively expressing the luciferase gene implanted within the interscapular region of animals was used to test the ability of the virus to inactivate breast tumor cells in vivo. Seventy-two mice bearing MM4L breast cancer xenografts were randomly divided into three groups and given two rounds of three consecutive intratumoral injections of OncSyn, inactivated OncSyn, or phosphate-buffered saline 3 days apart. A single round of virus injections resulted in a drastic reduction of tumor sizes ($p <or= 0.0001$) and diminution of chemiluminescence emitted by the cancer cells ($p <or= 0.0002$). This effect was enhanced by a second round of virus injections into the tumors 3 days after the first round ($p <or= 0.0001$). Systematic necropsy and pathological evaluation of the primary tumors revealed that the single round of injections resulted in extensive necrosis of tumor cells ($p <or= 0.0001$), which was enhanced by the second round of injections ($p <or= 0.0002$). Internal organs were not affected by virus inoculation. Mouse weights were not significantly impacted by any treatment during the course of the entire study ($p = 0.46$). These results show that the attenuated, fusogenic, and oncolytic HSV-1(F) virus strain OncSyn may effectively treat human breast tumors in vivo.

Jabbar, S. F., L. Abrams, et al. (2009). "Persistence of high-grade cervical dysplasia and cervical cancer requires the continuous expression of the human papillomavirus type 16 E7 oncogene." Cancer Res **69**(10): 4407-14.

Several mucosotropic human papillomaviruses (HPV), including HPV type 16 (HPV-16), are etiologic agents of a subset of anogenital cancers and head and neck squamous cell carcinomas. In mice, HPV-16 E7 is the most potent of the papillomaviral oncogenes in the development of cervical disease. Furthermore, interfering specifically with the expression of E7 in HPV-positive cell lines derived from human cervical cancers inhibits their ability to proliferate, indicating that the expression of E7 is important in maintaining the transformed phenotype in vitro. To assess the temporal role of E7 in maintaining HPV-associated tumors and precancerous lesions in vivo, we generated Bi-L E7 transgenic mice that harbor a tetracycline-inducible transgene that expresses both HPV-16 E7 and firefly luciferase. When we crossed Bi-L E7 mice to a K5-tTA transgene-inducing line of mice, which expresses a tetracycline-responsive transactivator selectively in the stratified squamous epithelia, the resulting Bi-L E7/K5-tTA bitransgenic mice expressed E7 and luciferase in the skin and cervical epithelium, and doxycycline repressed this expression. Bitransgenic mice displayed several overt and acute epithelial phenotypes previously shown to be associated with the expression of E7, and these phenotypes were reversed on treatment with doxycycline. Repressing the expression of E7 caused the regression of high-grade cervical dysplasia and established cervical tumors, indicating that they depend on the continuous expression of E7 for their persistence. These results suggest that E7 is a relevant target not only for anticancer therapy but also for the treatment of HPV-positive dysplastic cervical lesions.

Jensen, P. B., I. J. Christensen, et al. (1993). "Differential cytotoxicity of 19 anticancer agents in wild type and etoposide resistant small cell lung cancer cell lines." Br J Cancer **67**(2): 311-20.

A panel of six 'wild type' and three VP-16 resistant small cell lung cancer (SCLC) cell lines is used to evaluate to what extent in vitro sensitivity testing using a clonogenic assay can contribute to combine cytotoxic drugs to regimens with improved efficacy against SCLC. The resistant lines include (a) H69/DAU4, which is classical multidrug resistant (MDR) with a P-glycoprotein efflux pump (b) NYH/VM, which exhibits an altered topoisomerase II (topo II) activity and (c) H69/VP, which is cross-resistant to vincristine, exhibits a reduced drug accumulation as H69/DAU4 but is without P-glycoprotein. 19 anticancer agents were compared in the panel. The MDR lines demonstrated, as expected, cross-resistance to all topo II drugs, but also different patterns of collateral sensitivity to BCNU, cisplatin, ara-C, hydroxyurea, and to the topo I inhibitor camptothecin. The complete panel of nine cell lines clearly demonstrated diverse sensitivity patterns to drugs with different modes of action. Correlation analysis showed high correlation coefficients (CC) among drug analogues (e.g. VP-16/VM-26 0.99, vincristine/vindesine 0.89), and between drugs with similar mechanisms of action (e.g. BCNU/Cisplatin

0.89, VP-16/Doxorubicin 0.92), whereas different drug classes demonstrated low or even negative CC (e.g. BCNU/VP-16 -0.21). When the CC of the 19 drug patterns to VP-16 were plotted against the CC to BCNU, clustering was observed between drugs acting on microtubules, on topo II, alkylating agents, and antimetabolites. In this plot, camptothecin and ara-C patterns were promising by virtue of their lack of cross-resistance to alkylating agents and topo II drugs. Thus, the differential cytotoxicity patterns on this panel of cells can (1) give information about drug mechanism of action, (2) enable the selection and combination of non-cross-resistant drugs, and (3) show where new drugs 'fit in' among established agents.

Kim, K. Y., K. C. Choi, et al. (2005). "Extracellular signal-regulated protein kinase, but not c-Jun N-terminal kinase, is activated by type II gonadotropin-releasing hormone involved in the inhibition of ovarian cancer cell proliferation." J Clin Endocrinol Metab **90**(3): 1670-7.

Although a novel second form of GnRH (GnRH-II) has been reported to have an antiproliferative effect on gynecologic cancer cells, its biological mechanism remains to be elucidated. We have previously demonstrated that GnRH-II activates p38 MAPK. There is accumulating evidence that activation of MAPKs by GnRH-I and -II is important for cell proliferation, differentiation, and apoptosis. In the present study, we further investigated the involvement of GnRH-II in the inhibition of cell proliferation and activation of ERK1/2 and c-Jun N-terminal protein kinase/stress-activated protein kinase (JNK/SAPK) in ovarian cancer cells, OVCAR-3. The [(3)H]thymidine incorporation and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays revealed that treatment with GnRH-II suppresses cell proliferation of ovarian cancer cells. Western blot analysis demonstrated that ERK1/2 was activated by GnRH-II (100 nm). Moreover, PD98059 (10 mum), an inhibitor of a MAPK/ERK kinase, reversed the activation of ERK1/2 induced by GnRH-II. The activation of ERK1/2 by GnRH-II subsequently phosphorylated Elk-1 as a downstream pathway, which was blocked by PD98059. On the other hand, it is not likely that GnRH-II activates the JNK/SAPK pathway. Taken together, these results indicate that the ERK1/2 pathway is involved in the effect of GnRH-II on antiproliferation and may be an important target for ovarian cancer therapy.

Kuball, J., S. F. Wen, et al. (2002). "Successful adenovirus-mediated wild-type p53 gene transfer in patients with bladder cancer by intravesical vector instillation." J Clin Oncol **20**(4): 957-65.

PURPOSE: To study safety, feasibility, and biologic activity of adenovirus-mediated p53 gene transfer in patients with bladder cancer. PATIENTS AND METHODS: Twelve patients with histologically confirmed bladder cancer scheduled for cystectomy were treated on day 1 with a single intratumoral injection of SCH 58500 (rAd/p53) at cystoscopy at one dose level (7.5 x 10(11) particles) or a single intravesical instillation of SCH 58500 with a transduction-enhancing agent (Big CHAP) at three dose levels (7.5 x 10(11) to 7.5 x 10(13) particles). Cystectomies were performed in 11 patients on day 3, and transgene expression, vector distribution, and biologic markers of transgene activity were assessed by molecular and immunohistochemical methods in tumors and normal bladder samples. RESULTS: Specific transgene expression was detected in tissues from seven of eight assessable patients treated with intravesical instillation of SCH 58500 but in none of three assessable patients treated with intratumoral injection of SCH 58500. Induction of RNA and protein expression of the p53 target gene p21/WAF1 was demonstrated in samples from patients treated with SCH 58500 instillation at higher dose levels. Distribution studies after intravesical instillation of SCH 58500 revealed both high transduction efficacy and vector penetration throughout the whole urothelium and into submucosal tumor cells. No dose-limiting toxicity was observed, and side effects were local and of transient nature. CONCLUSION: Intravesical instillation of SCH 58500 combined with a transduction-enhancing agent is safe, feasible, and biologically active in patients with bladder cancer. Studies to evaluate the clinical efficacy of this treatment in patients with localized high-risk bladder cancer are warranted.

Lakka, S. S., R. Rajagopal, et al. (2001). "Adenovirus-mediated antisense urokinase-type plasminogen activator receptor gene transfer reduces tumor cell invasion and metastasis in non-small cell lung cancer cell lines." Clin Cancer Res **7**(4): 1087-93.

The urokinase-type plasminogen activator (uPA) and its receptor (uPAR) play an important role in the proteolytic cascade involved in the metastasis of lung and other cancers. We report that the reduction in uPAR levels produced by an antisense strategy using an adenovirus construct (Ad-uPAR) in H1299 cells, an invasive human lung cancer cell line that produces high levels of uPAR, resulted in a decrease of uPAR levels to 80-90% of those seen in cells infected with mock or adenovirus (Ad)-cytomegalovirus vector controls. In addition, increasing the multiplicity of infection from 25 to 200 caused a corresponding

decrease in the level of uPAR protein within 5 days of treatment, as shown by Western blot analysis. Furthermore, the in vitro translation of total RNA levels of Ad-uPAR-infected H1299 cells in a rabbit reticulocyte lysate system caused a 50-70% decrease in uPAR immunoprecipitate in Ad-uPAR-infected cells relative to the levels in cells of mock and vector controls. The Matrigel invasion assay showed the invasion of H1299 cells and A549 cells infected with Ad-uPAR to be decreased by 70% relative to mock- and vector-infected controls. Infection of tumor cells with Ad-uPAR before implantation significantly reduced the incidence of lung metastasis by 85% as compared with the control virus-infected cells injected into nude mice through the tail vein. Our collective results show that the uPAR system is a potential target of treatment for lung cancers.

Li, H., M. Zhao, et al. (2004). "Characterization of a new type HPV16 E7 variant isolated from cervical cancer highest incidence area in Hubei Province of China." Eksp Onkol **26**(1): 48-54.

AIM: To investigate the variation and biological properties of HPV16 E7 isolated from cervical cancer biopsy samples from highest incidence area in HuBei province of China. METHODS: HVP16 E7 sequences isolated from the cervical cancer biopsies of 10 local patients were amplified, sequenced and compared with prototype E7 gene. Then the variant gene was cloned into different vectors to study the antigenicity, expression and immunogenicity of its protein by Western blot, immunofluorescence and genetic immunization in vitro or in vivo. RESULTS: The results showed that 7 of 10 samples had the same mutations which led to a nonsense mutation at codon 43 of E7 sequence. The truncated E7 protein could be recognized by standard E7 monoclonal antibody in Western blot and expressed in NIH3T3 cells. In the blood sera of mice immunized intramuscularly by the plasmid DNA expressing the variant E7 gene specific E7 antibodies could be detected at week 2, 3, 5 and 6 after inoculation. However, no specific lymphoproliferation after E7 protein stimulation in vitro was detected by MTT colorimetric assay in comparison to the prototype E7 protein. CONCLUSION: HPV16 E7 gene may show variation in China and the variant protein could be expressed and induce host humoral immune response, but could not elicit special cellular-immune response against it. These data might hold the key for future development of HPV16 vaccine in HuBei province of China.

Li, T., Y. H. Ling, et al. (2008). "Tumor dependence on the EGFR signaling pathway expressed by the p-EGFR:p-AKT ratio predicts erlotinib sensitivity in human non-small cell lung cancer (NSCLC) cells expressing wild-type EGFR gene." J Thorac Oncol **3**(6): 643-7.

INTRODUCTION: This study was undertaken to identify molecular determinants of tumor dependency on the epidermal growth factor receptor (EGFR) signaling pathway for predicting clinical benefit from erlotinib monotherapy in non-small cell lung cancer (NSCLC) patients with tumors expressing wild-type EGFR gene. METHODS: The effect of erlotinib on the total and phosphorylated protein expression of EGFR and key downstream signaling molecules was determined by immunoblots in a panel of NSCLC cells expressing wild-type EGFR gene. The parameters that correlate with cell sensitivity and resistance to erlotinib was analyzed. RESULTS: Individual assessment of total or phosphorylated protein expression of EGFR or a downstream signaling molecule does not correlate with sensitivity to erlotinib in these NSCLC tumors. Resistance of NSCLC cells to erlotinib is associated with failed inhibition of at least one phosphorylated downstream signaling molecule. The dependency of NSCLC cells on the activated EGFR axis was measured by the ratio of p-EGFR to a phosphorylated downstream protein. A high ratio should indicate that activation of a downstream signaling molecule primarily results from the activation of upstream EGFR; and a low ratio should indicate that activation of a downstream signaling molecule primarily results from the activation of a upstream receptors other than EGFR. The p-EGFR:p-AKT ratio was 10-fold higher in erlotinib-sensitive cells than erlotinib-resistant cells ($p = 0.03$). It was the best predictor of erlotinib sensitivity among all parameters analyzed in this panel of NSCLC cell lines. CONCLUSIONS: The p-EGFR:p-AKT ratio deserves further investigation as a predictive parameter for clinical response to erlotinib in NSCLC tumors expressing wild-type EGFR gene.

Lin, C. C., M. C. Yen, et al. (2008). "Delivery of noncarrier naked DNA vaccine into the skin by supersonic flow induces a polarized T helper type 1 immune response to cancer." J Gene Med **10**(6): 679-89.

BACKGROUND: DNA vaccine is a new and powerful approach to generate immunological responses against infectious disease and cancer. The T helper type (Th)1 immune response is usually required for generating effective anti-tumor responses. A microparticulate bombardment system can induce an immune response using very low amounts of DNA. Using nozzle aerodynamics, a low pressure gene gun has been developed to decrease the noise associated with high pressure gene guns. Particles are propelled by supersonic flow through this novel nozzle. To test

whether this gun could inoculate a DNA vaccine that stimulates an anti-tumor Th1 immune response, we examined the effect of direct delivery of naked DNA (i.e. without any carrier) on the anti-tumor immune response of mice. METHODS: The luciferase reporter plasmid DNA was delivered using a low-pressure biolistic device and expressed in C3H/HeN, BALB/c, and C57BL/6 mice. RESULTS: Plasmid DNA expression was mainly in the epidermis. Noncarrier naked neu DNA vaccine and gold particle-coated neu DNA vaccine (at 1 microg per mouse) had similar anti-tumor effects in C3H mice. However, cytokine profile examination showed the Th1-bias of the response induced by naked DNA vaccine and the Th2-bias of the response induced by coated DNA vaccine. CONCLUSIONS: A shift in the immune response to favour enhanced tumor rejection can be achieved by skin delivery of naked DNA vaccine.

Liu, D. W., Y. P. Tsao, et al. (2000). "Recombinant adeno-associated virus expressing human papillomavirus type 16 E7 peptide DNA fused with heat shock protein DNA as a potential vaccine for cervical cancer." J Virol **74**(6): 2888-94.

In this study, we explore a potential vaccine for human papillomavirus (HPV)-induced tumors, using heat shock protein as an adjuvant, a peptide vaccine for safety, and adeno-associated virus (AAV) as a gene delivery vector. The tumor vaccine was devised by constructing a chimeric gene which contained HPV type 16 E7 cytotoxic T-lymphocyte (CTL) epitope DNA (M. C. Feltkamp, H. L. Smits, M. P. Vierboom, R. P. Minnaar, B. M. de Jongh, J. W. Drijfhout, J. ter Schegget, C. J. Melief, and W. M. Kast, Eur. J. Immunol. 23:2242-2249, 1993) fused with the heat shock protein gene as a tumor vaccine delivered via AAV. Our results demonstrate that this vaccine can eliminate tumor cells in syngeneic animals and induce CD4- and CD8-dependent CTL activity in vitro. Moreover, studies with knockout mice with distinct T-cell deficiencies confirm that CTL-induced tumor protection is CD4 and CD8 dependent. Taken together, the evidence indicates that this chimeric gene delivered by AAV has potential as a cervical cancer vaccine.

Loupakis, F., A. Ruzzo, et al. (2009). "KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer." Br J Cancer **101**(4): 715-21.

BACKGROUND: KRAS codons 12 and 13 mutations predict resistance to anti-EGFR monoclonal antibodies (moAbs) in metastatic colorectal cancer. Also, BRAF V600E mutation has been associated with resistance. Additional KRAS mutations are described in CRC. METHODS: We investigated the role of KRAS codons 61 and 146 and BRAF V600E mutations in predicting resistance to cetuximab plus irinotecan in a cohort of KRAS codons 12 and 13 wild-type patients. RESULTS: Among 87 KRAS codons 12 and 13 wild-type patients, KRAS codons 61 and 146 were mutated in 7 and 1 case, respectively. None of mutated patients responded vs 22 of 68 wild type (P=0.096). Eleven patients were not evaluable. KRAS mutations were associated with shorter progression-free survival (PFS, HR: 0.46, P=0.028). None of 13 BRAF-mutated patients responded vs 24 of 74 BRAF wild type (P=0.016). BRAF mutation was associated with a trend towards shorter PFS (HR: 0.59, P=0.073). In the subgroup of BRAF wild-type patients, KRAS codons 61/146 mutations determined a lower response rate (0 vs 37%, P=0.047) and worse PFS (HR: 0.45, P=0.023). Patients bearing KRAS or BRAF mutations had poorer response rate (0 vs 37%, P=0.0005) and PFS (HR: 0.51, P=0.006) compared with KRAS and BRAF wild-type patients. CONCLUSION: Assessing KRAS codons 61/146 and BRAF V600E mutations might help optimising the selection of the candidate patients to receive anti-EGFR moAbs.

Mariot, P., K. Vanoverberghe, et al. (2002). "Overexpression of an alpha 1H (Cav3.2) T-type calcium channel during neuroendocrine differentiation of human prostate cancer cells." J Biol Chem **277**(13): 10824-33.

Neuroendocrine differentiation of prostate epithelial cells is usually associated with an increased aggressivity and invasiveness of prostate tumors and a poor prognosis. However, the molecular mechanisms involved in this process remain poorly understood. We have investigated the possible expression of voltage-gated calcium channels in human prostate cancer epithelial LNCaP cells and their modulation during neuroendocrine differentiation. A small proportion of undifferentiated LNCaP cells displayed a voltage-dependent calcium current. This proportion and the calcium current density were significantly increased during neuroendocrine differentiation induced by long-term treatments with cyclic AMP permeant analogs or with a steroid-reduced culture medium. Biophysical and pharmacological properties of this calcium current suggest that it is carried by low-voltage activated T-type calcium channels. Reverse transcriptase-PCR experiments demonstrated that only a single type of LVA calcium channel mRNA, an alpha(1H) calcium channel mRNA, is expressed in LNCaP cells. Quantitative real-time reverse transcriptase-PCR revealed that alpha(1H) mRNA was overexpressed during neuroendocrine differentiation. Finally, we show that this calcium

channel promotes basal calcium entry at resting membrane potential and may facilitate neurite lengthening. This voltage-dependent calcium channel could be involved in the stimulation of mitogenic factor secretion and could therefore be a target for future therapeutic strategies.

Martinet, N., L. Bonnard, et al. (2003). "In vivo transglutaminase type 1 expression in normal lung, preinvasive bronchial lesions, and lung cancer." Am J Respir Cell Mol Biol **28**(4): 428-35.

Transglutaminase type 1 (TGase 1) is a member of a class of enzymes that catalyze the cross-linking of proteins, a characteristic feature of epidermal differentiation and squamous metaplasia. The role of TGase 1 has been extensively studied in epidermis but not in the lung. Using a polyclonal anti-TGase 1 antibody prepared in our laboratory (TGase-lac), TGase 1 expression in normal bronchial epithelium, bronchial preinvasive lesions, and lung cancer was characterized. The specificity of the antibody was confirmed by the presence in squamous differentiated bronchial cells of specific 106-kD and 92-kD bands by Western blotting. In addition, immunohistochemistry displayed a recognized pattern of labeling in both normal and tumor cells beneath the cytoplasmic membrane and within the cytosol. TGase 1 was shown to be expressed by cells from bronchial epithelium and bronchial preinvasive lesions, strongly expressed in most non-small-cell lung cancer tumor cells and in apoptotic bodies, but weakly expressed in small-cell lung cancer. The distribution of TGase 1 mRNA correlated with the immunohistochemical profile. These observations suggest that TGase 1 expression is a normal feature of bronchial epithelium and is linked to the process of squamous differentiation occurring in preinvasive lesions. Its role in lung cancer remains to be clarified.

Matsubara, H., T. Maeda, et al. (2001). "Combinatory anti-tumor effects of electroporation-mediated chemotherapy and wild-type p53 gene transfer to human esophageal cancer cells." Int J Oncol **18**(4): 825-9.

Delivery of electric pulses to an established solid tumor augments the permeability of cell membrane and increases the susceptibility of tumors to an anti-cancer agent that is administered in the vicinity of tumors. Forced expression of the wild-type p53 gene in tumor cells that have non-functional p53 gene(s) can also enhance their sensitivity to a DNA-damaging agent. To investigate the feasibility of electroporation-mediated therapy for cancer, electric pulses were delivered to human esophageal tumors developed in nude mice after they received an anti-cancer agent and/or plasmid DNA containing the wild-type p53 gene. The growth of esophageal tumors was suppressed with electroporation-mediated chemotherapy compared with the treatment with an anti-cancer agent or electroporation alone. Intratumoral injection of the wild-type p53 gene into p53-mutated esophageal tumors followed by electroporation also inhibited tumor growth. When mice were administered with the wild-type p53 gene and an anti-cancer agent, subsequent electroporation produced a synergistic therapeutic effect. Combinatory transfer of plasmid DNA and a pharmacological agent by electroporation is thereby a possible therapeutic strategy for the treatment of solid tumors.

Miyake, H., I. Hara, et al. (1999). "Synergistic enhancement of resistance to cisplatin in human bladder cancer cells by overexpression of mutant-type p53 and Bcl-2." J Urol **162**(6): 2176-81.

PURPOSE: The objective of this study was to characterize the effect of mutant-type p53 and Bcl-2 expression on the sensitivity to cisplatin in a human bladder cancer cell line both in vitro and in vivo. MATERIALS AND METHODS: We transfected mutant-type p53 cDNA, Bcl-2 cDNA, or both cDNAs into KoTCC-1, a human bladder cancer cell line that does not express mutant-type p53 or Bcl-2 protein. The effects of the overexpression of mutant-type p53, Bcl-2, or both on the sensitivity to cisplatin and the apoptotic features in vitro were evaluated by the MTT assay, staining with Hoechst 33258 and a DNA fragmentation assay. We then examined the in vivo effects of cisplatin treatment on the transfectants by subcutaneous and intraperitoneal tumor cell injection models in athymic nude mice. RESULTS: The introduction of mutant-type p53 or Bcl-2 conferred resistance to cisplatin on KoTCC-1 cells through the inhibition of apoptosis. This phenotype was more remarkable in the cell line transfected with both mutant-type p53 and Bcl-2 than in the cell lines transfected with either mutant-type p53 or Bcl-2 alone. Furthermore, the KoTCC-1 cells transfected with both mutant-type p53 and Bcl-2 exhibited significantly higher resistance to cisplatin treatment than cells transfected with mutant-type p53 or Bcl-2 alone in experimental models in vivo. CONCLUSIONS: These findings suggest that the overexpression of both mutant-type p53 and Bcl-2 in bladder cancer cells synergistically interferes with the therapeutic effect of cisplatin through the inhibition of the apoptotic pathway.

Muenscher, A., H. H. Feucht, et al. (2009). "Integration of human papilloma virus type 26 in laryngeal cancer of a child." Auris Nasus Larynx **36**(2): 232-4.

Squamous cell carcinoma (SCC) in larynx is rare with children and adolescents. Usually larynx cancer is common with male smokers in the 7th decade. Among patients with no history of tobacco and/or alcohol consumption several factors have can play a role in the outbreak of laryngeal cancer: such as individual predisposition, radiation, gastroesophageal reflux, viral infection, dietary factors and environmental influences. In literature only few cases of laryngeal cancer with children are reported. Recent studies show that the most frequent laryngeal malignancy is the embryonal rhabdomyosarcoma. Besides the recurrent respiratory papillomatosis (RRP) based on an infection with human papilloma virus (HPV) types 6 and 11 (low risk) and types 16 and 18 (high risk) is known for a possible malignant transformation towards a SCC. HPV type 26 is only reported as low risk type HPV associated with cervical cancer. Final diagnosis often takes a long time. Initial symptoms such as hoarseness, cough or shortness of breath are often referred to more typical pediatric diseases or laryngeal development.

Namoto, M., Y. Yonemitsu, et al. (1998). "Heterogeneous induction of apoptosis in colon cancer cells by wild-type p53 gene transfection." Int J Oncol **12**(4): 777-84.

To examine the effects of wild-type (wt)-p53 gene transfer on cancer cell growth and apoptosis induction, we transduced human wt-p53 cDNA into three colon cancer cell lines either with or without a mutation of the p53 gene using the HVJ-cationic liposome method. Wt-p53 gene transfer, thus, induced an apparent growth arrest in all cell lines, but its enhancement of the apoptotic rate varied (from about 4 to 70 times). The simultaneous doxorubicin treatment was able to enhance growth arrest and the apoptosis induction rate. These findings suggest that wt-p53 gene transfer using HVJ-cationic liposomes seems to be a potentially effective therapeutic strategy, however wt-p53 gene transfer still appears to be more effective in combination with other cytotoxic treatments.

Neumann, J., E. Zeindl-Eberhart, et al. (2009). "Frequency and type of KRAS mutations in routine diagnostic analysis of metastatic colorectal cancer." Pathol Res Pract **205**(12): 858-62.

Mutation analysis of the KRAS oncogene is now established as a predictive biomarker in colorectal cancer (CRC). Large prospective clinical trials have shown that only CRCs with wild-type KRAS respond to anti-epidermal growth factor receptor (EGFR) treatment. Therefore, mutation analysis is mandatory before treatment, and reliable benchmarks for the frequency and types of KRAS mutations have to be established for routinely testing large numbers of metastatic CRCs. A thousand and eighteen cases (879 primary tumors and 139 metastases) of metastatic colorectal cancer were analyzed for the KRAS mutational status of codons 12 and 13 of the KRAS gene by genomic sequencing in a routine setting. Results were analyzed separately for specimens derived from primary tumors and metastases. KRAS mutations in codons 12 and 13 were present in 39.3% of all analyzed CRCs. The most frequent types of mutations were glycine to aspartate on codon 12 (p.G12D, 36.0%), glycine to valine on codon 12 (pG12V, 21.8%), and glycine to aspartate on codon 13 (p.G13D, 18.8%). They account for 76.6% of all mutations and prevail in primary tumors and distant metastases, indicating a robustness of the KRAS mutational status during neoplastic dissemination. The frequency of KRAS mutations and the preponderance of three types of mutations in codons 12 and 13 in a large, unselected cohort of metastatic CRC confirm the previous data of small and selected CRC samples. Thus, a mutation frequency of 40% and a cluster of three mutation types (p.G12D, pG12V, and p.G13D) in primaries and metastases can be defined as benchmarks for routine KRAS analyses.

Ng, A. B. and N. B. Atkin (1973). "Histological cell type and DNA value in the prognosis of squamous cell cancer of uterine cervix." Br J Cancer **28**(4): 322-31.

Based on the evaluation of 362 cases of squamous cell carcinoma of the uterine cervix, the distribution of the tumours in relation to their modified Broders' grade, histological cell type as proposed by Wentz and Reagan, and the clinical stage of disease was evaluated. The morphological characteristics of the 3 cell types-large cell non-keratinizing, keratinizing, and small cell cancers-were described. The 5 year survival in relation to Broders' grade, cell type, extent and DNA values of the malignant cells were evaluated and compared. Broders' grading system was not useful in predicting the biological behaviour of cervical squamous cancer. The histological cell type and extent of the tumour were important factors in prognosis. The 5 year survival for large cell cancer was 51.8%, keratinizing cancer 34.7% and small cell cancer 10.0%. The 5 year survival was 63.3% for stage I neoplasms, 52.9% for stage II neoplasms, 30.7% for stage III neoplasms and 15.0% for stage IV neoplasms. When the DNA values of neoplastic cells were considered in relation to cell type and extent of disease, the biological behaviour of cervical squamous cell cancers was determined more accurately. The 5 year survival of women with cervical cancer in which the DNA values of the

neoplastic cells exceeded 155 was more favourable than those with DNA values of less than 155. This difference in 5 year survival was evident for comparable cell type and clinical stage of disease.

Nguyen, K. H., P. Hachem, et al. (2005). "Adenoviral-E2F-1 radiosensitizes p53wild-type and p53null human prostate cancer cells." Int J Radiat Oncol Biol Phys **63**(1): 238-46.

PURPOSE: E2F-1 is a transcription factor that enhances the radiosensitivity of various cell lines by inducing apoptosis. However, there are conflicting data concerning whether this enhancement is mediated via p53 dependent pathways. Additionally, the role of E2F-1 in the response of human prostate cancer to radiation has not been well characterized. In this study, we investigated the effect of Adenoviral-E2F-1 (Ad-E2F-1) on the radiosensitivity of p53wild-type (LNCaP) and p53null (PC3) prostate cancer cell lines. METHODS AND MATERIALS: LNCaP and PC3 cells were transduced with Ad-E2F-1, Adenoviral-Luciferase (Ad-Luc) control vector, or Adenoviral-p53 (Ad-p53). Expression of E2F-1 and p53 was examined by Western blot analysis. Annexin V and caspase 3 + 7 assays were performed to estimate the levels of apoptosis. Clonogenic survival assays were used to determine overall cell death. Statistical significance was determined by analysis of variance, using the Bonferroni method to correct for multiple comparisons. RESULTS: Western blot analysis confirmed the efficacy of transductions with Ad-E2F-1 and Ad-p53. Ad-E2F-1 transduction significantly enhanced apoptosis and decreased clonogenic survival in both cell lines. These effects were compounded by the addition of RT. Although E2F-1-mediated radiosensitization was independent of p53 status, this effect was more pronounced in p53wild-type LNCaP cells. When PC3 cells were treated with Ad-p53 in combination with RT and Ad-E2F-1, there was at least an additive reduction in clonogenic survival. CONCLUSIONS: Our results suggest that Ad-E2F-1 significantly enhances the response of p53wild-type and p53null prostate cancer cells to radiation therapy, although radiosensitization is more pronounced in the presence of p53. Ad-E2F-1 may be a useful adjunct to radiation therapy in the treatment of prostate cancer.

Nicoletti, M. I., T. G. Myers, et al. (2001). "Wild-type p53 marginally induces endogenous MDR-1 mRNA without causing a measurable drug resistance in human cancer cells." Int J Oncol **18**(2): 375-81.

The notion that wt p53 downregulates MDR-1 links p53 mutations to multidrug resistant phenotype. Alternatively, it has been envisioned that wt p53 protects cells against DNA damaging drugs by inducing MDR-1. Opposing conclusions on the relationship between MDR-1 and p53 have been predominantly based on the effects of p53 on MDR-1 promoter-constructs. We found that introduction of wt p53 slightly induced MDR-1 mRNA in three cell lines having endogenous mt p53. Wt p53-mediated induction of endogenous MDR-1 may represent a rudiment of cellular protection against toxic compounds earlier in evolution. Marked induction of p21WAF1/CIP1 (p21) mRNA was observed in all cell lines; and lower levels of wt p53 were required to induce p21 than MDR-1. Pgp was undetectable and wt p53 did not increase resistance to an MDR-1 substrate, suggesting the changes in MDR-1 mRNA may be functionally insignificant. Unlike endogenous MDR-1, the expression of an MDR-1 promoter (-434/+147 fragment) - luciferase construct was unchanged or even inhibited by wt p53 that may be secondary to wt p53-mediated cytotoxicity. Thus, partial promoter constructs may not accurately represent endogenous MDR-1.

Ogawa, N., T. Fujiwara, et al. (1997). "Novel combination therapy for human colon cancer with adenovirus-mediated wild-type p53 gene transfer and DNA-damaging chemotherapeutic agent." Int J Cancer **73**(3): 367-70.

Alteration of the wild-type (wt) p53 gene by mutation, deletion or re-arrangement is a major factor in the development of human colon cancer. Recent studies have demonstrated that p53 might be an essential component of the apoptotic pathway triggered by DNA-damaging stimuli such as chemotherapeutic agents and ionizing radiation. We examined the anti-tumor effects of adenovirus-mediated wt-p53 gene transfer in combination with a chemotherapeutic drug on the human colon cancer cell line WiDr, which is homozygous for a mutation in the p53 gene. Treatment with the chemotherapeutic drug cisplatin following infection with a replication-deficient, recombinant adenoviral vector expressing wt-p53 (termed AdCMVp53) significantly suppressed the growth of WiDr cells compared to single treatments alone. To evaluate the in vivo efficacy of AdCMVp53 and cisplatin given sequentially, WiDr cells were inoculated s.c. in nu/nu mice. After 3 days, AdCMVp53 was injected s.c. into the area where tumor cells were implanted, followed by i.p. administration of cisplatin. Analysis of initial growth inhibition at 21 days demonstrated a profound therapeutic cooperativity, though administration of either AdCMVp53 or cisplatin alone was followed only by a slowing of growth. Our results suggest that gene therapy using wt-p53-expressing adenovirus in combination with a chemotherapeutic DNA-damaging drug could be a useful strategy for treating human colon cancer.

Onuki, M., K. Matsumoto, et al. (2009). "Human papillomavirus infections among Japanese women: age-related prevalence and type-specific risk for cervical cancer." Cancer Sci **100**(7): 1312-6.

To obtain baseline data for human papillomavirus (HPV) screening and vaccination in Japan, we analyzed HPV DNA data from 2282 Japanese women (1517 normal cytology, 318 cervical intraepithelial neoplasia [CIN] grade 1, 307 CIN2-3, and 140 invasive cervical cancer [ICC]) that visited the University of Tsukuba Hospital or Ibaraki Seinan Medical Center Hospital for screening or treatment of cervical diseases between 1999 and 2007. An L1-based PCR method was used for individual HPV genotyping. The most common HPV types in ICC were, in order of decreasing prevalence, HPV16 (40.5%), HPV18 (24.4%), HPV52 (8.4%), HPV58 (3.1%), and HPV33 (3.1%). Based on the comparison of HPV type distributions between normal cytology and CIN2-3 and ICC, estimated risk of disease progression varied considerably by genotype: HPV16, HPV18, HPV31, HPV33, HPV35, HPV52, and HPV58 (prevalence ratio, 1.92; 95% confidence interval 1.58-2.34); other oncogenic types (0.31, 95% confidence interval 0.19-0.50); and non-oncogenic types (0.09, 95% confidence interval 0.03-0.43). HPV16 and/or HPV18, including coinfections with other types, contributed to 67.1% of ICC and 36.2% of CIN2-3 among Japanese women. More importantly, the overall prevalence of HPV16 and/or HPV18 varied greatly according to the women's age: highest in women aged 20-29 years (ICC, 90.0%; CIN2-3, 53.9%), decreasing with age thereafter, and lowest in women aged 60 years or older (ICC, 56.3%; CIN2-3, 25.0%). In conclusion, type-specific HPV testing may help identify Japanese women at high risk of progression to CIN2-3 and cancer. In Japan, current HPV vaccines are estimated to provide approximately 70% protection against ICC and may be more useful in reducing the incidence of cervical cancer and precancer in young women of reproductive age.

Osaki, S., Y. Nakanishi, et al. (2000). "Alteration of drug chemosensitivity caused by the adenovirus-mediated transfer of the wild-type p53 gene in human lung cancer cells." Cancer Gene Ther **7**(2): 300-7.

The aim of the present study is to identify the optimal anticancer agents for use in combination with gene therapy using wild-type (wt) p53 gene transfer. We used adenoviral vectors expressing human wt p53 (AdCAp53) and investigated the effects of wt p53 gene transfer in combination with 12 anticancer agents on a human pulmonary squamous cell carcinoma cell line, NCI-H157, and a human pulmonary large cell carcinoma cell line, NCI-H1299. Solutions containing anticancer agents at various concentrations were added followed by the addition of recombinant adenovirus solutions; after a 5-day incubation period, the anticancer activity was then evaluated by a 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carbo xanilide assay. Each 50% inhibitory concentration was calculated based on the dose-response curves. The agents showing a high degree of effectiveness on NCI-H157 cells were cisplatin (CDDP), 5-fluorouracil (5-FU), bleomycin, and 7-ethyl-10-hydroxy-camptothecin (SN-38), an active metabolite of irinotecan (CPT-11); conversely, cyclophosphamide and paclitaxel showed a low degree of effectiveness. Based on these data, an isobologram was performed to investigate the interaction between AdCAp53 and some anticancer agents. A supra-additive effect was thus observed for 5-FU and SN-38 on NCI-H157 cells. An additive effect was also observed for CDDP, paclitaxel, bleomycin, and cyclophosphamide on NCI-H157 cells. CDDP, paclitaxel, 5-FU, and SN-38 had an additive effect on NCI-H1299 cells. No drug showed any subadditive or protective effects. These findings suggest that CPT-11 and 5-FU may thus be useful as possible anticancer agents for use in a combination therapy regimen using wt p53 gene transfer. CDDP and CPT-11 had a significant antitumoral effect on H157 cell xenografts of nude mice in vivo. These results indicate that CPT-11 as well as CDDP would be a candidate for the combination of chemotherapy and gene therapy for non-small cell lung cancer.

Peters, G., V. Fantl, et al. (1995). "Chromosome 11q13 markers and D-type cyclins in breast cancer." Breast Cancer Res Treat **33**(2): 125-35.

One in six primary human breast cancers has DNA amplification centered on the cyclin D1 gene (CCND1) on chromosome 11q13. This genetic abnormality is preferentially associated with estrogen-receptor positive tumors and may define a sub-class of patients with an adverse prognosis. Although CCND1 has the credentials of a cellular oncogene, being a target for chromosomal translocation and retroviral integration, the 11q13 amplicon encompasses several other markers and CCND1 is not the only candidate for the key gene on the amplified DNA. To assess their relative importance, we have constructed a physical map of the amplified DNA and compared the extent and frequency of amplification across the region. Since it is likely that the gene providing the selective force for amplification will be expressed at elevated levels, we have also examined expression of both RNA and protein. By these criteria, cyclin D1 remains the strongest candidate for the key oncogene on the amplicon and we are currently investigating the functional consequences of its over-expression.

Peters, G. J., B. van Triest, et al. (2000). "Molecular downstream events and induction of thymidylate synthase in mutant and wild-type p53 colon cancer cell lines after treatment with 5-fluorouracil and the thymidylate synthase inhibitor raltitrexed." Eur J Cancer **36**(7): 916-24.

Inhibition of the key enzyme in DNA synthesis, thymidylate synthase (TS), by 5-fluorouracil (5-FU) and the novel antifolate raltitrexed (Tomudex; ZD1694), induces dTTP depletion, resulting in DNA strand breaks, which can initiate pathways leading to an apoptotic mode of cell death. We studied 5-FU- and ZD1694-induced TS inhibition in relation to the expression of p53, p21, Bcl-2 and Bax in six colon carcinoma cell lines, two with a wild-type (wt) p53 (Lovo, LS174T) and four with a mutant (mt) p53 (WiDr, WiDr/F, HT29 and SW948) phenotype. In untreated cells, a reciprocal correlation between p53 and Bcl-2 was found: in cells with a low wt p53, Bcl-2 expression was present; whilst in cells with mt p53, Bcl-2 expression was not detectable. Exposure to 5-FU (50 and 100 microM) and ZD1694 (50 and 100 nM) for 24 and 48 h induced p53 and p21 expression in wt p53 cells, but not in mt p53 cells. TS was induced approximately 2-10-fold in all cell lines. TS induction was highest after ZD1694 exposure in the mt p53 cells HT29 and WiDr/F (6-10-fold). After 5-FU treatment, TS was present both as the free enzyme and in the ternary complex; however, predominantly as the ternary complex between TS, FdUMP and 5,10-methylenetetrahydrofolate. In wt p53 cells, both drugs increased Bax expression up to 5-fold, whereas in mt p53 cells, only a very slight induction was found. In wt p53 cells, Bcl-2 expression hardly changed after drug treatment. These results indicate a p53-independent induction of TS but a regulatory role of wt p53 in the synthesis of Bax in the colon carcinoma cell lines after TS inhibition.

Pledgie-Tracy, A., M. D. Sobolewski, et al. (2007). "Sulforaphane induces cell type-specific apoptosis in human breast cancer cell lines." Mol Cancer Ther **6**(3): 1013-21.

Sulforaphane, an isothiocyanate found in cruciferous vegetables, has been shown to induce phase 2 detoxication enzymes and inhibit the growth of chemically induced mammary tumors in rats, although the exact mechanisms of action of sulforaphane are not understood. In this study, we evaluated the effects of sulforaphane on cell growth and death in several human breast cancer cell lines and examined the hypothesis that sulforaphane acts as a histone deacetylase (HDAC) inhibitor in these cell lines. Sulforaphane treatment inhibited cell growth, induced a G(2)-M cell cycle block, increased expression of cyclin B1, and induced oligonucleosomal DNA fragmentation in the four human breast cancer cell lines examined, MDA-MB-231, MDA-MB-468, MCF-7, and T47D cells. Activation of apoptosis by sulforaphane in MDA-MB-231 cells seemed to be initiated through induction of Fas ligand, which resulted in activation of caspase-8, caspase-3, and poly(ADP-ribose) polymerase, whereas apoptosis in the other breast cancer cell lines was initiated by decreased Bcl-2 expression, release of cytochrome c into the cytosol, activation of caspase-9 and caspase-3, but not caspase-8, and poly(ADP-ribose) polymerase cleavage. Sulforaphane inhibited HDAC activity and decreased the expression of estrogen receptor-alpha, epidermal growth factor receptor, and human epidermal growth factor receptor-2 in each cell line, although no change in the acetylation of H3 or H4 was seen. These data suggest that sulforaphane inhibits cell growth, activates apoptosis, inhibits HDAC activity, and decreases the expression of key proteins involved in breast cancer proliferation in human breast cancer cells. These results support testing sulforaphane in vivo and warrant future studies examining the clinical potential of sulforaphane in human breast cancer.

Prabha, S. and V. Labhasetwar (2004). "Nanoparticle-mediated wild-type p53 gene delivery results in sustained antiproliferative activity in breast cancer cells." Mol Pharm **1**(3): 211-9.

Gene expression with nonviral vectors is usually transient and lasts for only a few days. Therefore, repeated injection of the expression vector is required to maintain a therapeutic protein concentration in the target tissue. Biodegradable nanoparticles (approximately 200 nm diameter) formulated using a biocompatible polymer, poly(D,L-lactide-co-glycolide) (PLGA), have the potential for sustained gene delivery. Our hypothesis is that nanoparticle-mediated gene delivery would result in sustained gene expression, and hence better efficacy with a therapeutic gene. In this study, we have determined the antiproliferative activity of wild-type (wt) p53 gene-loaded nanoparticles in a breast cancer cell line. Nanoparticles containing plasmid DNA were formulated using a multiple-emulsion-solvent evaporation technique. To understand the mechanism of sustained gene expression with nanoparticles, we monitored the intracellular trafficking of both the nanoparticles and the nanoparticle-entrapped DNA, and also determined p53 mRNA levels over a period of time. Cells transfected with wt-p53 DNA-loaded nanoparticles demonstrated a sustained and significantly greater antiproliferative effect than those with naked wt-p53 DNA or wt-p53 DNA complexed with a commercially available transfecting agent

(Lipofectamine). Cells transfected with wt-p53 DNA-loaded nanoparticles demonstrated sustained p53 mRNA levels compared to cells which were transfected with naked wt-p53 DNA or the wt-p53 DNA-Lipofectamine complex, thus explaining the sustained antiproliferative activity of nanoparticles. Studies with fluorescently labeled DNA using confocal microscopy and quantitative analyses using a microplate reader demonstrated sustained intracellular localization of DNA with nanoparticles, suggesting the slow release of DNA from nanoparticles localized inside the cells. Cells which were transfected with naked DNA demonstrated transient intracellular DNA retention. In conclusion, nanoparticle-mediated wt-p53 gene delivery results in sustained antiproliferative activity, which could be therapeutically beneficial in cancer treatment.

Preto, A., S. K. Singhrao, et al. (2004). "Telomere erosion triggers growth arrest but not cell death in human cancer cells retaining wild-type p53: implications for antitelomerase therapy." Oncogene **23**(23): 4136-45.

Telomerase activity in tumours is often associated with p53 mutation. Many antitelomerase therapies take advantage of the inability of cells expressing mutant p53 to undergo replicative senescence, since this allows telomere erosion to continue until 'crisis', hence providing the desired cytotoxic effect. However, some tumour types, including breast, melanomas and thyroid, retain wild-type p53 function and the effectiveness of antitelomerase therapies in such tumour cells have not been adequately addressed. To explore this, we made use of two thyroid cancer cell lines K1 and K2, which retain wt p53. Telomere erosion induced by the expression of a dominant-negative (DN) hTERT resulted in delayed onset of growth arrest in K1 and K2 cells, reminiscent of replicative senescence, with low levels of BrdU labelling and apoptosis, associated with high p21(WAF1) and senescence-associated beta galactosidase expression. In contrast, abrogation of p53 function by the expression of HPV16 E6 in K1 and K2 cells either at the same time as DNhTERT or just prior to the onset of senescence allowed cells to continue growing until 'crisis'. Likewise, microinjection of a p53 neutralizing antibody into 'senescent' K1 DNhTERT cells permitted re-entry into the cell cycle. We conclude that thyroid tumour cells with wild-type p53 retain an intact p53-mediated growth arrest response to telomere erosion. This raises the intriguing question of why, therefore, p53 mutation is not selected for in such cancers, and also calls into question the therapeutic value of telomerase inhibitors in such cases.

Putzer, B. M., J. L. Bramson, et al. (1998). "Combination therapy with interleukin-2 and wild-type p53 expressed by adenoviral vectors potentiates tumor regression in a murine model of breast cancer." Hum Gene Ther **9**(5): 707-18.

Although cytokine gene transfer for cancer treatment can stimulate immune recognition and tumor regression in animal models, there is still a need for improvements to these strategies. In this study, we examined the efficacy of a combination gene therapy using adenovirus (Ad) 5 vectors expressing human interleukin-2 and the wild-type (wt) human p53 gene under control of the human cytomegalovirus immediate early promoter (AdIL-2 and Adp53wt, respectively). Infected murine cell lines and primary mouse tumor cells secreted high levels of IL-2 and over expressed the p53 protein for at least 9 days. After infection of cells with Adp53wt, DNA synthesis was significantly inhibited and apoptosis was induced within 3-5 days. Both vectors were tested in a transgenic mouse mammary adenocarcinoma model for antitumor response. Following a single intratumoral injection of mice bearing PyMT induced tumors, the combination of Adp53wt (1 x 10(9) pfu) plus a relatively low dose of AdIL-2 (1.5 x 10(8) pfu) caused regressions in 65% of the treated tumors without toxicity. Fifty percent of the treated mice remained tumor free and were immune to rechallenge with fresh tumor cells. In contrast, injection of either vector alone at this does resulted in only a delay in tumor growth. Only mice co-injected with Adp53wt and AdIL-2 showed specific antitumor cytolytic T lymphocyte (CTL) activity, indicating that the immune response involved in tumor regression was promoted by the combination therapy. These results suggest that cancer treatment strategies involving combined delivery of immunomodulatory and antiproliferative genes may be highly effective.

Raigoso, P., A. Junco, et al. (2000). "Tissue-type plasminogen activator (tPA) content in colorectal cancer and in surrounding mucosa: relationship with clinicopathologic parameters and prognostic significance." Int J Biol Markers **15**(1): 44-50.

The aim of this study was to evaluate the cytosolic tissue-type plasminogen activator (tPA) content in colorectal cancer, its possible relationship with the clinicopathologic parameters of tumors, and its prognostic significance. We have therefore examined by immunoenzymatic assay the cytosolic tPA content in tumors and paired surrounding normal mucosa samples from 162 colorectal cancer patients. Cytosolic tPA levels were significantly higher in surrounding normal mucosa samples than in neoplastic tissues (4.01 +/- 5.07 vs 2.63 +/- 5.82 ng/mg protein; $p < 0.0001$). By contrast, no significant

correlation was found between tPA content and clinicopathologic tumor parameters such as location, Dukes' stage, histologic grade, and DNA content or S-phase fraction. However, the results indicated that a high cytosolic tPA content (> 0.75 ng/mg protein) in tumors predicted for a shorter relapse-free and overall survival (both $p < 0.05$) in 123 resectable colorectal cancer patients who were prospectively evaluated during a mean follow-up period of 32.2 months. This suggests that tPA may give additional information to that provided by other biochemical markers currently used in colorectal cancer.

Russo, A., V. Bazan, et al. (2005). "The TP53 colorectal cancer international collaborative study on the prognostic and predictive significance of p53 mutation: influence of tumor site, type of mutation, and adjuvant treatment." J Clin Oncol **23**(30): 7518-28.

PURPOSE: The aims of the TP53 Colorectal Cancer (CRC) International Collaborative Study were to evaluate the possible associations between specific TP53 mutations and tumor site, and to evaluate the prognostic and predictive significance of these mutations in different site, stage, and treatment subgroups. PATIENTS AND METHODS: A total of 3,583 CRC patients from 25 different research groups in 17 countries were recruited to the study. Patients were divided into three groups according to site of the primary tumor. TP53 mutational analyses spanned exons 4 to 8. RESULTS: TP53 mutations were found in 34% of the proximal colon tumors and in 45% of the distal colon and rectal tumors. They were associated with lymphatic invasion in proximal tumors. In distal colon tumors, deletions causing loss of amino acids were associated with worse survival. In proximal colon tumors, mutations in exon 5 showed a trend toward statistical significance ($P < .05$) when overall survival was considered. Dukes' C tumors with wild-type TP53 and those with mutated TP53 (proximal tumors) showed significantly better prognosis when treated with adjuvant chemotherapy. CONCLUSION: Analysis of TP53 mutations from a large cohort of CRC patients has identified tumor site, type of mutation, and adjuvant treatment as important factors in determining the prognostic significance of this genetic alteration.

Rutter, J. L., U. Benbow, et al. (1997). "Cell-type specific regulation of human interstitial collagenase-1 gene expression by interleukin-1 beta (IL-1 beta) in human fibroblasts and BC-8701 breast cancer cells." J Cell Biochem **66**(3): 322-36.

Interleukin-1 beta (IL-1 beta) is a potent cytokine that stimulates interstitial collagenase-1 (matrix metalloproteinase-1; MMP-1). In this study, we compared the mechanism(s) by which IL-1 beta induces collagenase gene expression in two very different cells, normal human foreskin fibroblasts (HFFs) and an aggressive breast cancer cell line, BC-8701 cells. Northern analysis showed that the time course of collagenase induction was distinct in the two cells: although both cells expressed low levels of MMP-1 constitutively, addition of IL-1 beta increased MMP-1 mRNA in HFFs by 1 h and levels remained high over a 24-h period. In contrast, MMP-1 levels in IL-1 beta-treated BC-8701 cells did not increase until 4 h, peaked by 12 h and then declined. To analyze the transcriptional response, we cloned and sequenced more than 4,300 bp of the human MMP-1 promoter, and from this promoter clone, we prepared a series of 5'-deletion constructs linked to the luciferase reporter and transiently transfected these constructs into both cell types to measure both basal and IL-1 beta induced transcription. When both cell types were uninduced, promoter fragments containing less than 2,900 bp gave only a minimal transcriptional response, while larger fragments showed increased transcriptional activity. With IL-1 beta treatment, significant responsiveness ($P < 0.001$) in HFFs was seen only with the larger fragments, while in the BC-8701 cells, all fragments were significantly induced with IL-1 beta. Finally, we found that IL-1 beta stabilized MMP-1 mRNA in normal fibroblasts, but not in BC-8701 breast cancer cells. We conclude that both the transcriptional and post-transcriptional regulation of MMP-1 gene expression by IL-1 beta is controlled by cell-type specific mechanisms, and we suggest that IL-1 induced MMP-1 expression in tumor cells and in neighboring stromal cells may amplify the invasive ability of tumor cells.

Salatino, M., R. Schillaci, et al. (2004). "Inhibition of in vivo breast cancer growth by antisense oligodeoxynucleotides to type I insulin-like growth factor receptor mRNA involves inactivation of ErbBs, PI-3K/Akt and p42/p44 MAPK signaling pathways but not modulation of progesterone receptor activity." Oncogene **23**(30): 5161-74.

The present study addresses the effect of targeting type I insulin-like growth factor receptor (IGF-IR) with antisense strategies in in vivo growth of breast cancer cells. Our research was carried out on C4HD tumors from an experimental model of hormonal carcinogenesis in which the synthetic progestin medroxyprogesterone acetate (MPA) induced mammary adenocarcinomas in Balb/c mice. We employed two different experimental strategies. With the first one we demonstrated that direct intratumor injection of phosphorothioate antisense oligodeoxynucleotides (AS[S]ODNs) to IGF-IR mRNA resulted in a significant inhibition of C4HD

tumor growth. In the second experimental strategy, we assessed the effect of intravenous (i.v.) injection of AS [S]ODN on C4HD tumor growth. This systemic treatment also resulted in significant reduction in tumor growth. The antitumor effect of IGF-IR AS[S]ODNs in both experimental protocols was due to a specific antisense mechanism, since growth inhibition was dose-dependent and no abrogation of tumor proliferation was observed in mice treated with phosphorothioate sense ODNs (S[S]ODNs). In addition, IGF-IR expression was inhibited in tumors from mice receiving AS[S]ODNs, as compared to tumors from control groups. We then investigated signal transduction pathways modulated in vivo by AS[S]ODNs treatment. Tumors from AS[S]ODN-treated mice of both intratumoral and intravenous protocols showed a significant decrease in the degree of insulin receptor substrate-1 (IRS-1) tyrosine phosphorylation. Activation of two of the main IGF-IR signaling pathways, phosphatidylinositol 3-kinase (PI-3K)/Akt and p42/p44 mitogen-activated protein kinases (MAPK) was abolished in tumors growing in AS[S]ODN-treated animals. Moreover, ErbB-2 tyrosine phosphorylation was blocked by in vivo administration of AS[S]ODNs. On the other hand, we found no regulation of either progesterone receptor expression or activity by in vivo AS[S]ODNs administration. Our results for the first time demonstrated that breast cancer growth can be inhibited by direct in vivo administration of IGF-IR AS[S]ODNs.

Santin, A. D., S. Bellone, et al. (2008). "Human papillomavirus type 16 and 18 E7-pulsed dendritic cell vaccination of stage IB or IIA cervical cancer patients: a phase I escalating-dose trial." J Virol **82**(4): 1968-79.

The safety and immunogenicity of the human papillomavirus type 16 (HPV16) or HPV18 (HPV16/18) E7 antigen-pulsed mature dendritic cell (DC) vaccination were evaluated for patients with stage IB or IIA cervical cancer. Escalating doses of autologous DC (5, 10, and 15 x 10(6) cells for injection) were pulsed with recombinant HPV16/18 E7 antigens and keyhole limpet hemocyanin (KLH; an immunological tracer molecule) and delivered in five subcutaneous injections at 21-day intervals to 10 cervical cancer patients with no evidence of disease after they underwent radical surgery. Safety, toxicity, delayed-type hypersensitivity (DTH) reaction, and induction of serological and cellular immunity against HPV16/18 E7 and KLH were monitored. DC vaccination was well tolerated, and no significant toxicities were recorded. All patients developed CD4(+) T-cell and antibody responses to DC vaccination, as detected by enzyme-linked immunosorbent spot (ELISpot) and enzyme-linked immunosorbent assays (ELISA), respectively, and 8 out of 10 patients demonstrated levels of E7-specific CD8(+) T-cell counts, detected by ELISpot during or immediately after immunization, that were increased compared to prevaccination baseline levels. The vaccine dose did not predict the magnitude of the antibody or T-cell response or the time to detection of HPV16/18 E7-specific immunity. DTH responses to intradermal injections of HPV E7 antigen and KLH were detected for all patients after vaccination. We conclude that HPV E7-loaded DC vaccination is safe and immunogenic for stage IB or IIA cervical cancer patients. Phase II E7-pulsed DC-based vaccination trials with cervical cancer patients harboring a limited tumor burden, or who are at significant risk of tumor recurrence, are warranted.

Schaber, J. D., H. Fang, et al. (1998). "Prolactin activates Stat1 but does not antagonize Stat1 activation and growth inhibition by type I interferons in human breast cancer cells." Cancer Res **58**(9): 1914-9.

Type I interferons (IFN alpha and IFN beta) are presently used in the adjuvant treatment of several human cancers. However, these cytokines have demonstrated only modest success in breast cancer therapy, and research efforts have focused on improving their efficacy. Recent progress in understanding the molecular mechanisms underlying the antiproliferative effects of IFNs has identified the cytoplasmic transcription factor Stat1 as a critical mediator. It is, therefore, possible that IFN-induced growth inhibition of mammary epithelial cells is counteracted by other cytokines that also use Stat1. One such candidate IFN-antagonist with particular relevance to breast cancer is the mammotropic hormone prolactin (PRL). The main goal of this study was to examine whether PRL would interfere with type I IFN (IFN alpha/beta) signal transduction by competing for limited cytoplasmic Stat factors. A second aim was to test whether pretreatment of mammary tumor cell lines with IFN gamma could enhance the effect of IFN alpha/beta. By analyzing the effect of PRL on IFN alpha/beta-induced tyrosine phosphorylation of Stat proteins and their binding to IFN-regulated genes, we now report that costimulation of PRL receptors did not interfere with IFN alpha/beta signals in several human breast cancer cell lines, including T47D, MCF-7, and BT-20. Specifically, PRL did not affect IFN alpha/beta-induced tyrosine phosphorylation or heterodimerization of Stat1 and Stat2 in any cell line. Instead, IFN alpha/beta- and PRL-induced tyrosine phosphorylation of Stat1 was additive and occurred without evidence of competition for limited

concentrations of cytoplasmic Stat1. A similar additive relationship was observed on IFN alpha/beta- and PRL-induced Stat3 tyrosine phosphorylation. Furthermore, electrophoretic mobility shift assays showed that type I IFNs induced predominantly Stat1-Stat2 or Stat1-Stat3 heteromeric complexes with various IFN-response elements of IFN-stimulated genes, whereas PRL induced Stat1 homodimers. Despite significant mutual use of Stats by IFNs and PRL, these results indicated a high degree of signaling specificity in the two receptor systems, and that cytoplasmic levels of Stat proteins were not limiting. Similarly, PRL did not interfere with the growth-inhibitory effect of IFN beta. On the other hand, the study indicated that pretreatment of human breast cancer cell lines with IFN gamma enhanced the growth-inhibitory action of type I IFNs, suggesting a possible avenue for improving the effect of type I IFNs in the treatment of breast cancer patients.

Schatten, H., M. Ripple, et al. (2000). "Androgen and taxol cause cell type-specific alterations of centrosome and DNA organization in androgen-responsive LNCaP and androgen-independent DU145 prostate cancer cells." J Cell Biochem **76**(3): 463-77.

We investigated the effects of androgen and taxol on the androgen-responsive LNCaP and androgen-independent DU145 prostate cancer cell lines. Cells were treated for 48 and 72 h with 0.05-1 nM of the synthetic androgen R1881 and with 100 nM taxol. Treatment of LNCaP cells with 0.05 nM R1881 led to increased cell proliferation, whereas treatment with 1 nM R1881 resulted in inhibited cell division, DNA cycle arrest, and altered centrosome organization. After treatment with 1 nM R1881, chromatin became clustered, nuclear envelopes convoluted, and mitochondria accumulated around the nucleus. Immunofluorescence microscopy with antibodies to centrosomes showed altered centrosome structure. Although centrosomes were closely associated with the nucleus in untreated cells, they dispersed into the cytoplasm after treatment with 1 nM R1881. Microtubules were only faintly detected in 1 nM R1881-treated LNCaP cells. The effects of taxol included microtubule bundling and altered mitochondria morphology, but not DNA organization. As expected, the androgen-independent prostate cancer cell line DU145 was not affected by R1881. Treatment with taxol resulted in bundling of microtubules in both cell lines. Additional taxol effects were seen in DU145 cells with micronucleation of DNA, an indication of apoptosis. Simultaneous treatment with R1881 and taxol had no additional effects on LNCaP or DU145 cells. These results suggest that LNCaP and DU145 prostate cancer cells show differences not only in androgen responsiveness but in sensitivity to taxol as well.

Schuler, M., C. Rochlitz, et al. (1998). "A phase I study of adenovirus-mediated wild-type p53 gene transfer in patients with advanced non-small cell lung cancer." Hum Gene Ther **9**(14): 2075-82.

Mutations of the tumor suppressor gene p53 are the most common genetic alterations observed in human cancer. Loss of wild-type p53 function impairs cell cycle arrest as well as repair mechanisms involved in response to DNA damage. Further, apoptotic pathways as induced by radio- or chemotherapy are also abrogated. Gene transfer of wild-type p53 was shown to reverse these deficiencies and to induce apoptosis in vitro and in preclinical in vivo tumor models. A phase I dose escalation study of a single intratumoral injection of a replication-defective adenoviral expression vector encoding wild-type p53 was carried out in patients with incurable non-small cell lung cancer. All patients enrolled had p53 protein overexpression as a marker of mutant p53 status in pretreatment tumor biopsies. Treatment was performed either by bronchoscopic intratumoral injection or by CT-guided percutaneous intratumoral injection of the vector solution. Fifteen patients were enrolled in two centers, and were treated at four different dose levels ranging from 10(7) to 10(10) PFU (7.5 x 10(9) to 7.5 x 10(12) particles). No clinically significant toxicity was observed. Successful transfer of wild-type p53 was achieved only with higher vector doses. Vector-specific wild-type p53 RNA sequences could be demonstrated in posttreatment biopsies of six patients. Transient local disease control by a single intratumoral injection of the vector solution was observed in four of those six successfully transduced patients. There was no evidence of clinical responses at untreated tumor sites. Wild-type p53 gene therapy by intratumoral injection of a replication-defective adenoviral expression vector is safe, feasible, and biologically effective in patients with advanced non-small cell lung cancer.

Seth, P., D. Katayose, et al. (1997). "A recombinant adenovirus expressing wild type p53 induces apoptosis in drug-resistant human breast cancer cells: a gene therapy approach for drug-resistant cancers." Cancer Gene Ther **4**(6): 383-90.

The cytotoxicity of a recombinant adenovirus expressing the wild type tumor suppressor gene p53 (AdWTp53) was studied in two human breast cancer MCF-7 sublines selected for resistance to adriamycin (MCF-Adr) and mitoxantrone (MCF-Mito). Although the levels of wild type p53 protein following infection with AdWTp53 are comparable in all cell lines, the two drug-resistant MCF-7 sublines were 300- and 18-

fold more sensitive to killing by AdWTp53 compared with the drug-sensitive parental MCF-7 cell lines. In each cell line, AdWTp53 infection led to cell cycle arrest, and reduction of Cdk2 and cyclin B1-Cdc2 activity. Nucleosomal DNA fragmentation analysis (as a function of apoptosis) following AdWTp53 infection revealed that, while the parental MCF-7 cells failed to undergo apoptosis, both drug-resistant cell lines showed distinct DNA laddering. In MCF-Adr cells, a combination treatment of AdWTp53 and adriamycin was much more toxic than either of the reagents used individually. Finally, exposure of a mixed population of MCF-Adr and CD34+ cells to AdWTp53 selectively prevented MCF-Adr cell colony formation, while there was no inhibition of CFU-GM colony formation from CD34+ cells. These findings suggest that some drug-resistant human breast cancers may be effectively treated with adenovirus expressing wild type p53.

Sharma, K. and C. B. Srikant (1998). "Induction of wild-type p53, Bax, and acidic endonuclease during somatostatin-signaled apoptosis in MCF-7 human breast cancer cells." Int J Cancer **76**(2): 259-66.

Somatostatin (SST) analogs inhibit tumor cell growth by exerting direct anti-proliferative effects with cytostatic (growth arrest) or cytotoxic (apoptosis) consequences. The SST analog SMS 201-995 (octreotide, OCT) inhibits growth of MCF-7 human breast adenocarcinoma cells, which express multiple SSTRs. Its action has been reported to result in either apoptosis or growth arrest, but the underlying mechanisms have not been elucidated in this tumor cell model. Here, we report that OCT elicits cytotoxic response in these cells, leading to apoptosis, which is associated with a rapid, time-dependent induction of wild-type p53 and an increase in Bax. There was no G1 cell-cycle arrest in these cells during OCT treatment as suggested by the decrease in G1/S ratio and the lack of induction of pRb and p21. Additionally, we demonstrate that OCT-induced DNA fragmentation in this cell line is due to selective activation of a cation-insensitive acidic endonuclease. Our data provide a rationale for utilizing SST analogs to treat SSTR-positive breast cancer cells expressing wild-type p53.

Steiner, M. S., Y. Zhang, et al. (2000). "Adenoviral vector containing wild-type p16 suppresses prostate cancer growth and prolongs survival by inducing cell senescence." Cancer Gene Ther **7**(3): 360-72.

It is estimated that there will be >184,500 new cases of prostate cancer and 42,000 prostate cancer deaths in the United States this year. In the majority of patients diagnosed with prostate cancer, the disease will be too advanced for cure with standard medical treatment. New therapeutic strategies against advanced prostate cancer are desperately needed. As alterations in tumor-suppressor gene p16 are common in prostate cancer, one novel approach is gene therapy using a replication-deficient, E1/E3-deleted adenovirus type 5 containing a p16 under the control of a truncated Rous sarcoma virus promoter (AdRSVp16). In vitro, PC-3 cells that had been stably transfected with p16 expression vector under the control of an inducible promoter had a 70% reduction in cell number compared with the parental and control vector-transfected PC-3 cells. Similarly, AdRSVp16 significantly inhibited the growth of PPC-1 and PC-3 prostate cancer cells in culture. Furthermore, PPC-1 tumors grown in nude mice treated by a single injection of AdRSVp16 had a marked reduction in tumor size compared with untreated control-treated or viral control-treated PPC-1 tumors. Animals bearing tumors treated with AdRSVp16 also had longer survival. Adenovirally mediated expression of transgene was detected in xenograft tumors for at least 2 weeks. Taken together, these results suggest that AdRSVp16 should be considered for prostate cancer gene therapy in human clinical trials.

Stoff-Khalili, M. A., A. A. Rivera, et al. (2005). "A human adenoviral vector with a chimeric fiber from canine adenovirus type 1 results in novel expanded tropism for cancer gene therapy." Gene Ther **12**(23): 1696-706.

The development of novel therapeutic strategies is imperative for the treatment of advanced cancers like ovarian cancer and glioma, which are resistant to most traditional treatment modalities. In this regard, adenoviral (Ad) cancer gene therapy is a promising approach. However, the gene delivery efficiency of human serotype 5 recombinant adenoviruses (Ad5) in cancer gene therapy clinical trials to date has been limited, mainly due to the paucity of the primary Ad5 receptor, the coxsackie and adenovirus receptor (CAR), on human cancer cells. To circumvent CAR deficiency, Ad5 vectors have been retargeted by creating chimeric fibers possessing the knob domains of alternate human Ad serotypes. Recently, more radical modifications based on 'xenotype' knob switching with non-human adenovirus have been exploited. Herein, we present the characterization of a novel vector derived from a recombinant Ad5 vector containing the canine adenovirus serotype 1 (CAV-1) knob (Ad5Luc1-CK1), the tropism of which has not been previously described. We compared the function of this vector with our other chimeric viruses displaying the CAV-2 knob (Ad5Luc1-CK2) and Ad3 knob (Ad5/3Luc1). Our data demonstrate that the CAV-1 knob can alter

Ad5 tropism through the use of a CAR-independent entry pathway distinct from that of both Ad5Luc1-CK2 and Ad5/3-Luc1. In fact, the gene transfer efficiency of this novel vector in ovarian cancer cell lines, and more importantly in patient ovarian cancer primary tissue slice samples, was superior relative to all other vectors applied in this study. Thus, CAV-1 knob xenotype gene transfer represents a viable means to achieve enhanced transduction of low-CAR tumors.

Sun, Y., C. Qian, et al. (2000). "Gene transfer to liver cancer cells of B7-1 plus interleukin 12 changes immunoeffector mechanisms and suppresses helper T cell type 1 cytokine production induced by interleukin 12 alone." Hum Gene Ther **11**(1): 127-38.

To investigate the cooperative effect of B7-1 and IL-12 in the induction of antitumor activity, we have developed retroviral vectors encoding human B7-1, murine IL-12, or both B7-1 and IL-12 coordinately. Murine transformed liver cells (BNL) were engineered to stably express B7-1, IL-12, or both by infection with corresponding retroviruses. No tumor was observed in 20, 75, and 95% of mice receiving, respectively, B7-1-, IL-12-, and B7-1/IL-12-modified tumor cells after 250 days of inoculation. In contrast, injection of parental BNL or BNL/Neo cells resulted in lethal tumor progression in all mice. Protection against rechallenge with parental tumor cells was observed only in mice who had rejected BNL/IL-12, but not in animals that rejected BNL/B7-1 or BNL/B7-1-IL-12. Growth of parental tumor cells was significantly delayed by simultaneous injection in a distant site of irradiated tumor cells engineered to express IL-12 or both B7-1 and IL-12 but not B7-1 alone. BNL/B7-1 and BNL/B7-1-IL-12 showed similar efficacy in these experiments. Antitumor immunity induced by B7, with or without IL-12, was found to depend mainly on CD4+ T cells with a minor contribution of a non-T cell mechanism; whereas the effect of IL-12 was dependent on CD8+ T cells and on non-T cell effectors. Immunization of mice with IL-12-modified BNL cells induced secretion of a Thl pattern of cytokines while immunization with cells expressing both IL-12 and B7-1 resulted in inhibition of IFN-gamma production. Immunization with BNL/B7-1-IL-12 cells in the presence of anti-human B7-1 MAb resulted in restoration of IFN-gamma production to the levels found in animals injected with BNL/IL-12 cells. To summarize, in our model coexpression of B7-1 and IL-12 in tumor cells does not result in improved antitumoral activity as compared with expression of IL-12 alone. This may be related to the fact that B7-1 changes the mechanisms of antitumor immunity and inhibits IFN-gamma production induced by IL-12 in vivo.

Tahara, I., K. Miyake, et al. (2007). "Systemic cancer gene therapy using adeno-associated virus type 1 vector expressing MDA-7/IL24." Mol Ther **15**(10): 1805-11.

Melanoma differentiation-associated gene-7/interleukin-24 (mda-7/IL24), selectively induces apoptosis in cancer cells without harming normal cells. It also exerts immunomodulatory and antiangiogenic effects, as well as potent antitumor bystander effects, making it an ideal candidate for a new anticancer gene therapy. Here, we examined the feasibility of adeno-associated virus type 1 (AAV1) vector-mediated systemic gene therapy using mda-7/IL24. In vitro studies showed that medium conditioned by AAV1-mda7-transducedC2C12 cells induces tumor cell-specific apoptosis and inhibits angiogenesis in a human umbilical vein endothelial cell tube formation assay. To assess the in vivo effects of AAV1-mediated systemic delivery of MDA-7/IL24, we generated a subcutaneous tumor model by injecting Ehrlich ascites tumor cells into the dorsum of DDY mice. A single intravenous injection of AAV1-mda7 (2.0 x 10(11) viral genomes) significantly inhibited tumor growth. In addition, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL), and immunohistochemical analyses showed significant induction of tumor-cell-specific apoptosis and reduction of microvessel formation within the tumors, and there was a significant increase in survival among the AAV1-mda7-treated mice. These results clearly demonstrate that continuous systemic delivery of MDA-7/IL24 can serve as an effective treatment for cancer. Thus, AAV1 vector-mediated systemic delivery of MDA-7/IL24 represents a potentially important new approach to anticancer therapy.

Thomadaki, H., A. Scorilas, et al. (2008). "The role of cordycepin in cancer treatment via induction or inhibition of apoptosis: implication of polyadenylation in a cell type specific manner." Cancer Chemother Pharmacol **61**(2): 251-65.

PURPOSE: Most anticancer drugs show their antiproliferative and cytotoxic activity via induction of apoptosis. In the present study we assessed the implication and role of cordycepin, a polyadenylation-specific inhibitor and a well-known chemotherapeutic drug, in apoptosis, induced by the anticancer drug etoposide. METHODS: For this purpose, a variety of leukemia and lymphoma cell lines (U937, K562, HL-60, Daudi, Molt-4) were treated with the anticancer drugs etoposide and/or cordycepin and assessed for poly(A) polymerase (PAP) activity and isoforms by the highly sensitive PAP activity assay and western blotting, respectively. Induction of apoptosis was determined by endonucleosomal DNA cleavage,

DAPI staining, caspase-6 activity assay and DeltaPsi m reduction, whereas cytotoxicity and cell cycle status were assessed by Trypan blue staining, MTT assay and flow cytometry. RESULTS AND CONCLUSIONS: The results showed that PAP changes in all cell lines, in response to apoptosis induced by etoposide, in many cases even prior to hallmarks of apoptosis (endonucleosomal cleavage of DNA, DeltaPsi(m) reduction). A further elucidation to this apoptosis-polyadenylation correlation was added, by cell treatment with cordycepin, resulting in either suppression (U937, K562) or induction (HL-60) of the apoptotic process, according to the cell type. However, inhibition of polyadenylation did not influence the cell lines Daudi and Molt-4 used, where alternative apoptotic pathways are induced through cleavage of DNA into high molecular weight fragments.

Thottassery, J. V., L. Westbrook, et al. (2006). "c-Abl-independent p73 stabilization during gemcitabine- or 4'-thio-beta-D-arabinofuranosylcytosine-induced apoptosis in wild-type and p53-null colorectal cancer cells." Mol Cancer Ther **5**(2): 400-10.

Nucleoside anticancer drugs like gemcitabine (2'-deoxy-2',2'-difluorocytidine) are potent inducers of p53, and ectopic expression of wild-type p53 sensitizes cells to these agents. However, it is also known that nucleosides are efficient activators of apoptosis in tumor cells that do not express a functional p53. To clarify this issue, we examined the effects of gemcitabine and 4'-thio-beta-d-arabinofuranosylcytosine (T-ara-C) on p73, a structural and functional homologue of p53, whose activation could also account for nucleoside-induced apoptosis because no functionally significant mutations of p73 have been reported in cancers. Acute treatment of HCT 116 colon carcinoma cells with gemcitabine or T-ara-C induced marked cytotoxicity and cleavage of caspase-3 and poly(ADP-ribose) polymerase. T-ara-C and gemcitabine markedly induced p53 accumulation as well as increased levels of phospho-p53 (Ser15/Ser20/Ser46) and induced its binding to a consensus p53 response element. Despite robust activation of p53 by T-ara-C and gemcitabine, we found that wild-type and p53-/- HCT 116 cells exhibited almost equivalent sensitivity towards these nucleosides. Examination of p73 revealed that T-ara-C and gemcitabine markedly increased p73 protein levels and p73 DNA-binding activities in both p53-/- and wild-type cells. Furthermore, T-ara-C- and gemcitabine-induced increases in p73 levels occur due to a decrease in p73 protein turnover. RNA interference studies show that nucleoside-induced p73 increases are independent of c-Abl, a nucleoside-activated kinase recently implicated in p73 stabilization. HCT 116 lines, wherein the downstream p53/p73 targets Bax and PUMA (p53 up-regulated modulator of apoptosis) were deleted, were less sensitive to T-ara-C and gemcitabine. Together, these studies indicate that c-Abl-independent p73 stabilization pathways could account for the p53-independent mechanisms in nucleoside-induced apoptosis.

Toita, R., J. H. Kang, et al. (2009). "Protein kinase C alpha-specific peptide substrate graft-type copolymer for cancer cell-specific gene regulation systems." J Control Release **139**(2): 133-9.

We recently proposed a novel gene regulation system responding to specifically and abnormally activated intracellular enzymes in diseased cells. In the present study, we focused on protein kinase C (PKC)alpha, which is hyper-activated in most tumor cells, as a trigger for transgene regulation. We prepared cationic copolymers comprising hydrophilic and neutral polymers in main chains and cationic peptide substrates with different contents in side chains. Our copolymer with high peptide content (>3 mol%) condensed with pDNA more weakly than with poly(L-lysine) (pLL) having a similar molecular weight, but gene suppression was nearly identical to that of pLL, probably due to the steric hindrance of the main chains in our copolymer. Steric hindrance of the main chains barely affected the phosphorylation reaction of the pendant peptide. In cell and mouse experiments, higher gene expression was observed in complexes of pDNA with copolymers pended PKC alpha-specific substrate peptide than that in complexes with negative copolymers pended peptide substituted phosphorylation site of serine residues with alanine. These results indicate that our system can recognize intracellular PKC alpha as a trigger to regulate transgene expression, and may be useful for tumor gene therapy.

Tolcher, A. W., D. Hao, et al. (2006). "Phase I, pharmacokinetic, and pharmacodynamic study of intravenously administered Ad5CMV-p53, an adenoviral vector containing the wild-type p53 gene, in patients with advanced cancer." J Clin Oncol **24**(13): 2052-8.

PURPOSE: The purpose of this study was to assess the feasibility of administering Ad5CMV-p53, an adenoviral vector containing the wild-type p53 gene to patients with advanced malignancies, characterize the pertinent pharmacokinetic parameters, identify evidence of viral uptake in both normal and tumor tissue, and seek evidence of antitumor activity. METHODS: Patients were treated

with escalating doses of Ad5CMV-p53 intravenously over 30 minutes on days 1, 2, and 3, every 28 days. The clearance of circulating Ad5CMV-p53 (INGN 201) DNA was characterized in the plasma and paired tumor and skin biopsies were performed in patients treated at the two highest dose levels to assess vector uptake into tissues. RESULTS: Seventeen patients received 36 courses of Ad5CMV-p53 at doses ranging from 3 x 10(10) to 3 x 10(12) virus particles (vp). Fatigue, nausea, vomiting, and fever were common, but rarely severe. Abnormalities of coagulation parameters, including decreases in fibrinogen and increases in fibrin degradation products at 3 x 10(12)vp, precluded additional dose escalation. Ad5CMV-p53 DNA could be detected in the plasma by polymerase chain reaction assay in the majority of patients at 14 days and 28 days at doses of 3 x 10(10) and higher. Six patients treated at 1 x 10(12)vp and 3 x 10(12)vp dose levels had Ad5CMV-p53 DNA detected within paired tumor tissue collected day 4. CONCLUSION: Ad5CMV-p53 can be safely and repetitively administered up to 1 x 10(12)vp intravenously daily for 3 consecutive days. The absence of severe toxicities, the presence of circulating adenovirus 24 hours after administration, and detectable p53 transgene within tumor tissue distant from the site of administration demonstrates that systemic therapy with this adenoviral vector containing p53 is feasible.

Tomita, S., S. Deguchi, et al. (1999). "Analyses of microsatellite instability and the transforming growth factor-beta receptor type II gene mutation in sporadic breast cancer and their correlation with clinicopathological features." Breast Cancer Res Treat **53**(1): 33-9.

To determine the incidence of microsatellite instability (MSI) and its relationship with both clinicopathologic parameters and patient survival, 101 cases of breast cancer were investigated. In addition, transforming growth factor-beta (TGF-beta) receptor type II (RII) gene mutation was also examined to clarify the relation to MSI in breast cancer development. MSI and RII gene mutation were screened by single strand conformation polymorphism (SSCP). The mutations of the RII gene were confirmed by a direct sequence. An association between the MSI status and the clinicopathological features was examined to assess the potential of the MSI status as a prognostic indicator in sporadic breast cancer cases. MSI was detected in 12 of 101 (11.9%) breast cancer cases. The positive MSI breast cancer cases showed relatively more advanced disease than negative MSI cases, and also exhibited relatively poorer prognoses. No RII gene mutations were observed in any of the breast cancer cases. Our data suggest that the MSI status may thus be a useful indicator for the prognosis of sporadic breast cancer cases. However, the breast seems to be an infrequent target organ for cancer development through RII gene mutations. As a result, tumor progression through this pathway appears to be related to organ specificity. For positive MSI breast cancers, other target genes therefore still need to be identified.

Tortora, G. and F. Ciardiello (2003). "Antisense targeting protein kinase A type I as a drug for integrated strategies of cancer therapy." Ann N Y Acad Sci **1002**: 236-43.

We have studied the role of protein kinase A (PKA) and its type I isoform (PKAI) in the transduction of mitogenic signaling, apoptosis, and angiogenesis. We have contributed to the development of selective inhibitors of PKAI, including a hybrid DNA/RNA mixed backbone oligonucleotide (AS-PKAI). We, and others, have demonstrated that AS-PKAI has a cooperative antitumor effect with a selected class of cytotoxic drugs and with radiotherapy in vitro and in vivo and that these effects can also be obtained following oral adinistration. Previously, we developed a series of therapeutic models based on the pleiotropic role played by PKAI in cell proliferation, apoptosis, and angiogenesis. On the basis of our former demonstration of functional and structural interactions of PKAI and the activated epidermal growth factor receptor (EGFR), we have shown that the combined blockade of both signaling molecules by AS-PKAI and either the monoclonal antibody C225 (erbitux) or the small molecule ZD1839 (gefitinib), results in a marked cooperative antitumor effect in a variety of human tumor models. A further cooperative antitumor effect can be obtained when AS-PKAI is used in combination with both EGFR inhibitors and either cytotoxic drugs or radiotherapy. The antitumor activity is associated with inhibition of growth factors and angiogenic factors production and to induction of apoptosis. In light of the recently demonstrated role of PKAI on the bcl-2-dependent apoptotic pathway, we have recently shown a synergistic antitumor, antiangiogenic, and proapoptotic effect of AS-PKAI in combination with antisense bcl-2 (oblimersen) or with a bispecific bcl-2/bcl-xL second generation antisense. A connection between COX-2, EGFR and PKAI was established, and we demonstrated that the combination of AS-PKAI with gefitinib and a COX-2 inhibitor, all adminstered orally, can result in a potent antitumor and antiangiogenic activity. These studies support the development of AS-PKAI as a novel anticancer agent and suggest its potentially relevant role when integrated with conventional treatments

and/or other signaling inhibitors in novel therapeutic strategies.

Troester, M. A., K. A. Hoadley, et al. (2004). "Cell-type-specific responses to chemotherapeutics in breast cancer." Cancer Res **64**(12): 4218-26.

Recent microarray studies have identified distinct subtypes of breast tumors that arise from different cell types and that show statistically significant differences in patient outcome. To gain insight into these differences, we identified in vitro and in vivo changes in gene expression induced by chemotherapeutics. We treated two cell lines derived from basal epithelium (immortalized human mammary epithelial cells) and two lines derived from luminal epithelium (MCF-7 and ZR-75-1) with chemotherapeutics used in the treatment of breast cancer and assayed for changes in gene expression using DNA microarrays. Treatment doses for doxorubicin and 5-fluorouracil were selected to cause comparable cytotoxicity across all four cell lines. The dominant expression response in each of the cell lines was a general stress response; however, distinct expression patterns were observed. Both cell types induced DNA damage-response genes such as p21(waf1), but the response in the luminal cells showed higher fold changes and included more p53-regulated genes. Luminal cell lines repressed a large number of cell cycle-regulated genes and other genes involved in cellular proliferation, whereas the basal cell lines did not. Instead, the basal cell lines repressed genes that were involved in differentiation. These in vitro responses were compared with expression responses in breast tumors sampled before and after treatment with doxorubicin or 5-fluorouracil/mitomycin C. The in vivo data corroborated the cell-type-specific responses to chemotherapeutics observed in vitro, including the induction of p21(waf1). Similarities between in vivo and in vitro responses help to identify important response mechanisms to chemotherapeutics.

Vannucchi, S., M. V. Chiantore, et al. (2007). "Perspectives in biomolecular therapeutic intervention in cancer: from the early to the new strategies with type I interferons." Curr Med Chem **14**(6): 667-79.

Interferon (IFN) was the first cytokine produced by recombinant DNA technology used in wide-spread clinical treatment of infectious diseases as well as malignancies. The IFN clinical potential was clearly realized from the outset. However, IFN represents one of the most controversial drugs of our time, as remarkable cycles of promise and disappointment have affected its development and use. Considerable evidence regarding anti-tumor activities of IFNs has been reported. In this paper we focus on molecular bases of the IFN system that may relate to its antitumor activities. Many of the numerous genes transcriptionally activated by IFNs have been shown to encode proteins that activate immune recognition of tumor cells, directly or indirectly exert tumor suppressor activity and/or control tumor cell cycle and programmed cell death. In addition, a physiological relevant function for endogenous type I IFN in cancer immunoediting process and a new way to IFN clinical use based on gene therapy or vaccine-like approaches have recently been suggested. The identification of selected tissue-specific and/or tumor-specific target pathways as well as of different type I IFN tumor escape and resistance mechanisms may provide novel approaches in the search for new IFN-based therapeutic strategies to circumvent cancer disease or improve clinical outcome. Promising IFN treatment has been recently defined by using novel pharmaceutical preparations with a more favourable pharmacokinetic response, also in combination with other bioreagents or other modalities of therapy. Translational research, linking both basic and clinical research, will lead to a new rationale for the use of IFN in cancer therapy.

Vernon, S. D., E. R. Unger, et al. (1997). "Association of human papillomavirus type 16 integration in the E2 gene with poor disease-free survival from cervical cancer." Int J Cancer **74**(1): 50-6.

To determine the clinical relevance of human papillomavirus (HPV) integration and E2 function suggested by in vitro studies, we investigated 50 patients with HPV 16-positive primary cervical carcinoma (stage Ib-IV) diagnosed and treated at one institution. The physical state of HPV was determined by colorimetric in situ hybridization and was not found to vary by stage. Overall, 62% of tumors had integrated HPV, 16% had episomal and 22% had both integrated and episomal. The E1/E2 region was evaluated by 8 separate polymerase chain reactions, which resulted in overlapping products. There was no significant variation in ability to amplify the E1/E2 region with stage. E1/E2 amplification correlated with physical state. Nearly all tumors with episomal or mixed HPV 16 DNA amplified all 8 E1/E2 fragments. Half of the tumors with integrated HPV 16 DNA failed to amplify one or more E1/E2 fragments. Disruptions were most frequent in the E2 region. For all 46 patients receiving curative therapy, the Kaplan-Meier estimate of disease-free survival was determined for those whose primary tumors had amplifiable E2 compared with those lacking one or more E2 DNA fragments. Disruption of E2 was associated with significantly shortened disease-free survival.

Vijayalingam, S., T. Subramanian, et al. (2009). "Down-regulation of multiple cell survival proteins in head and neck cancer cells by an apoptogenic mutant of adenovirus type 5." Virology **392**(1): 62-72.

Head and neck squamous cell carcinomas (HNSCC) are one of the leading causes of cancer deaths world wide. Up-regulation of the epidermal growth factor receptor (EGFR) and BCL-2 family anti-apoptosis proteins in these cancers is linked to aggressive tumor growth, metastasis and chemoresistance. Infection of two HNSCC cell lines, SCC25 and CAL27 by an Ad5 mutant (lp11w) defective in coding for the viral anti-apoptosis protein, E1B-19K efficiently induced apoptotic cell death. In cells infected with lp11w there was a dramatic down-regulation of EGFR by apoptosis-dependent and -independent mechanisms. The levels of the anti-apoptotic proteins BCL-2, BCL-xL and MCL-1 were also down-regulated in lp11w-infected cells compared to uninfected or Ad5-RM infected cells. Infection with lp11w also enhanced sensitivity of the HNSCC cells to the chemotherapeutic drug cisplatin. Our results suggest that adenoviral vectors defective in E1B-19K would be valuable for efficient down-regulation of cell survival proteins and EGFR in epithelial cancers and could be exploited as oncolytic agents to treat HNSCCs.

Vikhanskaya, F., C. Falugi, et al. (2002). "Human papillomavirus type 16 E6-enhanced susceptibility to apoptosis induced by TNF in A2780 human ovarian cancer cell line." Int J Cancer **97**(6): 732-9.

In our study, we show that expression of HPV-16 E6 sensitizes TNF-induced cytotoxicity of human ovarian cancer cell line A2780. This effect is not related to a different number of TNF receptors present on cell membrane. The major induction of massive apoptosis induced by TNF is not p53- and p21(waf-1)-dependent but it is principally related to NF-kappaB inhibition in A2780/E6 cells. Consistently to NF-kappaB inhibition a rapidly release of cytochrome c and severe induction of DNA fragmentation are seen in A2780/E6 cells. Also in human colon cancer cell line HCT-116/E6 the expression of HPV-16 E6 enhances TNF-cytotoxicity. This effect is not present in the HCT-116/mu-p53 clone (transfected with a dominant-negative mutated p53 transgene). Thus, taken together all these observations suggest that HPV-16 E6 sensitizes A2780 and HCT-116 cells to TNF; this effect is not p53-dependent, but it is essentially mediated through an inhibition in activating NF-kappaB activities.

Vitale, G., C. H. van Eijck, et al. (2007). "Type I interferons in the treatment of pancreatic cancer: mechanisms of action and role of related receptors." Ann Surg **246**(2): 259-68.

OBJECTIVE: We evaluated the role of type I interferons (IFNs) and IFN receptors in the regulation of cell growth in 3 human pancreatic adenocarcinoma cell lines (BxPC-3, MiaPaCa-2, and Panc-1). BACKGROUND: Chemotherapy and radiotherapy have a marginal role in the management of pancreatic adenocarcinoma. The addition of IFN-alpha showed promising results in early clinical trials. METHODS: Cell proliferation and apoptosis were evaluated by DNA measurement and DNA fragmentation, respectively. Type I IFN receptor (IFNAR-1 and IFNAR-2 subunits) was determined by quantitative RT-PCR and immunocytochemistry. Cell cycle distribution was evaluated by propidium iodide staining and flow-cytometric analysis. RESULTS: The incubation with IFN-beta for 6 days showed a potent inhibitory effect on the proliferation of BxPC-3 (IC(50), 14 IU/mL) and MiaPaCa-2 (IC(50), 64 IU/mL). The inhibitory effect of IFN-beta was stronger than IFN-alpha in all 3 cell lines and mainly modulated by the stimulation of apoptosis, although cell cycle arrest was induced as well. The expression of the type I IFN receptors was significantly higher in BxPC-3 (the most sensitive cell line to IFN) and mainly localized on the membrane, whereas in Panc-1 (the most resistant cell line) about 60% to 70% of cells were negative for IFNAR-2c with a mainly cytoplasmic staining for IFNAR-2c. CONCLUSION: The antitumor activity of IFN-beta is more potent than IFN-alpha in pancreatic cancer cell lines through the induction of apoptosis. Further studies should investigate in vivo whether the intensity and distribution of IFNAR-1 and IFNAR-2c may predict the response to therapy with IFN-alpha and IFN-beta in pancreatic cancer.

Watanabe, T. and B. A. Sullenger (2000). "Induction of wild-type p53 activity in human cancer cells by ribozymes that repair mutant p53 transcripts." Proc Natl Acad Sci U S A **97**(15): 8490-4.

Several groups have attempted to develop gene therapy strategies to treat cancer via introduction of the wild-type (wt) p53 cDNA into cancer cells. Unfortunately, these approaches do not result in regulated expression of the p53 gene and do not reduce expression of the mutant p53 that is overexpressed in cancerous cells. These shortcomings may greatly limit the utility of this gene replacement approach. We describe an alternative strategy with trans-splicing ribozymes that can simultaneously reduce mutant p53 expression and restore wt p53 activity in various human cancers. The ribozyme accomplished such conversion by repairing defective p53 mRNAs with high fidelity and specificity. The

corrected transcripts were translated to produce functional p53 that can transactivate p53-responsive promoters and down-modulate expression of the multidrug resistance (MDR1) gene promoter. The level of wt p53 activity generated was significant, resulting in a 23-fold induction of a p53-responsive promoter and a 3-fold reduction in MDR1 promoter expression in transfected cancer cells. Once efficient delivery systems are developed, this strategy should prove useful for making human cancers more responsive to p53 activity and more sensitive to chemotherapeutic agents.

Wiman, K. G. (2007). "Restoration of wild-type p53 function in human tumors: strategies for efficient cancer therapy." Adv Cancer Res **97**: 321-38.

The p53 tumor suppressor gene is mutated in around 50% of all human tumors. Most mutations inactivate p53's specific DNA binding, resulting in failure to activate transcription of p53 target genes. As a consequence, mutant p53 is unable to trigger a p53-dependent biological response, that is cell cycle arrest and apoptosis. Many tumors express high levels of nonfunctional mutant p53. Several strategies for restoration of wild-type p53 function in tumors have been designed. Wild-type p53 reconstitution by adenovirus-mediated gene transfer has shown antitumor efficacy in clinical trials. Screening of chemical libraries has allowed identification of small molecules that reactivate mutant p53 and trigger mutant p53-dependent apoptosis. These novel strategies raise hopes for more efficient cancer therapy.

Wu, H., S. Wang, et al. (2008). "Reversal of the malignant phenotype of ovarian cancer A2780 cells through transfection with wild-type PTEN gene." Cancer Lett **271**(2): 205-14.

OBJECTIVE: PTEN (phosphatase and tensin homologue deleted on chromosome 10) is a tumor suppressor gene identified on human chromosome 10q23. Substantial studies have demonstrated that PTEN can inhibit cell proliferation, migration and invasion of many cancer cells. The purpose of this study was to determine whether upregulation of PTEN gene by transfection wild-type PTEN gene to ovarian cancer cells can inhibit growth and migration and to explore the potential for PTEN gene therapy of ovarian cancers. METHOD: Wild-type and phosphatase-inactive (C124A) PTEN plasmids were transfected into ovarian epithelial cancer A2780 cells, and their effects on cell apoptosis, cell proliferation, cell migration and cell invasion were analyzed by flow cytometry analysis, TUNEL assay, MTT assay, wound-healing assay and transwell assay. RESULTS: Both wild-type and mutant PTEN can upregulate the expression of PTEN gene dramatically; however, it is wild-type PTEN not phosphatase-inactive PTEN that can induce apoptosis and decrease cell migration, invasion and proliferation in ovarian cancer cells. CONCLUSION: These results demonstrated that PTEN had played an important role in the cell proliferation, cell migration and invasion dependent on its phosphatase activity. Enhanced expression of PTEN by gene transfer is sufficient to reverse the malignant phenotype of ovarian cancer cells and transfection of ovarian cancer cells with wild-type PTEN gene might be another novel approach for therapeutic intervention in ovarian cancer.

Yamashita, K., H. L. Park, et al. (2006). "PGP9.5 methylation in diffuse-type gastric cancer." Cancer Res **66**(7): 3921-7.

Diffuse-type gastric cancer (DGC) is the most deadly form of gastric cancer and is frequently accompanied by peritoneal dissemination and metastasis. The specific molecular events involved in DGC pathogenesis remain elusive. Accumulating evidence of epigenetic inactivation in tumor suppressor genes led us to conduct a comprehensive screen to identify novel methylated genes in human cancers using pharmacologic unmasking and subsequent microarray analysis. We compared differential RNA expression profiles of DGC and intestinal-type gastric cancer (IGC) cell lines treated with 5-aza-2'-deoxycytidine using microarrays containing 22,284 genes. We identified 16 methylated genes, including many novel genes, in DGC cell lines and studied PGP9.5 with particular interest. In primary gastric cancers, PGP9.5 was found to be more frequently methylated in DGCs (78%) than in IGCs (36%; DGC versus IGC, $P < 0.05$). Furthermore, real-time methylation-specific PCR analysis of PGP9.5 showed relatively higher methylation levels in DGC than in IGC. Our data thus implicate a molecular event common in the DGC phenotype compared with IGC.

Yang, B., J. R. Eshleman, et al. (1996). "Wild-type p53 protein potentiates cytotoxicity of therapeutic agents in human colon cancer cells." Clin Cancer Res **2**(10): 1649-57.

Wild-type p53 is induced by DNA damage. In different cell types, this induction is suggested either to facilitate DNA repair by inducing a cell cycle pause or to potentiate cell death via apoptosis. Wild-type p53 in different cell types has similarly been associated with either enhancement of or increased resistance to the cytotoxicity of many cancer therapeutic agents. We have constructed a colorectal cancer cell line bearing, in addition to endogenous mutant p53 alleles, an exogenous wild-type p53 allele

that is under the regulatable control of the lac repressor. Induction of wild-type p53 by isopropyl-beta-thiogalactopyranoside in these cells induces a reversible growth arrest but does not induce cell death. However, we find that the induction of wild-type p53 powerfully potentiates the cytotoxicity of both irradiation and 5-fluorouracil, two agents that are used clinically in the treatment of colorectal cancer. We also find that induction of wild-type p53 potentiates the cytotoxicity of topotecan, a member of the camptothecin family of drugs that also has clinical activity against colon cancer. These findings suggest that the common loss of wild-type p53 in many colorectal cancers may play a role in the clinical resistance of these tumors to anticancer agents. Although some cancer cells may not be directly killed by p53 gene therapy, our findings suggest that genetic alteration of some cancers to induce wild-type p53 may increase their sensitivity to cytotoxic gene therapy.

Yoon, S. S., N. M. Carroll, et al. (1998). "Cancer gene therapy using a replication-competent herpes simplex virus type 1 vector." Ann Surg **228**(3): 366-74.

OBJECTIVE: The authors investigate the efficacy of hrR3, a viral vector derived from herpes simplex virus type 1 (HSV 1), in destroying colon carcinoma cells in vitro and in vivo. The effect of adding the prodrug ganciclovir in combination with hrR3 infection also is assessed. SUMMARY BACKGROUND DATA: Most cancer gene therapy strategies use viral vectors that are incapable of replication. The HSV 1 vector hrR3 is capable of replication, and its replication is cytotoxic to cells. hrR3 also possesses the HSV-thymidine kinase gene, which converts ganciclovir into a toxic metabolite. Thus, the addition of ganciclovir to hrR3-infected cells may enhance the ability of hrR3 to destroy tumor cells. To increase specificity for tumor cells, hrR3 has a mutated ribonucleotide reductase gene and replicates selectively in cells with high levels of endogenous rbonucleotide reductase. Actively dividing cells such as tumor cells have high levels of endogenous ribonucleotide reductase for synthesis of DNA precursors. The authors are interested in the use of HSV 1 vectors to treat liver metastases from colorectal cancer. METHODS: Ribonucleotide reductase expression in several colon carcinoma cell lines and in primary cultures of human hepatocytes was determined by Western blot analysis. hrR3-mediated cytotoxicity in the colon carcinoma cell lines was determined using an in vitro assay. The human colon carcinoma cell line HT29 was injected into the flanks of nude mice followed by intratumoral injection of hrR3. Tumor growth rate was assessed with and without the addition of intraperitoneal ganciclovir. RESULTS: Ribonucleotide reductase levels in colon carcinoma cell lines are much higher than in primary cultures of human hepatocytes. hrR3 efficiently destroys colon carcinoma cell lines in vitro. A single intratumoral injection of hrR3 into HT29 flank tumors significantly reduces tumor growth rate, and the administration of ganciclovir has no additive effect. CONCLUSIONS: The inherent cytotoxicity of hrR3 replication effectively destroys colon carcinoma cells in vitro and in vivo. This cytotoxicity is not enhanced in vivo by the addition of ganciclovir. In the future, more efficacious and selective HSV 1 vectors may be useful in the treatment of cancer.

Zhang, Z. W., S. E. Patchett, et al. (2000). "Topoisomerase I inhibitor (camptothecin)-induced apoptosis in human gastric cancer cells and the role of wild-type p53 in the enhancement of its cytotoxicity." Anticancer Drugs **11**(9): 757-64.

Camptothecin (CPT), a human topoisomerase I inhibitor, blocks DNA replication in human cancer cells. It represents a promising new class of chemotherapeutic agents with broad anti-tumor activity. However, its effect on gastric cancer cells remains unknown. We examined cell growth, apoptosis and cell cycle phase distribution in gastric cancer cells by exposing these cells to CPT for up to 72 h. Cell viability was determined by the Trypan blue exclusion assay. Cell cycle phase distribution and apoptosis were measured using flow cytometry, fluorescence microscopy and DNA ladder assay. Exposure of exponentially growing gastric AGS cancer cells to CPT induced time-dependent apoptosis and growth inhibition. Serum starvation-synchronized AGS cells (about 60% cells in G0/G1 phase) showed similar cellular responses. Analysis of cell cycle phase distribution of AGS cells treated with CPT for up to 72 h showed no obvious differences compared to untreated control cells. Although the induction of apoptosis was noticed in gastric cancer cell lines both with and without p53, cells lacking p53 showed less apoptosis compared to those cell lines possessing p53. Our data show that CPT is capable of inducing gastric cancer cell growth inhibition and apoptosis. Wild-type p53 may enhance the cytotoxicity of CPT against gastric carcinoma.

References

1. Amado, R. G., M. Wolf, et al. (2008). "Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer." J Clin Oncol **26**(10): 1626-34.
2. Amantini, C., P. Ballarini, et al. (2009). "Triggering of transient receptor potential vanilloid type 1 (TRPV1) by capsaicin induces Fas/CD95-mediated apoptosis of urothelial cancer cells in an ATM-dependent manner." Carcinogenesis **30**(8): 1320-9.
3. Awata, H., K. Hirose, et al. (2002). "Heterogeneity of apoptosis and proliferative activity within superficial spreading type early gastric cancer." Oncol Rep **9**(1): 153-8.

4. Bahn, D. K., P. Silverman, et al. (2004). "In treating localized prostate cancer the efficacy of cryoablation is independent of DNA ploidy type." Technol Cancer Res Treat **3**(3): 253-7.
5. Bartlett, J. M., A. Munro, et al. (2008). "Type 1 receptor tyrosine kinase profiles identify patients with enhanced benefit from anthracyclines in the BR9601 adjuvant breast cancer chemotherapy trial." J Clin Oncol **26**(31): 5027-35.
6. Bossi, G. and A. Sacchi (2007). "Restoration of wild-type p53 function in human cancer: relevance for tumor therapy." Head Neck **29**(3): 272-84.
7. Bouvet, M., R. J. Bold, et al. (1998). "Adenovirus-mediated wild-type p53 tumor suppressor gene therapy induces apoptosis and suppresses growth of human pancreatic cancer [seecomments]." Ann Surg Oncol **5**(8): 681-8.
8. Brouwer, E., M. J. Havenga, et al. (2007). "Human adenovirus type 35 vector for gene therapy of brain cancer: improved transduction and bypass of pre-existing anti-vector immunity in cancer patients." Cancer Gene Ther **14**(2): 211-9.
9. Burfeind, P., C. L. Chernicky, et al. (1996). "Antisense RNA to the type I insulin-like growth factor receptor suppresses tumor growth and prevents invasion by rat prostate cancer cells in vivo." Proc Natl Acad Sci U S A **93**(14): 7263-8.
10. Cascallo, M., J. Calbo, et al. (2000). "Modulation of drug cytotoxicity by reintroduction of wild-type p53 gene (Ad5CMV-p53) in human pancreatic cancer." Cancer Gene Ther **7**(4): 545-56.
11. Chang, J., C. Lee, et al. (2000). "Over-expression of ERT(ESX/ESE-1/ELF3), an ets-related transcription factor, induces endogenous TGF-beta type II receptor expression and restores the TGF-beta signaling pathway in Hs578t human breast cancer cells." Oncogene **19**(1): 151-4.
12. Chang, J., K. Park, et al. (1997). "Expression of transforming growth factor beta type II receptor reduces tumorigenicity in human gastric cancer cells." Cancer Res **57**(14): 2856-9.
13. Czegledy, J., M. Evander, et al. (1994). "Human papillomavirus type 18 E6* mRNA in primary tumors and pelvic lymph nodes of Hungarian patients with squamous cervical cancer." Int J Cancer **56**(2): 182-6.
14. Das, G. C., D. Holiday, et al. (2001). "Taxol-induced cell cycle arrest and apoptosis: dose-response relationship in lung cancer cells of different wild-type p53 status and under isogenic condition." Cancer Lett **165**(2): 147-53.
15. Day, J. M., P. A. Foster, et al. (2008). "17beta-hydroxysteroid dehydrogenase Type 1, and not Type 12, is a target for endocrine therapy of hormone-dependent breast cancer." Int J Cancer **122**(9): 1931-40.
16. Dominguez, G., J. M. Silva, et al. (2001). "Wild type p73 overexpression and high-grade malignancy in breast cancer." Breast Cancer Res Treat **66**(3): 183-90.
17. Fechner, G., F. G. Perabo, et al. (2003). "Preclinical evaluation of a radiosensitizing effect of gemcitabine in p53 mutant and p53 wild type bladder cancer cells." Urology **61**(2): 468-73.
18. Fernando, G. J., B. Murray, et al. (1999). "Expression, purification and immunological characterization of the transforming protein E7, from cervical cancer-associated human papillomavirus type 16." Clin Exp Immunol **115**(3): 397-403.
19. Ferrandina, G., G. Almadori, et al. (1998). "Growth-inhibitory effect of tamoxifen and quercetin and presence of type II estrogen binding sites in human laryngeal cancer cell lines and primary laryngeal tumors." Int J Cancer **77**(5): 747-54.
20. Fujii, T., Y. Matsushima, et al. (1995). "Serum antibody against unfused recombinant E7 protein of human papillomavirus type 16 in cervical cancer patients." Jpn J Cancer Res **86**(1): 28-34.
21. Fujiwara, T., E. A. Grimm, et al. (1993). "A retroviral wild-type p53 expression vector penetrates human lung cancer spheroids and inhibits growth by inducing apoptosis." Cancer Res **53**(18): 4129-33.
22. Gao, N., Y. D. Hu, et al. (2001). "The exogenous wild-type p14ARF gene induces growth arrest and promotes radiosensitivity in human lung cancer cell lines." J Cancer Res Clin Oncol **127**(6): 359-67.
23. Gingras, S., R. Moriggl, et al. (1999). "Induction of 3beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerase type 1 gene transcription in human breast cancer cell lines and in normal mammary epithelial cells by interleukin-4 and interleukin-13." Mol Endocrinol **13**(1): 66-81.
24. Gonzalez-Zuloeta Ladd, A. M., A. Arias Vasquez, et al. (2007). "Differential roles of Angiotensinogen and Angiotensin Receptor type 1 polymorphisms in breast cancer risk." Breast Cancer Res Treat **101**(3): 299-304.
25. Gorka, M., W. M. Daniewski, et al. (2005). "Autophagy is the dominant type of programmed cell death in breast cancer MCF-7 cells exposed to AGS 115 and EFDAC, new sesquiterpene analogs of paclitaxel." Anticancer Drugs **16**(7): 777-88.
26. Graniela Sire, E. A., F. Vikhanskaya, et al. (1995). "Sensitivity and cellular response to different anticancer agents of a human ovarian cancer cell line expressing wild-type, mutated or no p53." Ann Oncol **6**(6): 589-93.
27. Han, B., M. Nakamura, et al. (2006). "Calcitonin inhibits invasion of breast cancer cells: involvement of urokinase-type plasminogen activator (uPA) and uPA receptor." Int J Oncol **28**(4): 807-14.
28. Hasegawa, Y., N. Emi, et al. (1993). "Gene transfer of herpes simplex virus type I thymidine kinase gene as a drug sensitivity gene into human lung cancer cell lines using retroviral vectors." Am J Respir Cell Mol Biol **8**(6): 655-61.
29. Hu, Y., R. K. Le Leu, et al. (2008). "The potential of sphingomyelin as a chemopreventive agent in AOM-induced colon cancer model: wild-type and p53+/- mice." Mol Nutr Food Res **52**(5): 558-66.
30. Husain, I., J. L. Mohler, et al. (1994). "Elevation of topoisomerase I messenger RNA, protein, and catalytic activity in human tumors: demonstration of tumor-type specificity and implications for cancer chemotherapy." Cancer Res **54**(2): 539-46.
31. Hwang, R. F., E. M. Gordon, et al. (1998). "Gene therapy for primary and metastatic pancreatic cancer with intraperitoneal retroviral vector bearing the wild-type p53 gene." Surgery **124**(2): 143-50; discussion 150-1.
32. Ikegami, S., T. Tadakuma, et al. (2005). "Selective gene therapy for prostate cancer cells using liposomes conjugated with IgM type monoclonal antibody against prostate-specific membrane antigen." Hum Cell **18**(1): 17-23.
33. Ip, C., S. P. Briggs, et al. (1996). "The efficacy of conjugated linoleic acid in mammary cancer prevention is independent of the level or type of fat in the diet." Carcinogenesis **17**(5): 1045-50.
34. Israyelyan, A. H., J. M. Melancon, et al. (2007). "Effective treatment of human breast tumor in a mouse xenograft model with herpes simplex virus type 1 specifying the NV1020 genomic deletion and the gBsyn3 syncytial mutation enabling high viral replication and spread in breast cancer cells." Hum Gene Ther **18**(5): 457-73.
35. Jabbar, S. F., L. Abrams, et al. (2009). "Persistence of high-grade cervical dysplasia and cervical cancer requires the continuous expression of the human papillomavirus type 16 E7 oncogene." Cancer Res **69**(10): 4407-14.
36. Jensen, P. B., I. J. Christensen, et al. (1993). "Differential cytotoxicity of 19 anticancer agents in wild type and etoposide resistant small cell lung cancer cell lines." Br J Cancer **67**(2): 311-20.

37. Kim, K. Y., K. C. Choi, et al. (2005). "Extracellular signal-regulated protein kinase, but not c-Jun N-terminal kinase, is activated by type II gonadotropin-releasing hormone involved in the inhibition of ovarian cancer cell proliferation." J Clin Endocrinol Metab **90**(3): 1670-7.
38. Kuball, J., S. F. Wen, et al. (2002). "Successful adenovirus-mediated wild-type p53 gene transfer in patients with bladder cancer by intravesical vector instillation." J Clin Oncol **20**(4): 957-65.
39. Lakka, S. S., R. Rajagopal, et al. (2001). "Adenovirus-mediated antisense urokinase-type plasminogen activator receptor gene transfer reduces tumor cell invasion and metastasis in non-small cell lung cancer cell lines." Clin Cancer Res **7**(4): 1087-93.
40. Li, H., M. Zhao, et al. (2004). "Characterization of a new type HPV16 E7 variant isolated from cervical cancer highest incidence area in Hubei Province of China." Eksp Onkol **26**(1): 48-54.
41. Li, T., Y. H. Ling, et al. (2008). "Tumor dependence on the EGFR signaling pathway expressed by the p-EGFR:p-AKT ratio predicts erlotinib sensitivity in human non-small cell lung cancer (NSCLC) cells expressing wild-type EGFR gene." J Thorac Oncol **3**(6): 643-7.
42. Lin, C. C., M. C. Yen, et al. (2008). "Delivery of noncarrier naked DNA vaccine into the skin by supersonic flow induces a polarized T helper type 1 immune response to cancer." J Gene Med **10**(6): 679-89.
43. Liu, D. W., Y. P. Tsao, et al. (2000). "Recombinant adeno-associated virus expressing human papillomavirus type 16 E7 peptide DNA fused with heat shock protein DNA as a potential vaccine for cervical cancer." J Virol **74**(6): 2888-94.
44. Loupakis, F., A. Ruzzo, et al. (2009). "KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer." Br J Cancer **101**(4): 715-21.
45. Mariot, P., K. Vanoverberghe, et al. (2002). "Overexpression of an alpha 1H (Cav3.2) T-type calcium channel during neuroendocrine differentiation of human prostate cancer cells." J Biol Chem **277**(13): 10824-33.
46. Martinet, N., L. Bonnard, et al. (2003). "In vivo transglutaminase type 1 expression in normal lung, preinvasive bronchial lesions, and lung cancer." Am J Respir Cell Mol Biol **28**(4): 428-35.
47. Matsubara, H., T. Maeda, et al. (2001). "Combinatory anti-tumor effects of electroporation-mediated chemotherapy and wild-type p53 gene transfer to human esophageal cancer cells." Int J Oncol **18**(4): 825-9.
48. Miyake, H., I. Hara, et al. (1999). "Synergistic enhancement of resistance to cisplatin in human bladder cancer cells by overexpression of mutant-type p53 and Bcl-2." J Urol **162**(6): 2176-81.
49. Muenscher, A., H. H. Feucht, et al. (2009). "Integration of human papilloma virus type 26 in laryngeal cancer of a child." Auris Nasus Larynx **36**(2): 232-4.
50. Namoto, M., Y. Yonemitsu, et al. (1998). "Heterogeneous induction of apoptosis in colon cancer cells by wild-type p53 gene transfection." Int J Oncol **12**(4): 777-84.
51. Neumann, J., E. Zeindl-Eberhart, et al. (2009). "Frequency and type of KRAS mutations in routine diagnostic analysis of metastatic colorectal cancer." Pathol Res Pract **205**(12): 858-62.
52. Ng, A. B. and N. B. Atkin (1973). "Histological cell type and DNA value in the prognosis of squamous cell cancer of uterine cervix." Br J Cancer **28**(4): 322-31.
53. Nguyen, K. H., P. Hachem, et al. (2005). "Adenoviral-E2F-1 radiosensitizes p53wild-type and p53null human prostate cancer cells." Int J Radiat Oncol Biol Phys **63**(1): 238-46.
54. Nicoletti, M. I., T. G. Myers, et al. (2001). "Wild-type p53 marginally induces endogenous MDR-1 mRNA without causing a measurable drug resistance in human cancer cells." Int J Oncol **18**(2): 375-81.
55. Onuki, M., K. Matsumoto, et al. (2009). "Human papillomavirus infections among Japanese women: age-related prevalence and type-specific risk for cervical cancer." Cancer Sci **100**(7): 1312-6.
56. Osaki, S., Y. Nakanishi, et al. (2000). "Alteration of drug chemosensitivity caused by the adenovirus-mediated transfer of the wild-type p53 gene in human lung cancer cells." Cancer Gene Ther **7**(2): 300-7.
57. Peters, G. J., B. van Triest, et al. (2000). "Molecular downstream events and induction of thymidylate synthase in mutant and wild-type p53 colon cancer cell lines after treatment with 5-fluorouracil and the thymidylate synthase inhibitor raltitrexed." Eur J Cancer **36**(7): 916-24.
58. Peters, G., V. Fantl, et al. (1995). "Chromosome 11q13 markers and D-type cyclins in breast cancer." Breast Cancer Res Treat **33**(2): 125-35.
59. Pledgie-Tracy, A., M. D. Sobolewski, et al. (2007). "Sulforaphane induces cell type-specific apoptosis in human breast cancer cell lines." Mol Cancer Ther **6**(3): 1013-21.
60. Prabha, S. and V. Labhasetwar (2004). "Nanoparticle-mediated wild-type p53 gene delivery results in sustained antiproliferative activity in breast cancer cells." Mol Pharm **1**(3): 211-9.
61. Preto, A., S. K. Singhrao, et al. (2004). "Telomere erosion triggers growth arrest but not cell death in human cancer cells retaining wild-type p53: implications for antitelomerase therapy." Oncogene **23**(23): 4136-45.
62. Putzer, B. M., J. L. Bramson, et al. (1998). "Combination therapy with interleukin-2 and wild-type p53 expressed by adenoviral vectors potentiates tumor regression in a murine model of breast cancer." Hum Gene Ther **9**(5): 707-18.
63. Raigoso, P., A. Junco, et al. (2000). "Tissue-type plasminogen activator (tPA) content in colorectal cancer and in surrounding mucosa: relationship with clinicopathologic parameters and prognostic significance." Int J Biol Markers **15**(1): 44-50.
64. Russo, A., V. Bazan, et al. (2005). "The TP53 colorectal cancer international collaborative study on the prognostic and predictive significance of p53 mutation: influence of tumor site, type of mutation, and adjuvant treatment." J Clin Oncol **23**(30): 7518-28.
65. Rutter, J. L., U. Benbow, et al. (1997). "Cell-type specific regulation of human interstitial collagenase-1 gene expression by interleukin-1 beta (IL-1 beta) in human fibroblasts and BC-8701 breast cancer cells." J Cell Biochem **66**(3): 322-36.
66. Salatino, M., R. Schillaci, et al. (2004). "Inhibition of in vivo breast cancer growth by antisense oligodeoxynucleotides to type I insulin-like growth factor receptor mRNA involves inactivation of ErbBs, PI-3K/Akt and p42/p44 MAPK signaling pathways but not modulation of progesterone receptor activity." Oncogene **23**(30): 5161-74.
67. Santin, A. D., S. Bellone, et al. (2008). "Human papillomavirus type 16 and 18 E7-pulsed dendritic cell vaccination of stage IB or IIA cervical cancer patients: a phase I escalating-dose trial." J Virol **82**(4): 1968-79.
68. Schaber, J. D., H. Fang, et al. (1998). "Prolactin activates Stat1 but does not antagonize Stat1 activation and growth inhibition by type I interferons in human breast cancer cells." Cancer Res **58**(9): 1914-9.
69. Schatten, H., M. Ripple, et al. (2000). "Androgen and taxol cause cell type-specific alterations of centrosome and DNA organization in androgen-responsive LNCaP and androgen-independent DU145 prostate cancer cells." J Cell Biochem **76**(3): 463-77.
70. Schuler, M., C. Rochlitz, et al. (1998). "A phase I study of adenovirus-mediated wild-type p53 gene transfer in patients

with advanced non-small cell lung cancer." Hum Gene Ther **9**(14): 2075-82.
71. Seth, P., D. Katayose, et al. (1997). "A recombinant adenovirus expressing wild type p53 induces apoptosis in drug-resistant human breast cancer cells: a gene therapy approach for drug-resistant cancers." Cancer Gene Ther **4**(6): 383-90.
72. Sharma, K. and C. B. Srikant (1998). "Induction of wild-type p53, Bax, and acidic endonuclease during somatostatin-signaled apoptosis in MCF-7 human breast cancer cells." Int J Cancer **76**(2): 259-66.
73. Steiner, M. S., Y. Zhang, et al. (2000). "Adenoviral vector containing wild-type p16 suppresses prostate cancer growth and prolongs survival by inducing cell senescence." Cancer Gene Ther **7**(3): 360-72.
74. Stoff-Khalili, M. A., A. A. Rivera, et al. (2005). "A human adenoviral vector with a chimeric fiber from canine adenovirus type 1 results in novel expanded tropism for cancer gene therapy." Gene Ther **12**(23): 1696-706.
75. Sun, Y., C. Qian, et al. (2000). "Gene transfer to liver cancer cells of B7-1 plus interleukin 12 changes immunoeffector mechanisms and suppresses helper T cell type 1 cytokine production induced by interleukin 12 alone." Hum Gene Ther **11**(1): 127-38.
76. Tahara, I., K. Miyake, et al. (2007). "Systemic cancer gene therapy using adeno-associated virus type 1 vector expressing MDA-7/IL24." Mol Ther **15**(10): 1805-11.
77. Thomadaki, H., A. Scorilas, et al. (2008). "The role of cordycepin in cancer treatment via induction or inhibition of apoptosis: implication of polyadenylation in a cell type specific manner." Cancer Chemother Pharmacol **61**(2): 251-65.
78. Thottassery, J. V., L. Westbrook, et al. (2006). "c-Abl-independent p73 stabilization during gemcitabine- or 4'-thio-beta-D-arabinofuranosylcytosine-induced apoptosis in wild-type and p53-null colorectal cancer cells." Mol Cancer Ther **5**(2): 400-10.
79. Toita, R., J. H. Kang, et al. (2009). "Protein kinase C alpha-specific peptide substrate graft-type copolymer for cancer cell-specific gene regulation systems." J Control Release **139**(2): 133-9.
80. Tolcher, A. W., D. Hao, et al. (2006). "Phase I, pharmacokinetic, and pharmacodynamic study of intravenously administered Ad5CMV-p53, an adenoviral vector containing the wild-type p53 gene, in patients with advanced cancer." J Clin Oncol **24**(13): 2052-8.
81. Tomita, S., S. Deguchi, et al. (1999). "Analyses of microsatellite instability and the transforming growth factor-beta receptor type II gene mutation in sporadic breast cancer and their correlation with clinicopathological features." Breast Cancer Res Treat **53**(1): 33-9.
82. Tortora, G. and F. Ciardiello (2003). "Antisense targeting protein kinase A type I as a drug for integrated strategies of cancer therapy." Ann N Y Acad Sci **1002**: 236-43.
83. Troester, M. A., K. A. Hoadley, et al. (2004). "Cell-type-specific responses to chemotherapeutics in breast cancer." Cancer Res **64**(12): 4218-26.
84. Vannucchi, S., M. V. Chiantore, et al. (2007). "Perspectives in biomolecular therapeutic intervention in cancer: from the early to the new strategies with type I interferons." Curr Med Chem **14**(6): 667-79.
85. Vernon, S. D., E. R. Unger, et al. (1997). "Association of human papillomavirus type 16 integration in the E2 gene with poor disease-free survival from cervical cancer." Int J Cancer **74**(1): 50-6.
86. Vijayalingam, S., T. Subramanian, et al. (2009). "Down-regulation of multiple cell survival proteins in head and neck cancer cells by an apoptogenic mutant of adenovirus type 5." Virology **392**(1): 62-72.
87. Vikhanskaya, F., C. Falugi, et al. (2002). "Human papillomavirus type 16 E6-enhanced susceptibility to apoptosis induced by TNF in A2780 human ovarian cancer cell line." Int J Cancer **97**(6): 732-9.
88. Vitale, G., C. H. van Eijck, et al. (2007). "Type I interferons in the treatment of pancreatic cancer: mechanisms of action and role of related receptors." Ann Surg **246**(2): 259-68.
89. Watanabe, T. and B. A. Sullenger (2000). "Induction of wild-type p53 activity in human cancer cells by ribozymes that repair mutant p53 transcripts." Proc Natl Acad Sci U S A **97**(15): 8490-4.
90. Wiman, K. G. (2007). "Restoration of wild-type p53 function in human tumors: strategies for efficient cancer therapy." Adv Cancer Res **97**: 321-38.
91. Wu, H., S. Wang, et al. (2008). "Reversal of the malignant phenotype of ovarian cancer A2780 cells through transfection with wild-type PTEN gene." Cancer Lett **271**(2): 205-14.
92. Yamashita, K., H. L. Park, et al. (2006). "PGP9.5 methylation in diffuse-type gastric cancer." Cancer Res **66**(7): 3921-7.
93. Yang, B., J. R. Eshleman, et al. (1996). "Wild-type p53 protein potentiates cytotoxicity of therapeutic agents in human colon cancer cells." Clin Cancer Res **2**(10): 1649-57.
94. Yoon, S. S., N. M. Carroll, et al. (1998). "Cancer gene therapy using a replication-competent herpes simplex virus type 1 vector." Ann Surg **228**(3): 366-74.
95. Zhang, Z. W., S. E. Patchett, et al. (2000). "Topoisomerase I inhibitor (camptothecin)-induced apoptosis in human gastric cancer cells and the role of wild-type p53 in the enhancement of its cytotoxicity." Anticancer Drugs **11**(9): 757-64.
96. PubMed (2011). http://www.ncbi.nlm.nih.gov/pubmed.
97. Cancer. Wikipedia. (2011) http://en.wikipedia.org/wiki/Cancer.

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