Cervical Cancer

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Abstract: Cancer is the cells that grow out of control. Cancer cells can also invade other tissues. Growing out of control and invading other tissues are what makes a cell a cancer cell. Involved in more than 100 diseases, cancers can cause serious illness and death. Normally, the cells become cancer cells because of DNA damage. This material is a literature collection of the researches on the cervical cancer.
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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.

Literatures

Abu, J., M. Batuwangala, et al. (2005). "Retinoic acid and retinoid receptors: potential chemopreventive and therapeutic role in cervical cancer." Lancet Oncol **6**(9): 712-20.

Retinoids are natural and synthetic derivatives of vitamin A, which can be obtained from animal products (milk, liver, beef, fish oils, and eggs) and vegetables (carrots, mangos, sweet potatoes, and spinach). Retinoids regulate various important cellular functions in the body through specific nuclear retinoic-acid receptors and retinoid-X receptors, which are encoded by separate genes. Retinoic-acid receptors specifically bind tretinoin and alitretinoin, whereas retinoid-X receptors bind only alitretinoin. Retinoids have long been established as crucial for several essential life processes-healthy growth, vision, maintenance of tissues, reproduction, metabolism, tissue differentiation (normal, premalignant cells, and malignant cells), haemopoiesis, bone development, spermatogenesis, embryogenesis, and overall survival. Therefore, deficiency of vitamin A can lead to various unwanted biological effects. Several experimental and epidemiological studies have shown the antiproliferative activity of retinoids and their potential use in cancer treatment and chemoprevention. Emerging clinical trials have shown the chemotherapeutic and chemopreventive potential of retinoids in cancerous and precancerous conditions of the uterine cervix. In this review, we explore the potential chemopreventive and therapeutic roles of retinoids in preinvasive and invasive cervical neoplasia.

Ahn, W. S., S. M. Bae, et al. (2004). "Anti-cancer effect of adenovirus p53 on human cervical cancer cell growth in vitro and in vivo." Int J Gynecol Cancer **14**(2): 322-32.

To evaluate anti-tumor effects of recombinant adenovirus p53, time-course p53, E6 expression, and cell growth inhibition were investigated in vitro and in vivo using cervical cancer cell lines such as CaSki, SiHa, HeLa, HeLaS3, C33A, and HT3. The cell growth inhibition was studied via cell count assay, MTT assay and neutral red assay. After transfecting AdCMVp53 into SiHa cells-xenografted nude mice, the transduction efficiency and anti-tumor effect were investigated for a month. The results showed that adenoviral p53 expression induced significant growth suppression on the cancer cells, in which E6 transcript was strongly repressed, and that the expression of p53 and E6 were remarkably dependent on each cell type. The transduction efficiency was highly maintained in vivo as well as in vitro, and the size of tumor was remarkably decreased in comparison with AdCMVLacZ control. The results suggest that the adenovirus-mediated p53 gene transfection was done very effectively in vitro and in vivo experiment, and the cell growth was suppressed via p53-dependent apoptotic cell death, and that the anti-tumor effect could be related to E6 and p53 expression pattern.

Ahn, W. S., S. M. Bae, et al. (2004). "Recombinant adenovirus-p53 gene transfer and cell-specific growth suppression of human cervical cancer cells in vitro and in vivo." Gynecol Oncol **92**(2): 611-21.

Ahn et al investigated the time-course expression patterns of p53 and E6 on cervical cancer cells to obtain a molecular level understanding of cell-dependent tumor growth suppression effects of recombinant adenovirus expressing p53 in vitro and in vivo. Four human papillomavirus (HPV)-infected human cervical cancer cell lines (HPV 16-positive cells, CaSki and SiHa cells; and HPV 18-positive cells, HeLa and HeLaS3 cells) were used. Also, HPV negative C33A and HT3 cell line that has a mutation on p53 gene were used. After infection with AdCMVp53, the cell growth inhibition was studied via cell count assay, MTT assay, and Neutral red assay. After transfecting AdCMVp53 and AdCMVLacZ into the cancer cells-xenografted nude mice, antitumor effects were investigated for 1 month, respectively. For each cervical cancer cell, IC50 was as follows; CaSki (68.5 multiplicity of infection, or MOI), SiHa (43.5 MOI), HeLa (31 MOI), HeLaS3 (42 MOI), C33A (21 MOI), and HT3 (62 MOI). In particular, complete inhibition of cell growth was observed at 125 MOI in both CaSki and SiHa cells. However, the complete inhibition was detected at 62.5 MOI in HeLa and HeLaS3. In contrast, at these MOI, no suppression of cell growth was observed when cells were infected with recombinant adenovirus expressing beta-gal as a negative control. The levels of p53 protein were notably expressed in CaSki and HeLa more than in SiHa and HeLaS3 on days 2 and 4. However, the p53 was only detected in HeLaS3 on day 6. In contrast, p53 expression was continually maintained in C33A and HT3 during the same periods. After transfection AdCMVp53 into CaSki- and SiHa-xenografted nude mice, the size of tumor was remarkably decreased in SiHa cells as compared to AdCMVLacZ transfection. CONCLUSION: The adenovirus-mediated p53 gene transfection was done effectively in vitro and in vivo. Also, the antitumor effects were accomplished via differential role of p53-specific apoptotic cell death, which is dependent upon the cervical cancer cell line.

Au, W. W., S. Abdou-Salama, et al. (2007). "Inhibition of growth of cervical cancer cells using a dominant negative estrogen receptor gene." Gynecol Oncol **104**(2): 276-80.

Estrogen stimulates human papilloma virus oncogene expression, promotes cervical cancer (CC) cell proliferation and prevents apoptosis. Therefore, blockage of estrogen function may have therapeutic application to CC. CasKi CC cells were transfected with an adenovirus expressing a dominant negative estrogen receptor gene (Ad-ER-DN) and their responses were investigated by RT-PCR, Flow Cytometry and Western blot assays. RESULT: Transfected cells showed disturbance of cell colony morphology, reduced HPV E6 and E7 mRNA, interruption of cell proliferation, reduced cyclin D1 protein and expression of apoptosis. CONCLUSION: We report, for the first time, the use of Ad-ER-DN to block estrogen receptors which led to dramatic changes in CC cells that are consistent with the possible reactivation of cellular p53 and Rb function. Their reactivation most likely allowed the recognition of existing chromosome abnormalities as a serious stress signal and the initiation of a cascade of cellular events in response to the stress, including the activation of the core apoptotic machinery which led to self-destruction of the CC cells.

Bae, S. M., H. J. Min, et al. (2006). "Protein expression profile using two-dimensional gel analysis in squamous cervical cancer patients." Cancer Res Treat **38**(2): 99-107.

Screening in cervical cancer is now progressing to discover candidate genes and proteins that may serve as biological markers and that play a role in tumor progression. We examined the protein expression patterns of the squamous cell carcinoma (SCC) tissues from Korean women with using two-dimensional polyacrylamide gel electrophoresis (2-DE) and matrix assisted laser desorption/ionization-time of flight (MALDI- TOF) mass spectrometer. Normal cervix and SCC tissues were solubilized and 2-DE was performed using pH 3 approximately 10 linear IPG strips of 17 cm length. The protein expression was evaluated using PDQuest 2-D software. The differentially expressed protein spots were identified with a MALDI-TOF mass spectrometer, and the peptide mass spectra identifications were performed using the Mascot program and by searching the Swiss-prot or NCBInr databases. A total of 35 proteins were detected in SCC. 17 proteins were up-regulated and 18 proteins were down-regulated. Among the proteins that were identified, 12 proteins (pigment epithelium derived factor, annexin A2 and A5, keratin 19 and 20, heat shock protein 27, smooth muscle protein 22 alpha, alpha-enolase, squamous cell carcinoma antigen 1 and 2, glutathione S-transferase and apolipoprotein a1) were protein previously known to be involved in tumor, and 21 proteins were newly identified in this study. CONCLUSION: 2-DE offers the total protein expression profiles of SCC tissues; further characterization of these differentially expressed proteins will give a chance to identify the badly needed tumor-specific diagnostic markers for SCC.

Banno, K., M. Yanokura, et al. (2007). "Epigenetic inactivation of the CHFR gene in cervical cancer contributes to sensitivity to taxanes." Int J Oncol **31**(4): 713-20.

A relationship between inactivation of mitotic checkpoint genes and sensitivity of cancer cells to anticancer agents has been reported. We investigated the effect of epigenetic inactivation by aberrant hypermethylation of the mitotic checkpoint gene CHFR (checkpoint with forkhead and ring finger) on the sensitivity of cervical cancer cells to taxanes. Methylation-specific PCR (MSP) of cervical smears showed aberrant methylation of CHFR in 12.3% (2/14) of adenocarcinoma specimens. In contrast, aberrant DNA methylation was not detected in normal cervical cells or squamous cell carcinoma cells. Aberrant methylation of CHFR was also analyzed in 6 human cervical carcinoma-derived cell lines and was observed in SKG-IIIb and HeLa cells. These cell lines showed high sensitivity to taxanes, but became taxane-resistant upon treatment with 5-azacytidine. Furthermore, suppression of CHFR expression in siRNA-transfected SKG-IIIa cells caused increased sensitivity to taxanes. In conclusion, aberrant methylation of the CHFR gene may be useful as a molecular marker for selection of therapy for patients with cervical adenocarcinoma with a poor prognosis, and may also suggest a new therapeutic strategy of targeting CHFR in cervical cancer. To our knowledge, this study is the first to examine epigenetic inactivation by aberrant hypermethylation of CHFR in cervical cancer.

Bauerschmitz, G. J., A. Kanerva, et al. (2004). "Evaluation of a selectively oncolytic adenovirus for local and systemic treatment of cervical cancer." Int J Cancer **111**(2): 303-9.

Treatment options for disseminated cervical cancer remain inadequate. Here, we investigated a strategy featuring Ad5-Delta 24 RGD, an oncolytic adenovirus replication-competent selectively in cells defective in the Rb-p16 pathway, such as most cervical cancer cells. The viral fiber contains an alpha(v)beta(3) and alpha(v)beta(5) integrin-binding RGD-4C motif, allowing coxsackie-adenovirus receptor-independent infection. These integrins have been reported to be frequently upregulated in cervical cancer. Oncolysis of cervical cancer cells was similar to a wild-type control in vitro. In an animal model of cervical cancer, the therapeutic efficacy of Ad5-Delta 24 RGD could be demonstrated for both intratumoral and intravenous application routes. Biodistribution was determined following intravenous administration to mice. Further preclinical safety data were obtained by demonstrating lack of replication of the agent in human peripheral blood mononuclear cells. These results suggest that Ad5-Delta 24 RGD could be useful for local or systemic treatment of cervical cancer in patients with disease resistant to currently available modalities.

Belcastro, M., M. R. Miller, et al. (2004). "C/EBPbeta activity and HPV-16 E6/E7 mRNA expression are not altered by imiquimod (ALDARA) in human cervical cancer cells in vitro." Gynecol Oncol **92**(2): 660-8.

The purpose of this study was to determine the potential relationship between imiquimod and C/EBPbeta by investigating the extent to which imiquimod could alter C/EBPbeta binding activity to known sequences of the HPV-16 NCR, which could lead to the repression of HPV-16 E6/E7 oncogene expression, possibly impacting a major mechanism by which HPV causes cellular transformation. The effect of imiquimod treatment on C/EBPbeta binding activity to its consensus sequence as well as to two specific regions of the HPV-16 NCR was determined by electromobility shift assay (EMSA) in CaSki cervical cancer cells. HPV-16 E6/E7 gene expression was evaluated by RNase protection assay (RPA) in CaSki and in W12-E cells treated with imiquimod. In addition, C/EBPbeta mRNA expression and protein production in response to imiquimod were evaluated by reverse transcription polymerase chain reaction (RT-PCR) and Western blotting, respectively, in the cervical cancer cell lines, CaSki, HeLa, and C33A. C/EBPbeta binding activity, mRNA expression, and protein production remained unchanged with imiquimod treatment. Initially, HPV-16 E6/E7 expression appeared to be increased with imiquimod treatment in CaSki cells, but this effect was not reproducible. HPV-16 E6/E7 expression was not reproducibly altered with imiquimod treatment in W12-E cells. CONCLUSION: While these results indicate that imiquimod does not alter C/EBPbeta binding activity, nor does it appear to decrease HPV-16 E6/E7 oncogene expression in vitro, it remains possible that imiquimod may have utility in treating cervical dysplasia or cervical cancer via another mechanism.

Borkamo, E. D., B. C. Schem, et al. (2009). "cDNA microarray analysis of serially sampled cervical cancer specimens from patients treated with thermochemoradiotherapy." Int J Radiat Oncol Biol Phys **75**(5): 1562-9.

To elucidate changes in gene expression after treatment with regional thermochemoradiotherapy in locally advanced squamous cell cervical cancer. Tru-Cut biopsy specimens were serially collected from 16 patients. Microarray gene expression levels before and 24 h after the first and second trimodality treatment sessions were compared. Pathway and network analyses were conducted by use of Ingenuity Pathways Analysis (IPA; Ingenuity Systems, Redwood City, CA). Single gene expressions were analyzed by quantitative real-time reverse

transcription-polymerase chain reaction. We detected 53 annotated genes that were differentially expressed after trimodality treatment. Central in the three top networks detected by IPA were interferon alfa, interferon beta, and interferon gamma receptor; nuclear factor kappaB; and tumor necrosis factor, respectively. These genes encode proteins that are important in regulation cell signaling, proliferation, gene expression, and immune stimulation. Biological processes over-represented among the 53 genes were fibrosis, tumorigenesis, and immune response. CONCLUSIONS: Microarrays showed minor changes in gene expression after thermochemoradiotherapy in locally advanced cervical cancer. We detected 53 differentially expressed genes, mainly involved in fibrosis, tumorigenesis, and immune response. A limitation with the use of serial biopsy specimens was low quality of ribonucleic acid from tumors that respond to highly effective therapy. Another "key limitation" is timing of the post-treatment biopsy, because 24 h may be too late to adequately assess the impact of hyperthermia on gene expression.

Branca, M., C. Giorgi, et al. (2006). "Over-expression of topoisomerase IIalpha is related to the grade of cervical intraepithelial neoplasia (CIN) and high-risk human papillomavirus (HPV), but does not predict prognosis in cervical cancer or HPV clearance after cone treatment." Int J Gynecol Pathol **25**(4): 383-92.

One of the pathways leading to cervical cancer is a loss of normal cell cycle control. Topoisomerase IIalpha and IIbeta are important nuclear proteins controlling the G2/M checkpoint, and shown to be over-expressed in many human cancers. Their links to oncogenic human papillomavirus (HPV) types and their prognostic value in cervical cancer are practically unexplored. As part of our HPV-PathogenISS study, a series of 150 squamous cell carcinomas (SCC) and 152 CIN lesions were examined using immunohistochemical (IHC) staining for topoisomerase IIalpha (topo IIalpha), and tested for HPV using PCR with three primer sets (MY09/11, GP5/GP6, SPF). Follow-up data were available from all SCC patients, and 67 CIN lesions had been monitored with serial PCR for HPV clearance/persistence after cone treatment. Topo IIalpha expression increased with increasing grade of CIN ($p = 0.0001$), with the most dramatic up-regulation upon progression from CIN2 to CIN3 and peaking in SCC (OR 16.23; 95%CI 7.89-33.38). Topo IIalpha up-regulation was also significantly associated with HR-HPV detection in univariate analysis (OR = 3.07; 95%CI 1.70-5.52), but was confounded by the histological grade (Mantel-Haenszel common OR = 1.622; 95%CI 0.782-3.365), and by entering both p16(INK4a) (9) and Survivin (33) in the multivariate regression model. Topo IIalpha did not predict clearance/persistence of HR-HPV after treatment of CIN, and it was not a prognostic factor in cervical cancer in either univariate or multivariate analysis. CONCLUSIONS: Over-expression of topo IIalpha is significantly associated with progression from CIN2 to CIN3, being a late marker of cell proliferation. Its close association with HR-HPV is plausibly explained by the fact that E7 oncoproteins of these HR-HPV (but not LR-HPV) block the normal pRb-mediated inhibition of topo IIalpha by degrading the wild-type Rb.

Cane, S., E. Bignotti, et al. (2004). "The novel serine protease tumor-associated differentially expressed gene-14 (KLK8/Neuropsin/Ovasin) is highly overexpressed in cervical cancer." Am J Obstet Gynecol **190**(1): 60-6.

Serine proteases are redundant enzymes implicated in the extracellular modulation required for tumor growth and invasion. Tumor-associated differentially expressed gene-14 (TADG-14) is a novel transmembrane serine protease recently reported by our group to be highly overexpressed in ovarian carcinomas. The goal of this study was to investigate the frequency of expression of the TADG-14 gene in human cervical tumors. STUDY DESIGN: TADG-14 expression was evaluated in 19 cervical cancer cell lines (11 primary and 8 established cell lines) as well as in 8 normal cervical keratinocyte cultures by reverse transcriptase polymerase chain reaction. In addition, to validate gene expression data at the protein level, TADG-14 expression was evaluated by immunohistochemistry on paraffin-embedded tissue from which all 11 primary tumor cell lines were established. TADG-14 was found to be highly expressed in 82% (9/11) primary cervical cancer cell lines and in 87% (7/8) established cervical cancer cell lines by reverse transcriptase-polymerase chain reaction. Expression of TADG-14 by primary squamous cervical tumors was 100% (6/6), whereas 60% (3/5) of primary adenocarcinomas expressed TADG-14. In contrast, none of the normal cervical keratinocyte control cultures (n=4) or flash frozen normal cervical biopsy specimens (n=4) expressed TADG-14. Immunohistochemistry staining of paraffin-embedded cervical cancer specimens confirmed TADG-14 expression in tumor cells and its absence on normal cervical epithelial cells. CONCLUSION: Cervical cancer expressed a high level of TADG-14, suggesting that this protease may play an important role in invasion and metastasis. Because TADG-14 appears only in abundance in tumor tissue and contains a secretion signal sequence, suggesting that TADG-14 is secreted, it may prove to be a useful diagnostic tool for the early detection of

recurrent/persistent cervical cancer after standard treatment or as a novel molecular target for cervical cancer therapy.

Chao, A., T. H. Wang, et al. (2008). "Analysis of functional groups of differentially expressed genes in the peripheral blood of patients with cervical cancer undergoing concurrent chemoradiation treatment." Radiat Res **169**(1): 76-86.

Chao, A., Wang, T. H., Lee, Y. S., Hong, J. H., Tsai, C. N., Chen, C. K., Tsai, C. S., Chao, A. S. and Lai, C. H. Analysis of Functional Groups Differentially Expressed Genes in the Peripheral Blood of Patients with Cervical Cancer Undergoing Concurrent Chemoradiation Treatment. Radiat. Res. 169, 76-86 (2008). We prospectively investigated the gene expression profiles of cervical cancer patients undergoing concurrent chemoradiation treatment. Up-regulated genes associated with anemia were analyzed. Peripheral blood of 20 patients (bulky stage IB-IVA cervical squamous cell carcinomas) undergoing concurrent chemoradiation treatment at four times was collected. Total RNA extracted by the PAXgene Blood RNA System was analyzed with microarrays and MetaCoretrade mark functional network analyses. Fifty-three genes were significantly differentially expressed during concurrent chemoradiation treatment. Fetal and embryonic hemoglobin genes were up-regulated when patients had been severely myelosuppressed. Twenty-eight genes correlated significantly with the hemoglobin genes are involved in responses to hypoxia and oxygenation, TGF-beta signaling, cell cycle suppression, G-protein signaling, and transcriptional regulation. c-Myc has the highest rank in transcriptional co-regulation. In addition, IGKV1D-13 was significantly down-regulated in patients with severe hematological toxicity. These approaches identified biological processes in peripheral blood modulated by concurrent chemoradiation treatment and subsequent anemia.

Chen, D., T. H. Carter, et al. (2004). "Apoptosis in cervical cancer cells: implications for adjunct anti-estrogen therapy for cervical cancer." Anticancer Res **24**(5A): 2649-56.

BACKGROUND: Many tumors show dependence on estrogen for growth and establishment of drug resistance. We examined the effects of estrogen on cervical cancer cells exposed to apoptotic agents including drugs used for treatment. We tested the effect of estradiol on apoptosis in three cervical cancer cell lines. Apoptosis was measured by endonucleolytic degradation of DNA. Bcl-2 was measured by Western analysis. Estradiol reduced the percentage of cells undergoing apoptosis after exposure to the DNA-damaging agents UVB, mitomycin-C and cisplatin. Protection against taxol-induced apoptosis was marginal. Protection was independent of HPV gene expression, and not specific to apoptosis induced by DNA damage, since estradiol significantly reduced the number of apoptotic cells produced after exposure to indole-3-carbinol (I3C), a non-genotoxic phytochemical effective in preventing HPV-induced tumors. Higher concentrations of I3C overcame the anti-apoptotic effect of estradiol. Treatment with I3C resulted in loss of the survival protein Bcl-2, and estradiol partially reversed this effect. CONCLUSION: Estrogen protects cervical cancer cells treated with DNA-damaging agents; UVB, mitomycin-C and cisplatin, from apoptotic death. For I3C, which induces apoptosis and is anti-estrogenic, the amount of apoptosis versus survival and the level of Bcl-2 depend on the I3C/estradiol ratio.

Chung, H. H., M. K. Kim, et al. (2006). "XRCC1 R399Q polymorphism is associated with response to platinum-based neoadjuvant chemotherapy in bulky cervical cancer." Gynecol Oncol **103**(3): 1031-7.

OBJECTIVES: The aim of the study was to assess whether the genetic polymorphisms were associated with the tumor response in patients treated with platinum-based neoadjuvant chemotherapy (NAC) for bulky cervical cancer. We retrospectively reviewed the clinical data and recruited paraffin-embedded, formalin-fixed tissues of 36 patients with bulky cervical carcinoma. All patients underwent two or three cycles of platinum-based NAC followed by radical hysterectomy. We determined the genotypes of each single nucleotide polymorphism (SNP) of ERCC1, ERCC2, GGH, GSTP1, MTHFR, SLC19A1 and XRCC1 using single base primer extension assay. The response to platinum-based NAC was higher in patients with SNP of XRCC1 R399Q (P=0.015), and there was a significant increased chance of treatment response in women with the G/G genotype (OR 38.0; 95% CI 1.66-870.45; P=0.023). The probability of response was also higher in patients with SNP of SLC19A1 6318C/T (P=0.032). There were dose-dependent influence of the number of alleles on the response to platinum-based chemotherapy (chi2 test for linear-by-linear association; P=0.009 for XRCC1 R399Q and P=0.017 for SLC19A1 6318C/T, respectively). Moreover, the multifactor dimensionality reduction (MDR) analysis documented that the combinations of XRCC1 R399Q and GGH-401C/T genetic polymorphisms were significantly associated with response to chemotherapy (P<0.0001). CONCLUSIONS: Genetic polymorphism of XRCC1 R399Q is associated with response to platinum-based NAC in bulky cervical cancer, and MDR analysis

documented association between gene-gene interaction of XRCC1 R399Q and treatment response.

Chung, Y. M., B. G. Kim, et al. (2005). "Increased expression of ICAM-3 is associated with radiation resistance in cervical cancer." Int J Cancer **117**(2): 194-201.

To search for a marker that predicts the efficacy of radiation therapy in human cervical cancer, gene expression profiles between parental SiHa cervical cancer cells and radiation-resistant SiHa/R cells have been compared by the microarray technique. Microarray and Northern blot analyses demonstrated that the ICAM-3 expression was upregulated in SiHa/R cells. This increased expression of ICAM-3 in SiHa cells enhanced cell survival by about 34.3% after a 2 Gy dosage of radiation. In addition, SiHa/ICAM-3 cells showed a 2.45-fold higher level of FAK phosphorylation than that of the control cells. In tumor specimens, ICAM-3 staining was restricted to tumor stromal endothelial cells and lymphocytes. The overexpression of ICAM-3 was significantly more frequent in radiation-resistant cervical cancer specimens when compared with radiation-sensitive specimens (83.3% vs. 35.3%; $p = 0.015$). With these observations, we can suggest that an increased expression of ICAM-3 is associated with radiation resistance in cervical cancer cells and the expression of ICAM-3 can be used as a valuable biomarker to predict the radiation resistance in cervical cancer that occurs during radiotherapy.

Cui, Z. and L. Huang (2005). "Liposome-polycation-DNA (LPD) particle as a carrier and adjuvant for protein-based vaccines: therapeutic effect against cervical cancer." Cancer Immunol Immunother **54**(12): 1180-90.

With the successful identification of many tumor-specific antigens, tumor-associated antigens, and the potential of using unfractioned tumor cell derivatives as tumor antigens, a system and/or adjuvant that can deliver these antigens and help them to induce strong and effective anti-tumor immune responses is greatly needed. Previously, we reported that a MHC class I-restricted peptide epitope derived from human papillomavirus (HPV) 16 E7 protein, when incorporated into a clinically proven safe LPD (liposome-polycation-DNA) particle, was able to effectively eradicate tumors established in mice. Cervical cancer is the second most common cancer among women worldwide. HPV infection is clearly linked to this cancer. Vaccines based on the early (E) gene products of HPV could be effective in controlling it. However, besides the fact that epitope vaccines have many limitations particularly, concerning the diverse HLAs in humans, the use of the epitope as an antigen prevented us from fully characterizing the immune responses induced by the LPD as a vaccine carrier and/or adjuvant in previous studies. In the present study, by using the HPV 16 E7 protein as an antigen, we first showed that LPD, as a vaccine carrier and adjuvant induced strong and robust immune responses, both cellular and antibody. We then showed that immunization with LPD particles incorporated with either the wild type HPV 16 E7 protein or a potentially safer mutant induced strong immune responses that caused complete regressions of a model cervical cancer tumor established in murines. LPD could be a potent vaccine carrier and/or adjuvant for many antigens.

Dall, P., I. Herrmann, et al. (2005). "In vivo cervical cancer growth inhibition by genetically engineered cytotoxic T cells." Cancer Immunol Immunother **54**(1): 51-60.

The CD44 v7/8 splice variant that is frequently expressed in cervical carcinoma and rarely expressed in normal tissues displays promising properties as a target antigen for cancer immune therapy. In this study, cytotoxic T lymphocytes (CTLs) were genetically engineered to gain CD44v7/8 target specificity. Clone 96 (Cl96), an established murine cytotoxic T-cell line, and naive murine T cells were retrovirally transduced with a fusion gene construct encoding for the single chain fragment scFv of the monoclonal antibody VFF17 and for the zeta chain of the T-cell receptor (TCR). The therapeutic potential of genetically engineered T cells was tested in vitro and in vivo. Surface expression of the chimeric TCR on infected Cl96 and naive T cells was shown by FACS analysis. CD44v7/8-positive target cells were efficiently lysed by transduced Cl96 and naive T cells, demonstrating the functionality and specificity of the chimeric TCR. In a xenograft BALB/c mouse model, efficient growth retardation of CD44v7/8-positive tumours was mediated by genetically engineered Cl96(VFF17)cyYZ cells. CONCLUSIONS: We were able to reprogramme the target specificity of recombinant Cl96 and naive CTLs resulting in efficient cytolysis of CD44v7/8-positive cervical cancer cells. High transduction rates and the specific cytolysis of CD44v7/8-redirected CTLs are promising tools for an immune gene therapy approach for advanced cervical cancer.

Daniel, D., C. Chiu, et al. (2005). "CD4+ T cell-mediated antigen-specific immunotherapy in a mouse model of cervical cancer." Cancer Res **65**(5): 2018-25.

A major agenda for tumor immunology is the generation of specific immune responses leading to the destruction of incipient and frank neoplasia. In this report, we show that a novel HPV16 E7 fusion protein

can produce objective therapeutic responses against incipient cervical cancer in genetically engineered mice that express in the cervix the HPV16 early region genes implicated as causative agents in human cervical cancer. Although nonresponsive toward the HPV16 E7 oncoprotein in the CD8+ T-cell compartment by virtue of MHC haplotype, the mice were capable of mounting an induced CD4+ T-cell response against E7, and in addition developed spontaneous anti-E7 antibodies. HPV16/CD4-/- mice showed increased tumor burden indicative of CD4-mediated immune surveillance. Seeking to enhance the CD4 response, we immunized mice bearing incipient cervical cancer with a recombinant protein fusing E7 with a mycobacterial heat shock protein. The incidences of cervical carcinoma and of high-grade dysplasia (CIN 3) were consequently reduced by comparison to control mice. Thus, an HPV16 E7 immunogen holds promise for noninvasive treatment and prevention of human cervical cancer.

Das, S. and K. Somasundaram (2006). "Therapeutic potential of an adenovirus expressing p73 beta, a p53 homologue, against human papilloma virus positive cervical cancer in vitro and in vivo." Cancer Biol Ther **5**(2): 210-7.

Human papilloma virus (HPV) infection is the most important risk factor for cervical cancer development. p53 based gene therapy is not suitable for cervical cancer because HPV oncoprotein E6 inactivates p53 protein by targeting it for ubiquitin mediated degradation. Here we evaluated the efficiency of Ad-p73, a replication deficient adenovirus expressing p73beta a p53 homologue, to inhibit the growth of HPV positive cervical cancer cells in vitro using tissue culture system and in vivo using human xenografts in nude mice. Ad-p73, but not Ad-p53 (p53 adenovirus), inhibited the growth in vitro of three different HPV positive cervical cancer cell lines, HeLa, ME180, and SiHa, efficiently, which correlated with stable expression of functional p73 protein. However, the growth of a HPV negative cervical cancer cell line, C33A, was inhibited equally by both Ad-p73 and Ad-p53. In addition, we show that Ad-p73 preinfected HeLa cells and HCT116 E6 cells, an E6 stable cell line, failed to form tumors in nude mice unlike Ad-p53 or Ad-LacZ preinfected cells. Moreover, Ad-p73, but not Ad-p53, inhibited completely the growth of already established tumors of HeLa or HCT116 E6 cells. Furthermore, the ability of p73 to inhibit the growth of these tumors correlated with the stable expression of p73 protein with the concomitant induction of its target gene p21(WAF1/CIP1) and induction of apoptosis in tumor cells. These results suggest that Ad-p73 inhibits efficiently the growth in vitro and tumorigenicity and tumor growth in vivo of HPV positive cervical cancer cells and that p73-based approach should be explored as a potential therapeutic model for the treatment of cervical cancer.

de la Cruz-Hernandez, E., E. Perez-Cardenas, et al. (2007). "The effects of DNA methylation and histone deacetylase inhibitors on human papillomavirus early gene expression in cervical cancer, an in vitro and clinical study." Virol J **4**: 18.

BACKGROUND: The methylation status at the human papilloma virus (HPV) genome found in pre-invasive and invasive cervical lesions suggests that neoplastic transformation can be suppressed by gene hypermethylation, whereas hypomethylation accompanies or causes cancer progression; hence, epigenetic therapy aimed at reactivating cellular suppressor-gene expression has the potential to act as a tumor promoter by enhancing HPV oncoprotein expression in HPV-related malignancies. The objective of this study was to determine the influence of hydralazine and valproate on HPV oncogene expression in cervical cancer cell lines and the primary tumors of patients undergoing treatment with hydralazine and valproate. Overall, hydralazine and valproate either alone or combined exerted a growth inhibitory effect on cervical cancer cell lines. A cell line-specific up-regulating effect was observed on E6/E7 gene expression, which in general correlated with DNA hypomethylation and histone acetylation at the long control region (LCR). Nonetheless, E6/E7 expression was unchanged or decreased in the majority of patients with cervical cancer treated with hydralazine, valproate, or both. In some cervical cancer cell lines, these drugs led to increased transcription of p53, and increased its stabilization due to acetylation at lysines 273 and 282, which allowed a higher bax-protein transactivating effect. CONCLUSION: The results of this study demonstrate that hydralazine and valproate can be safely administered to HPV-related malignancies such as cervical cancer because they do not increase viral oncoprotein expression. Most importantly, the antitumor effect of hydralazine and valproate in cervical cancer may at least partially depend on an up-regulating effect on p53 gene and on the valproate-induced hyperacetylation of p53 protein, protecting it from degradation by E6.

Echchannaoui, H., M. Bianchi, et al. (2008). "Intravaginal immunization of mice with recombinant Salmonella enterica serovar Typhimurium expressing human papillomavirus type 16 antigens as a potential route of vaccination against cervical cancer." Infect Immun **76**(5): 1940-51.

Cervical cancer, the second leading cause of cancer deaths in women, is the consequence of high-risk human papillomavirus (HPV) infections. Toward the development of therapeutic vaccines that can induce both innate and adaptive mucosal immune responses, we analyzed intravaginal (ivag) vaccine delivery of live attenuated Salmonella enterica serovar Typhimurium expressing HPV16L1 as a model antigen. Innate immune responses were examined in cervicovaginal tissues by determining gene expression patterns by microarray analysis using nylon membranes imprinted with cDNA fragments coding for inflammation-associated genes. At 24 h, a wide range of genes, including those for chemokines and Th1- and Th2-type cytokine and chemokine receptors were up-regulated in mice ivag immunized with Salmonella compared to control mice. However, the majority of transcripts returned to their steady-state levels 1 week after immunization, suggesting a transient inflammatory response. Indeed, cervicovaginal histology of immunized mice showed a massive, but transient, infiltration of macrophages and neutrophils, while T cells were still increased after 7 days. Ivag immunization also induced humoral and antitumor immune responses, i.e., serum and vaginal anti-HPV16VLP antibody titers similar to those induced by oral immunization, and significant protection in tumor protection experiments using HPV16-expressing C3 tumor cells. These results show that ivag immunization with live attenuated Salmonella expressing HPV16 antigens modulates the local mucosal gene expression pattern into a transient proinflammatory profile, elicits strong systemic and mucosal immunity against HPV16, and confers protection against HPV16 tumor cells subcutaneously implanted in mice. Examination of the efficacy with which ivag HPV16E7E6 Salmonella induces regression of tumors located in cervicovaginal tissue is warranted.

Ferrandina, G., F. O. Ranelletti, et al. (2006). "Celecoxib up-regulates the expression of the zeta chain of T cell receptor complex in tumor-infiltrating lymphocytes in human cervical cancer." Clin Cancer Res **12**(7 Pt 1): 2055-60.

We evaluated the effects of celecoxib treatment on tumor-infiltrating lymphocyte (TIL) subsets [CD3(+), CD4(+),CD8(+), CD25(+), and T cell receptor (TCR)-zeta-expressing cells] and tryptase-positive mast cells in cervical tumors. Circulating levels of cytokines [interleukin (IL)-1beta, IL-10, tumor necrosis factor-alpha, IL-6, and IL-12] and angiogenesis-modulating factors (vascular endothelial growth factor and endostatin) have also been analyzed. EXPERIMENTAL DESIGN: Cervical tumor biopsies and blood samples were obtained at the time of diagnosis and after 10 days of celecoxib treatment (400 mg b.i.d., at 8:00 a.m. and 8:00 p.m.) in 27 cases. Immunohistochemistry and ELISA assays were used to assess the expression of biological factors in tumor tissue and circulating levels of cytokines and angiogenic molecules. We showed a statistically significant increase in the percentage of TIL expressing the TCR-zeta chain after celecoxib treatment: indeed, in cases exposed to celecoxib, the percentage of TCR-zeta(+) cells ranged from 5.0 to 50.0 (median, 22.5) with respect to baseline expression (range, 3.0-50.0; median, 10.0; P = 0.0016). There was no significant treatment-related difference in the percentage of CD3(+), CD4(+), CD8(+), and CD25(+) TIL as well as in tryptase-positive cells. IL-12 levels were significantly reduced in posttreatment samples with respect to baseline levels (P = 0.002). We also found a reduction in the circulating levels of vascular endothelial growth factor, and a statistically significant increase of serum endostatin levels (P = 0.035). CONCLUSIONS: We reported the first evidence in humans that celecoxib restores zeta expression by TIL in primary cervical tumors, suggesting that a positive modulation of immune function may serve as an additional mechanism supporting the antitumor effect of this class of drugs.

Fujii, T., M. Saito, et al. (2006). "Intratumor injection of small interfering RNA-targeting human papillomavirus 18 E6 and E7 successfully inhibits the growth of cervical cancer." Int J Oncol **29**(3): 541-8.

Human papillomavirus (HPV) 18 is related not only to squamous cell carcinoma of the cervix, but also to adenocarcinoma and small cell carcinoma of the cervix, in which prognosis is known to be poor. Small interfering RNA (siRNA) that targets HPV18 E6 and E7 was tested in HPV18-positive cell lines to investigate its effect and investigate its mechanism of action. Nude mice were also tested in a combination of siRNA and atelocollagen to determine whether it might be useful as a new molecule-targeting therapy for cervical cancer. siRNAs targeting HPV18 E6 and E7 were transfected into cervical cancer cells in vitro and they were investigated for cell growth inhibition, expression of E6 and E7 mRNA, expression of retinoblastoma protein, and senescence-associated beta-galactosidase staining. Sequence-specific siRNA inhibited cell growth. Decreased expression of E6 and E7 mRNA followed with E7 protein was observed in the transfected cells, but the expression of retinoblastoma protein and the beta-galactosidase staining increased, suggesting cell growth inhibitory effect through senescence. Treatment of xenografts established from SKG-II cells with siRNA specific for E6 and E7 obviously suppressed tumor growth in

vivo. These results indicate that atelocollagen-mediated delivery of siRNA HPV18 E6 and E7 can be used as a novel therapeutic approach for cervical cancer.

Green, K. L., T. D. Southgate, et al. (2006). "Diffusible VP22-E2 protein kills bystander cells and offers a route for cervical cancer gene therapy." Hum Gene Ther **17**(2): 147-57.

Human papillomaviruses (HPVs) are a causative agent of cervical cancer and are implicated in several other types of malignant disease including cancer of the vulva, oral cancer, and skin cancer. In HPV-transformed cells, expression of the viral E6 and E7 oncogenes increases cell proliferation and inhibits apoptosis. Expression of the viral E2 protein in HPV-transformed cells represses transcription of E6 and E7 and induces apoptosis and/or growth arrest. We have shown previously that herpes simplex virus type 1 (HSV-1) VP22-HPV E2 fusion proteins can traffic between cells and induce apoptosis. Here we show that replication-defective adenoviruses can be used to deliver VP22-E2 fusion proteins to target cells. We show that the use of adenoviral vectors to deliver VP22-E2 proteins leads to high levels of apoptosis. Interestingly, VP22-E2 proteins produced in adenovirus-infected cells are able to enter uninfected cells and induce apoptosis. Trafficking between cells and the induction of apoptosis in bystander cells are detectable in a three-dimensional tumor model. These results suggest that adenoviral vectors expressing VP22-E2 fusion proteins could be used to treat cervical cancer and other HPV-associated diseases.

Gu, W., L. Putral, et al. (2006). "Inhibition of cervical cancer cell growth in vitro and in vivo with lentiviral-vector delivered short hairpin RNA targeting human papillomavirus E6 and E7 oncogenes." Cancer Gene Ther **13**(11): 1023-32.

In this study, we investigated the suppressive effect of a short hairpin RNA delivered by a lentiviral vector (LV-shRNA) against human papillomavirus (HPV) type 18 E6 on the expression of the oncogenes E6 and E7 in cervical cancer HeLa cells both in vitro and in vivo. The LV-shRNA effectively delivered the shRNA to HeLa cells and lead to a dose-dependent reduction of E7 protein and the stabilization of E6 target proteins, p53 and p21. Low-dose infection of HeLa cells with LV-shRNA caused reduced cell growth and the induction of senescence, whereas a high-dose infection resulted in specific cell death via apoptosis. Transplant of HeLa cells infected with a low dose of LV-shRNA into Rag-/- mice significantly reduced the tumor weight, whereas transplant of cells infected with a high dose resulted in a complete loss of tumor growth. Systemic delivery of LV-shRNA into mice with established HeLa cell lung metastases led to a significant reduction in the number of tumor nodules. Our data collectively suggest that lentiviral delivery is an effective way to achieve stable suppression of E6/E7 oncogene expression and induce inhibition of tumor growth both in vitro and in vivo. These results encourage further investigation of this form of RNA interference as a promising treatment for cervical cancer.

Gu, W., L. Putral, et al. (2008). "siRNA and shRNA as anticancer agents in a cervical cancer model." Methods Mol Biol **442**: 159-72.

We describe the protocols of using siRNAs, or shRNAs delivered by a lentiviral vector, as a means to silence cancer-causing genes. We use cervical cancer as a model to demonstrate the inhibition of the human papillomavirus (HPV) oncogenes E6 and E7 in cervical cancer cells by RNAi and inhibition of the cell growth in vitro and tumor growth in mouse models. The protocols include methods on siRNA and shRNA design, production of lentiviral-vectored shRNA, transfection or transduction of cervical cancer cells with siRNA or shRNA, and detection of the inhibitory effects of siRNA or shRNA both in vitro and in vitro.

Gu, W., L. N. Putral, et al. (2007). "The development and future of oligonucleotide-based therapies for cervical cancer." Curr Opin Mol Ther **9**(2): 126-31.

Cervical cancer is an attractive model in which to test gene-specific therapies, because elimination of the HPV oncogenes E6 and E7 may result in cancer cell senescence. Oligonucleotide-based therapies tested over the years include antisense oligonucleotides, ribozymes and, more recently, small interfering RNA (siRNA)-based treatments. The development and use of these technologies are reviewed. siRNA-based therapies have been touted as potential treatments for cancers, genetic disorders and viral infections and have a number of advantages over antisense and ribozyme technologies. As with the older technologies, in vitro testing of siRNAs against cervical cancer has shown promising results, however, the issues that held up the clinical development of ribozymes and antisense are currently also challenging the siRNA field; these are target selection, specificity and delivery. If these issues can be overcome, a range of new and potent therapies for cervical cancer could become available.

Gupta, N., P. M. Martin, et al. (2006). "Down-regulation of BCRP/ABCG2 in colorectal and cervical cancer." Biochem Biophys Res Commun **343**(2): 571-7.

Expression of Breast Cancer Resistance Protein (BCRP/ABCG2) in tumor cells is associated with resistance to multiple chemotherapeutic agents. BCRP also protects against phototoxicity by mediating the efflux of protoporphyrins from cells. However, chemotherapy and photodynamic therapy are effective treatment options for cancer. Furthermore, protoporphyrins are essential, in the form of heme, for the synthesis of nitric oxide, over-production of which is associated with cancer. This raises the question as to whether the expression of this transporter is altered in cancer. To address this question, we investigated the expression of BCRP in colorectal cancer and cervical cancer. Paired normal and cancer tissues from colectomy specimens were used for the analysis of BCRP mRNA by RT-PCR and Northern blot. BCRP was analyzed by immunohistochemistry/immunofluorescence. Similar studies were also done with specimens of normal cervix and cancer cervix. A commercial dot blot was probed to quantify the expression of BCRP in paired normal and cancer cDNA samples from 154 patients with tumors in 19 different tissues. BCRP mRNA was present in normal colorectal tissue and showed a 6-fold decrease in cancer. BCRP was abundant in the normal colon and showed a decrease in colon cancer. The down-regulation of BCRP mRNA and protein was also evident in cervical cancer. There was also a decrease in BCRP mRNA in cancer in 12 of the 19 different tissues collected from 154 patients. These data show that cancer-associated down-regulation of BCRP is likely to be a common phenomenon in several tissues. Decreased expression of BCRP may have a role in tumorigenesis by allowing accumulation of genotoxins and over-production of nitric oxide.

Gurska, S., T. Farkasova, et al. (2007). "Radiosensitivity of cervical cancer cell lines: the impact of polymorphisms in DNA repair genes." Neoplasma **54**(3): 195-201.

The aim of this study was to evaluate radiosensitivity of cervical cancer cells in vitro and to assess the relationship between genetic polymorphisms in DNA repair genes and the response of cells to ionizing radiation. The alkaline comet assay as a predictive assay of radiosensitivity was used to examine the susceptibility of four human cervical cancer cell lines (CaSki, C-33A, HeLa and SiHa) to radiation damages. The initial DNA damage and the residual DNA damage at 15, 30, 45 and 60 min after irradiation were assessed. Genotypes of DNA repair genes (XRCC1, hOGG1, PARP, XPD, XRCC3 and XRCC4) were analyzed by PCR-RFLP assays. The comet data clearly indicate a variable but dose-dependent increase in the initial DNA damage in all cell lines. The highest slope of dose response curve was observed in C-33A cells and this cell line was assumed to be radiosensitive. All cell lines repaired DNA damage in a similar manner, the level of DNA strand breakage has returned near the background level within 45 min after irradiation. According to the genotype we found that C-33A cells are polymorphic in the majority of analyzed DNA repair genes. This pilot study indicated associations between polymorphisms in DNA repair genes and cell radiosensitivity.

Hamada, K., T. Shirakawa, et al. (2006). "Adenovirus-mediated transfer of human papillomavirus 16 E6/E7 antisense RNA and induction of apoptosis in cervical cancer." Gynecol Oncol **103**(3): 820-30.

In most cervical cancers, human papillomaviruses (HPVs) are identified. The E6 and E7 genes of HPVs encode proteins, that interfere with the function of the tumor suppressor proteins p53 and Rb. We are exploring the potential use of antisense HPV RNA transcripts for gene therapy for HPV-positive cervical cancers. Via a recombinant adenoviral vector, Ad5CMV-HPV 16 AS, we introduced the antisense RNA transcripts of the E6 and E7 genes of HPV type 16 into human cervical cancer SiHa cells harboring HPV 16. We then analyzed the effects of expression of these genes on cell and tumor growth. HPV 16 E6/E7 antisense RNA was detected for 14 days in Ad5CMV-HPV 16 AS-infected cells. After infection, E6 and E7 protein expression was suppressed, and p53 and Rb protein expression increased. The Ad5CMV-HPV 16 AS-infected cells underwent apoptosis in vitro and in vivo. Cell growth and tumorigenicity were greatly suppressed. Ad5CMV-HPV 16 AS treatment significantly reduced the volumes of established subcutaneous tumors. CONCLUSION: Transfection of cervical cancer cells with HPV 16 E6/E7 antisense RNA in a form such as Ad5CMV-HPV 16 AS might be a potentially useful approach to the therapy of HPV 16-positive cervical cancer.

Hamada, K., N. Ueda, et al. (2005). "The nude rat as an orthotopic model for cervical cancer." Gynecol Oncol **99**(3 Suppl 1): S159-65.

The purposes of this study were to establish intracervical tumors of the nude rat as an orthotopic experimental model for human cervical cancer and to preliminary evaluate the effects of the adenoviral vector, Ad5CMV-p53, on orthotopic cervical tumor size. Human cervical cancer SiHa and ME-180 cells were injected into the cervix of the nude rat. Four days later, 1 x 10(9) plaque forming units (PFU) of Ad5CMV-p53 were injected into the cervix. The rats were later sacrificed to determine cervical tumor size. Eight of ten nude rats developed SiHa cell tumors; all

ten nude rats developed ME-180 cell tumors. Four of ten SiHa cell tumors metastasized to the pelvic cavity; no ME-180 cell tumors did. The growth of Ad5CMV-p53-infected cells was greatly suppressed. The ad5CMV-p53 treatment significantly reduced both cell tumor volumes in nude rat cervixes. CONCLUSION: The nude rat cervix grows tumors similar to human cervical cancer tumors and makes an excellent experimental model. Transfection of cervical cancer cells with the wild-type p53 gene via Ad5CMV-p53 is a potential therapeutic approach to cervical cancer.

Harima, Y., A. Togashi, et al. (2004). "Prediction of outcome of advanced cervical cancer to thermoradiotherapy according to expression profiles of 35 genes selected by cDNA microarray analysis." Int J Radiat Oncol Biol Phys **60**(1): 237-48.

To identify a set of genes related to thermoradiosensitivity of cervical carcinoma and to establish a predictive method. A total of 19 patients with cervical cancer (1 with Stage IIIA, 11 with Stage IIIB, 5 with Stage IVA, and 2 with Stage IVB) who underwent definitive thermoradiotherapy between May 1995 and August 2001 were included in this study. We compared the expression profiles of 8 thermoradiosensitive and 11 thermoradioresistant tumors obtained by punch biopsy before treatment using a cDNA microarray consisting of 23,040 human genes. We selected 35 genes on the basis of a clustering analysis and confirmed the validity of these genes with a cross-validation test. Some of these genes were already known to be associated with apoptosis (BIK, TEGT, SSI-3), hypoxia-inducible genes (HIF1A, CA12), and tumor cell invasion and metastasis (CTSL, CTSB, PLAU, CD44). We developed a "predictive score" system that could clearly separate the thermoradiosensitive group from the thermoradioresistant group. CONCLUSION: These results from the treatment program between May 1995 and August 2001 showed that by using gene-expression profiles we can predict the outcome of thermoradiotherapy for advanced cervical carcinoma. A "predictive score" system was developed that could clearly separate the thermoradiosensitive group from the thermoradioresistant group. These results may eventually lead to the achievement of "personalized therapy" for this disease.

Hasina, R., A. L. Pontier, et al. (2006). "NOL7 is a nucleolar candidate tumor suppressor gene in cervical cancer that modulates the angiogenic phenotype." Oncogene **25**(4): 588-98.

Cervical cancer is associated with human papilloma virus infection. However, this infection is insufficient to induce transformation and progression. Loss of heterozygosity analyses suggest the presence of a tumor suppressor gene (TSG) on chromosome 6p21.3-p25. Here we report the cloning NOL7, its mapping to chromosome band 6p23, and localization of the protein to the nucleolus. Fluorescence in situ hybridization analysis demonstrated an allelic loss of an NOL7 in cultured tumor cells and human tumor samples. Transfection of NOL7 into cervical carcinoma cells inhibited their growth in mouse xenografts, confirming its in vivo tumor suppressor activity. The induction of tumor dormancy correlated with an angiogenic switch caused by a decreased production of vascular endothelial growth factor and an increase in the production of the angiogenesis inhibitor thrombospondin-1. These data suggest that NOL7 may function as a TSG in part by modulating the expression of the angiogenic phenotype.

He, J., C. Huang, et al. (2008). "Proteomic analysis of cervical cancer cells treated with suberonylanilide hydroxamic acid." J Biosci **33**(5): 715-21.

Suberonylanilide hydroxamic acid (SAHA)is an orally administered histone deacetylase inhibitor (HDACI) that has shown significant antitumour activity in a variety of tumour cells.To identify proteins involved in its antitumour activity,we utilized a proteomic approach to reveal protein expression changes in the human cervical cancer cell line HeLa following SAHA treatment.Protein expression profiles were analysed by 2-dimensional polyacrylamide gel electrophoresis (2-DE) and protein identification was performed on a MALDI-Q-TOF MS/MS instrument.As a result,a total of nine differentially expressed proteins were visualized by 2-DE and Coomassie brilliant blue (CBB) staining.Further,all the changed proteins were positively identified via mass spectrometry (MS)/MS analysis. Of these,PGAM1 was significantly downregulated in HeLa cells after treatment with SAHA. Moreover,PGAM1 has been proven to be downregulated in another cervical cancer cell line (CaSki) by western blot analysis.Together,using proteomic tools,we identified several differentially expressed proteins that underwent SAHA-induced apoptosis. These changed proteins may provide some clues to a better understanding of the molecular mechanisms underlying SAHA-induced apoptosis in cervical cancer.

Heideman, D. A., R. D. Steenbergen, et al. (2005). "Oncolytic adenovirus expressing a p53 variant resistant to degradation by HPV E6 protein exhibits potent and selective replication in cervical cancer." Mol Ther **12**(6): 1083-90.

Rationale in the development of novel treatment strategies for HPV-associated cancers is targeting on the basis of the presence of HPV in (pre)malignant cells. Here, we designed a new conditionally replicating adenovirus (CRAd) for selective and effective oncolytic replication in HPV-containing cells. As the backbone, we used the CRAd AdCB016, which replicates selectively in cells expressing HPV E6 and E7 proteins. To enhance its oncolytic potency, we armed AdCB016 with p53 variant mp53(268N), which is resistant to HPV E6-mediated degradation. The new CRAd AdCB016-mp53(268N) was analyzed for its lytic replication properties in cervical carcinoma cell lines, HPV-immortalized keratinocyte cell lines representing dysplastic cells, and primary human keratinocytes. AdCB016-mp53(268N) exhibited 10- to 1000-fold greater efficacy than AdCB016 on high-risk HPV-positive cervical carcinoma cells and HPV-immortalized keratinocytes. Importantly, infection with AdCB016-mp53(268N) did not affect primary nonmalignant human keratinocytes. This favorable efficacy and safety profile was confirmed in organotypic raft cultures. Our findings suggest that AdCB016-mp53(268N) is a promising new agent for treatment of HPV-associated human cancers.

Heo, M. Y., S. A. Salama, et al. (2008). "Abrogation of estrogen receptor signaling augments cytotoxicity of anticancer drugs on CaSki cervical cancer cells." Anticancer Res **28**(4B): 2181-7.

BACKGROUND: We have reported that a gene therapy approach, using a dominant-negative estrogen receptor blocker (DN) gene, can cause cell death in cervical cancer cells in vitro. We investigated the mechanisms for enhanced cell killing when DN was combined with cisplatin (CP) and paclitaxel (TX). Cells were transduced with DN at 24 h and/or treated with drugs at 48 h, and harvested at 48 and 72 h after transduction. Effects were determined using the MTT cytotoxic, and TUNEL and caspase-3 activity apoptotic assays. Each agent induced cytotoxic and apoptotic effects, and activated caspase-3. In the combined treatments, significant synergistic effects were observed based on the MTT and TUNEL assays, but with antagonistic caspase-3 activation effect. CONCLUSION: The enhanced cell killing effect was mediated by the initiation of new and multiple mechanisms, particularly via caspase-independent pathways.

Hsu, K. F., C. L. Wu, et al. (2008). "Conditionally replicating E1B-deleted adenovirus driven by the squamous cell carcinoma antigen 2 promoter for uterine cervical cancer therapy." Cancer Gene Ther **15**(8): 526-34.

Cervical cancer is the second most common type of malignant tumor among women worldwide. When the disease is confined locally, it can be controlled with surgical resection and radiotherapy. However, patients with recurrent or metastatic disease often have a poor prognosis. Measurement of serum levels of squamous cell carcinoma (SCC) antigens has been widely used as serological markers for SCC of uterine cervix. Recently, it has been demonstrated that cervical cancer patients with elevated squamous cell carcinoma antigen-2 (SCCA2) expression in tumor cells carry a poor prognosis. Here, by using a luciferase reporter assay, we show that SCCA2 promoter was active in SCCA2-producing human cervical cancer cell lines, including Cx, Cxwj, SiHa and HeLa cells, but relatively quiescent in normal cervical epithelial cells. We then developed a conditionally replicating adenovirus, designated Ad-KFH, under the transcriptional control of the SCCA2 promoter. This E1B-55 kDa-deleted oncolytic adenovirus replicated specifically in and lysed SCCA2-producing cervical cancer cells. Furthermore, in a peritoneal metastatic tumor model, Ad-KFH retarded Cxwj tumor growth in NOD/severe combined immunodeficient mice and prolonged survival of tumor-bearing mice, especially when combined with cisplatin. These results suggest that Ad-KFH may provide a new strategy of gene therapy for advanced or recurrent uterine cervical cancer.

Huang, A. C., S. C. Hsu, et al. (2009). "Involvement of matrix metalloproteinases in the inhibition of cell invasion and migration through the inhibition of NF-[kappa]B by the new synthesized ethyl 2-[N-p-chlorobenzyl-(2'-methyl)]anilino-4-oxo-4,5-dihydrofuran-3-carboxylate (JOTO1007) in human cervical cancer Ca ski cells." In Vivo **23**(4): 613-9.

JOTO1007 (ethyl 2-[N-p-chlorobenzyl-(2'-methyl)] anilino-4-oxo-4,5-dihydrofuran -3-carboxylate) has anticancer effects in human cervical cancer Ca Ski cells. However, its mechanism of action on the cell migration and invasion of human cervical cancer Ca Ski cells is not fully understood. In this study, firstly, the effects of JOTO1007 on the migration and invasion of Ca Ski cells were examined by using matrigel counting. The results showed that JOTO1007 suppressed the migration and invasion of the Ca Ski cells. Secondly, the effect of JOTO1007 on the levels of proteins associated with cell metastasis was examined using Western blotting. The results indicated that JOTO1007 inhibited the levels of son of sevenless homolog 1 (SOS-1), growth factor receptor-bound protein 2 (GRB2), Ras homolog gene family, member A (RhoA), Rho-associated, coiled-coil containing protein kinase 1 (ROCK-1), focal adhesion kinase (FAK), phosphorylated-c-jun (p-c-jun), nuclear

factor kappa B (NF-kappaB) p65, cyclooxygenase-2 (COX-2), extracellular signal-regulated kinases 1/2 (ERK1/2), matrix metalloproteinase-2 (MMP-2), MMP-7 and MMP-9 but promoted the levels of protein kinase C (PKC), phosphoinositide 3-kinases (PI3K), MAP kinase kinase kinase 3 (MEKK3), mitogen-activated protein kinase kinase 7 (MKK7), c-jun and inducible nitric oxide synthases (iNOS), while not affecting Ras, phosphorylated-ERK (p-ERK), p38 and c-jun N-terminal kinase 1/2 (JNK1/2), which finally led to the inhibition of migration and invasion of the Ca Ski cells in vitro. Overall, JOTO1007 inhibited NF-kappaB which then led to the inhibition of the MMP-2, -7 and -9 expression followed by the inhibition of migration and invasion in the Ca Ski cells.

Hung, C. F., A. Monie, et al. (2007). "DNA vaccines for cervical cancer: from bench to bedside." Exp Mol Med **39**(6): 679-89.

More than 99% of cervical cancers have been associated with human papillomaviruses (HPVs), particularly HPV type 16. The clear association between HPV infection and cervical cancer indicates that HPV serves as an ideal target for development of preventive and therapeutic vaccines. Although the recently licensed preventive HPV vaccine, Gardasil, has been shown to be safe and capable of generating significant protection against specific HPV types, it does not have therapeutic effect against established HPV infections and HPV-associated lesions. Two HPV oncogenic proteins, E6 and E7, are consistently co-expressed in HPV-expressing cervical cancers and are important in the induction and maintenance of cellular transformation. Therefore, immunotherapy targeting E6 and/or E7 proteins may provide an opportunity to prevent and treat HPV-associated cervical malignancies. It has been established that T cell-mediated immunity is one of the most crucial components to defend against HPV infections and HPV-associated lesions. Therefore, effective therapeutic HPV vaccines should generate strong E6/E7-specific T cell-mediated immune responses. DNA vaccines have emerged as an attractive approach for antigen-specific T cell-mediated immunotherapy to combat cancers. Intradermal administration of DNA vaccines via a gene gun represents an efficient way to deliver DNA vaccines into professional antigen-presenting cells in vivo. Professional antigen-presenting cells, such as dendritic cells, are the most effective cells for priming antigen-specific T cells. Using the gene gun delivery system, we tested several DNA vaccines that employ intracellular targeting strategies for enhancing MHC class I and class II presentation of encoded model antigen HPV-16 E7. Furthermore, we have developed a strategy to prolong the life of DCs to enhance DNA vaccine potency. More recently, we have developed a strategy to generate antigen-specific CD4(+) T cell immune responses to further enhance DNA vaccine potency. The impressive pre- clinical data generated from our studies have led to several HPV DNA vaccine clinical trials.

Iwakawa, M., T. Ohno, et al. (2007). "The radiation-induced cell-death signaling pathway is activated by concurrent use of cisplatin in sequential biopsy specimens from patients with cervical cancer." Cancer Biol Ther **6**(6): 905-11.

To identify changes in gene expression related to the concurrent use of platinum compounds with radiotherapy, in the treatment of cervical cancer. Biopsy specimens were obtained from 39 patients with squamous cell carcinoma of the uterine cervix, before and during fractionated radiotherapy. Twenty patients were treated with radiotherapy (RT) alone, while 19 received the same radiotherapy plus concomitant chemotherapy with cisplatin (CRT). Changes in gene expression induced by treatment were investigated using single-color oligo-microarrays consisting of 44K human sequences. Paraffin-embedded samples were used to examine apoptosis and the expression of protein by treatment-responsive genes. Changes in mRNA expression were assessed for these genes by real-time reverse transcriptase-polymerase chain reaction. Aberrant genomic change (detected using microarray-based comparative genomic hybridization), human papillomavirus infection, and p53 status were also evaluated. The expression of CDKN1A, BAX, TNFSF8, and RRM2B was consistently upregulated by CRT (9 Gy with a single administration of cisplatin). Similar expression changes were induced by RT (9 Gy) alone, although the variability between tumors was greater. Apoptotic cells were significantly increased in both groups. CRT significantly increased the numbers of cases with diffusely distributed CDKN1A-positive cells. Genetic losses at 2q33-ter and gains of 3q26-ter were detected in the samples with high frequency; 60% were positive for human papillomavirus DNA; and three tumors had deletions/mutations of the p53 gene. There was no difference in the incidence of these genomic changes between the groups, and no association was found with the changes in expression of CDKN1A, BAX, TNFSF8 or RRM2B. CONCLUSIONS: Using biopsy samples from pretreatment and midtreatment cervical tumors, we identified therapy-induced genes related to the cell death signaling pathway. CRT produced a homogenous pattern of changes in expression of known radiation-responsive genes.

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.

Jones, E. E. and S. I. Wells (2006). "Cervical cancer and human papillomaviruses: inactivation of retinoblastoma and other tumor suppressor pathways." Curr Mol Med **6**(7): 795-808.

Infection with human papillomaviruses (HPVs) is a major public health burden worldwide and is associated with benign and malignant lesions of the skin and genital tract. HPV causes cervical cancer, which represents the second most prevalent cancer in women worldwide. Functions of the viral oncogenes E6 and E7 are essential for carcinogenesis and for support of the viral life cycle. We will begin by discussing the relationship between HPV infection and disease, followed by a review of E6 and E7 activities and their respective cellular targets. Particular emphasis will be placed on established and newly discovered mechanisms by which E7 inhibits members of the cellular retinoblastoma protein family. We will then describe how current research links the above molecular interactions to malignant transformation as well as to aspects of the viral life cycle in vitro and in vivo. As a result of decades of intense HPV research, promising therapies to prevent infection and to treat HPV associated cancers are now on the horizon. We will conclude our review by a description of potential gene therapeutic and hormonal approaches and of new developments in the design of effective vaccines.

Jonson, A. L., L. M. Rogers, et al. (2008). "Gene silencing with siRNA targeting E6/E7 as a therapeutic intervention in a mouse model of cervical cancer." Gynecol Oncol **111**(2): 356-64.

Selective silencing of HPV oncogenes using short interfering RNA (siRNA) blocks E6/E7 expression and restores normal p53 and Rb function. Our objective was to determine if siRNA targeting E6/E7 would inhibit the growth of established tumors in a mouse model of cervical cancer. In vitro studies were performed using unique siRNA sequences to confirm their ability to target and reduce E6/E7 mRNA and restore functioning p53. Next, siRNA targeting lamin was injected daily for three days into tumors established from HPV 16 positive CaSki human cervical cancer cells. Immunohistochemistry and branched DNA gene quantification were used to determine distribution and duration of activity of these siRNA. For our therapeutic studies tumors were directly injected with siRNA targeting E6/E7, non-targeting control siRNA, or saline. In preliminary experiments injections were daily or every three days for a total of three doses. A second therapeutic experiment utilized every three day dosing for 35 days. Tumor volume, growth curves and E7 mRNA levels were assessed. The two most active siRNA sequences resulted in a 67% and 71% reduction in E6/E7 mRNA. Fluorescent lamin siRNA was visualized up to 120 h after the initial tumor injection and was evenly distributed throughout the tumors. IHC showed lamin expression to be inhibited by 68% and 75% when compared to controls at 54 and 120 h respectively. In our preliminary therapeutic intervention experiments there was no significant difference in tumor growth between the treatment groups when mice were treated with three daily injections ($p=0.41$). However, when treated every third day for three injections final tumor volume was less in animals injected with siRNA sequences A (78% reduction; $p<0.0001$) and G (60% reduction; $p=0.005$) compared to saline injection. Tumors showed a corresponding decrease in E6/E7 mRNA. Extended treatment with siRNA completely or nearly

eradicated tumors in 70% of the animals. CONCLUSION: Therapeutic siRNA targeting E6/E7 significantly inhibits tumor growth in this mouse model of cervical cancer. Further investigation is needed to determine optimal dosing and route of delivery.

Kanerva, A., S. Lavilla-Alonso, et al. (2008). "Systemic therapy for cervical cancer with potentially regulatable oncolytic adenoviruses." PLoS One **3**(8): e2917.

Clinical trials have confirmed the safety of selectively oncolytic adenoviruses for treatment of advanced cancers. However, increasingly effective viruses could result in more toxicity and therefore it would be useful if replication could be abrogated if necessary. We analyzed viruses containing the cyclooxygenase-2 (Cox-2) or vascular endothelial growth factor (VEGF) promoter for controlling replication. Anti-inflammatory agents can lower Cox-2 protein levels and therefore we hypothesized that also the promoter might be affected. As Cox-2 modulates expression of VEGF, also the VEGF promoter might be controllable. First, we evaluated the effect of anti-inflammatory agents on promoter activity or adenovirus infectivity in vitro. Further, we analyzed the oncolytic potency of the viruses in vitro and in vivo with and without the reagents. Moreover, the effect of on virus replication was analyzed. We found that RGD-4C or Ad5/3 modified fibers improved the oncolytic potency of the viruses in vitro and in vivo. We found that both promoters could be downregulated with dexamethasone, sodium salicylate, or salicylic acid. Oncolytic efficacy correlated with the promoter activity and in vitro virus production could be abrogated with the substances. In vivo, we saw good therapeutic efficacy of the viruses in a model of intravenous therapy of metastatic cervical cancer, but the inhibitory effect of dexamethasone was not strong enough to provide significant differences in a complex in vivo environment. Our results suggest that anti-inflammatory drugs may affect the replication of adenovirus, which might be relevant in case of replication associated side effects.

Kang, B. Y., H. You, et al. (2009). "Cervical cancer isolate PT3, super-permissive for adeno-associated virus replication, over-expresses DNA polymerase delta, PCNA, RFC and RPA." BMC Microbiol **9**: 79.

BACKGROUND: Adeno-associated virus (AAV) type 2 is an important virus due to its use as a safe and effective human gene therapy vector and its negative association with certain malignancies. AAV, a dependo-parvovirus, autonomously replicates in stratified squamous epithelium. Such tissue occurs in the nasopharynx and anogenitals, from which AAV has been clinically isolated. Related autonomous parvoviruses also demonstrate cell tropism and preferentially replicate in oncogenically transformed cells. Combining these two attributes of parvovirus tropism, squamous and malignant, we assayed if AAV might replicate in squamous cervical carcinoma cell isolates. Three primary isolates (PT1-3) and two established cervical cancer cell lines were compared to normal keratinocytes (NK) for their ability to replicate AAV. One isolate, PT3, allowed for high levels of AAV DNA replication and virion production compared to others. In research by others, four cellular components are known required for in vitro AAV DNA replication: replication protein A (RPA), replication factor C (RFC), proliferating cell nuclear antigen (PCNA), and DNA polymerase delta (POLD1). Thus, we examined PT3 cells for expression of these components by DNA microarray and real-time quantitative PCR. All four components were over-expressed in PT3 over two representative low-permissive cell isolates (NK and PT1). However, this super-permissiveness did not result in PT3 cell death by AAV infection. CONCLUSION: These data, for the first time, provide evidence that these four cellular components are likely important for AAV in vivo DNA replication as well as in vitro. These data also suggest that PT3 will be a useful reagent for investigating the AAV-permissive transcriptome and AAV anti-cancer effect.

Kawanaka, T., A. Kubo, et al. (2008). "Prognostic significance of HIF-2alpha expression on tumor infiltrating macrophages in patients with uterine cervical cancer undergoing radiotherapy." J Med Invest **55**(1-2): 78-86.

Hypoxia-inducible factor (HIF)-2alpha, a basic helix-loop-helix (bHLH)-PAS protein, is the principal regulator of the hypoxic transcriptional response. An immunohistochemical study reported strong HIF-2alpha expression in the cytoplasm of tumor infiltrative macrophages (TIMs). Thus we assessed the expression of HIF-2alpha in human cervical cancer tissue before radiation therapy and its relationship to the clinical outcome. Seventy three patients with histologically proven primary advanced squamous cell carcinoma of the uterine cervix underwent radiotherapy in Tokushima University Hospital after biopsy specimens were taken. Among 73 specimens stained for HIF-2alpha, 53 (72.6%) exhibited HIF-2alpha immunoreactivity in the TIMs. In only 5 of 73 cases, HIF-2alpha immunoreactivity was observed in the nuclei of tumor cells. The HIF-2alpha positive cell count ratio in TIMs was associated with disease-free survival (DFS) with the worst DFS (p=0.024) being in cases in the group with

a high positive cell count ratio. A high HIF-2alpha positive cell count ratio in TIMs increased the risk of local recurrence (p=0.0142). These findings might suggest that the ratio of the HIF-2alpha positive cell in TIMs may be a new predictive indicator for prognosis before radiation therapy for uterine cervical cancer.

Kim, S. H., C. I. Hwang, et al. (2007). "GADD153 mediates celecoxib-induced apoptosis in cervical cancer cells." Carcinogenesis **28**(1): 223-31.

Celecoxib, a selective cyclooxygenase 2 inhibitor, is known to have anti-inflammatory activity and to induce apoptosis in various types of cancer cells. Here, we examined the molecular mechanism of celecoxib-induced apoptosis in cervical cancer cell lines (HeLa, CaSki and C33A). Screening of a microarray cDNA-chip containing 225 different genes revealed that growth arrest and DNA damage inducible gene (GADD153), a transcription factor involved in apoptosis, showed the strongest differential expression following celecoxib treatment in all three cervical cancer cell lines. Notably, siRNA-induced silencing of GADD153 suppressed celecoxib-induced apoptosis in all the three cell lines, and exogenous expression of GADD153 triggered apoptosis in cervical cancer cells in the absence of other apoptotic stimuli. A luciferase reporter gene assay and mRNA stability tests revealed that expression of GADD153 was regulated at both the transcriptional and post-transcriptional levels following celecoxib treatment. The region between -649 and -249, containing an intact C/EBP-ATF binding site, was required for the basal activity and celecoxib-induced stimulation of GADD153 promoter activity. Also, mRNA stability test showed that celecoxib prolonged the half-life of GADD153 mRNA. In terms of signaling pathway, addition of the NF-kappaB inhibitor, N-tosyl-L phenylalanyl-chloromethyl ketone (TPCK), had no effect on GADD153 expression levels. Celecoxib treatment induced Bak expression, whereas cell treated with siGADD153 or TPCK showed lower levels of celecoxib-induced Bak up-regulation. These novel findings collectively suggest that GADD153 may play a key role in celecoxib-induced apoptosis in cervical cancer cells by regulating the expression of proapoptotic proteins such as Bak.

Kim, S. H., C. I. Hwang, et al. (2006). "GADD153 mediates celecoxib-induced apoptosis in cervical cancer cells." Carcinogenesis **27**(10): 1961-9.

Celecoxib, a selective cyclooxygenase-2 inhibitor, is known to possess anti-inflammatory activity and also induces apoptosis in various types of cancer cells. Here, we examined the molecular mechanism of celecoxib-induced apoptosis in cervical cancer cell lines (HeLa, CaSki and C33A). Screening of a cDNA microarray chip containing 225 different genes revealed that GADD153 (growth arrest and DNA damage inducible gene), a transcription factor involved in apoptosis, showed the strongest differential expression following celecoxib treatment in all three cervical cancer cell lines. Notably, siRNA-induced silencing of GADD153 suppressed celecoxib-induced apoptosis in all three cell lines, and exogenous expression of GADD153 triggered apoptosis in cervical cancer cells in the absence of other apoptotic stimuli. A luciferase reporter gene assay and mRNA stability tests revealed that the expression of GADD153 was regulated at both the transcriptional and post-transcriptional levels following celecoxib treatment. The region between -649 and -249, containing an intact C/EBP-ATF binding site, is required for celecoxib-induced stimulation of GADD153 promoter activity. In terms of signaling pathway, addition of the NF-kappaB inhibitor, N-tosyl-L-phenylalanyl-chloromethyl ketone, had no effect on GADD153 expression levels. Celecoxib treatment induced Bak expression, whereas cell transfected with siGADD153 showed lower levels of celecoxib-induced Bak upregulation. These novel findings collectively suggest that GADD153 may play a key role in celecoxib-induced apoptosis in cervical cancer cells by regulating the expression of proapoptotic proteins such as Bak.

Kim, Y. W., S. M. Bae, et al. (2004). "Comparison of As(2)O(3) and As(4)O(6) in the detection of SiHa cervical cancer cell growth inhibition pathway." Cancer Res Treat **36**(4): 255-62.

An arsenical compound, As(2)O(3), has been reported to be effective for treating acute leukemia and inducing apoptosis in many different tumor cells. In this study, the ability of As(4)O(6) to suppress cell growth and induce gene expression patterns was tested using a cDNA microarray in HPV16 immortalized cervical carcinoma cells, SiHa cells, along with As(2)O(3). A novel arsenical compound, As(4)O(6), was designed and its ability to induce cell growth inhibition as well as gene expression profiles along with As(2)O(3) in HPV16 infected SiHa cervical cancer cells was compared. Both As(2)O(3) and As(4)O(6) induced apoptosis in SiHa cells, as determined by DNA ladder formation. To further compare the gene expression profiles between these two drugs, a 384 cDNA microarray system was employed. Also, the gene expression profiles were classified into the Gene Ontology (GO) to investigate apoptosis-related cellular processes. As(4)O(6) was more effective i suppressing the growth of SiHa cells in vitro compared to As(2)O(3). In the case of treatment with As(2)O(3), 41 genes were up- or down-

regulated at least 2 fold compared to non-treatment. However, 65 genes were up- or down-regulated by As(4)O(6) treatment. In particular, 27 genes were commonly regulated by both arsenic compounds. Also, the GO analysis indicated that down-regulation of cell-regulatory functions, such as cell cycle, protein kinase activity and DNA repair, induced anti-tumor effect. CONCLUSION: These data support that As(4)O(6) could be more effective than As(2)O(3) in inhibiting the growth of HPV16 infected cervical cancer cells. This appears to be mediated through a unique, but overlapping regulatory mechanism(s), suggesting that the regulated genes and cellular processes could be further used as a new potential drug approach for treating cervical cancer in clinical settings.

Koivusalo, R., A. Mialon, et al. (2006). "Activation of p53 in cervical cancer cells by human papillomavirus E6 RNA interference is transient, but can be sustained by inhibiting endogenous nuclear export-dependent p53 antagonists." Cancer Res **66**(24): 11817-24.

p53 is degraded in cervical cancer cells by the human papillomavirus E6 and can be stabilized with short interfering RNA (siRNA) molecules targeting E6 mRNA. In this in vitro study, we show that E6 siRNA-induced p53 activation is transient in HeLa cervical cancer cells despite continuous suppression of E6 mRNA; activation can be sustained if the endogenous p53 antagonists COP1, MDM2, Pirh2, and c-Jun-NH(2)-kinase are also targeted by siRNAs or by inhibiting the nuclear export of p53 with leptomycin B. The direct targeting of any one of these four cellular p53 antagonists had no effect on p53 activity when E6 was intact, but inhibited the fading off of E6 siRNA-induced p53 activation in nonstress conditions. The effect was additive when multiple cellular antagonists were concomitantly inhibited, indicating that all these proteins degrade p53 when E6 is inactivated. The antiproliferative effect induced by E6 silencing was enhanced when the endogenous p53 antagonists were additionally targeted. In conclusion, if human papillomavirus E6 is inhibited under nonstress conditions, the subsequent p53 activation is quickly reversed by the endogenous p53 degenerative machinery. The present results indicate that several cellular p53 antagonists must be inhibited for sustained p53 activity if E6 siRNA therapy is attempted and if no combined genotoxic therapy is applied.

Kubota, H., T. Suzuki, et al. (2005). "Increased expression of GRP94 protein is associated with decreased sensitivity to X-rays in cervical cancer cell lines." Int J Radiat Biol **81**(9): 701-9.

Radiation therapy is one of the standard treatments for cervical cancer. Glucose regulated protein 94 (GRP94) is a molecular chaperone, which increases in amount after X-ray irradiation. This study examined the involvement of GRP94 in radio-resistance in human cervical cancer cells. Seven human cervical carcinoma cell lines (HeLa, SKG-I, SKG-IIIb, QG-U, Caski, SiHa and C33A) were examined for basal levels of GRP94 protein by western blotting analysis. Sensitivity to X-ray irradiation of these cell lines was determined with a colony survival assay. The suppression of GRP94 expression was performed using specific small-interfering RNA (siRNA) in HeLa and Caski cells. HeLa cells and QG-U cells, with higher basal levels of GRP94, exhibited a low sensitivity to X-ray cell killing. In HeLa cells, the sensitivity increased when protein GRP94 levels were reduced by specific siRNA transfection. However, a reduction in GRP94 protein had little effect on the X-ray sensitivity of Caski cells, which expressed low basal GRP94 protein levels but showed a low sensitivity to X-rays. CONCLUSIONS: High basal protein levels of GRP94 were correlated with a modest decrease in sensitivity to X-ray cell death in some cervical cancer cell lines. These results suggest that higher GRP94 protein expression is one of the molecular mechanisms causing resistance to radiation, and therefore GRP94 siRNA might be useful in tumor-specific gene therapy by reversing radio-resistance prior to radiation in cervical cancer.

Lea, J. S., N. Sunaga, et al. (2007). "Silencing of HPV 18 oncoproteins With RNA interference causes growth inhibition of cervical cancer cells." Reprod Sci **14**(1): 20-8.

Silencing the expression of human papillomavirus (HPV) oncoproteins should have therapeutic benefits for cervical cancer. The authors' objective was to study RNA interference of the HPV 18 E6/E7 bicistronic mRNA with E6 small interfering RNA (siRNA) and E7 siRNA and determine the effect of each siRNA on oncoprotein expression, resultant cell growth, and downstream molecular effects. RNA interference was used to knockdown HPV 18 E6 and E7 oncoproteins on the HPV 18 positive cervical cancer cell lines HeLa and C4I. Western blotting was used to assay for each oncoprotein expression and select downstream molecular targets. Cell cycle analyses, cell viability assays, and colony formation assays were performed to determine the effect of treatment by both HPV 18 E6 siRNA and E7 siRNA. The transfection reagent oligofectamine and Tax siRNA were used as negative controls. Transfection with E6 siRNA caused complete loss of E6 but not E7 oncoprotein. However, E7 siRNA induced complete loss of both E6 and E7 oncoproteins. E6 siRNA

mediated the reexpression of p53 protein and a moderate decrease in phosphorylated retinoblastoma protein expression (pRb), resulting in decreased colony formation. Transfection with E7 siRNA mediated a robust increase in p53 expression and complete loss of pRb, resulting in a marked decrease in colony formation compared to the E6 siRNA (P =.001). Flow cytometry revealed significantly increased apoptotic cells with E7 siRNA compared to E6 siRNA and control. RNA interference targeting the E7 portion of the bicistronic HPV 18 mRNA can silence both E6 and E7 oncoproteins and is most effective in cervical cancer growth inhibition.

Lee, C. M., C. B. Fuhrman, et al. (2006). "Phosphatidylinositol 3-kinase inhibition by LY294002 radiosensitizes human cervical cancer cell lines." Clin Cancer Res **12**(1): 250-6.

The phosphatidylinositol 3-kinase (PI3K) catalytic subunit is amplified in cervical cancers, implicating PI3K in cervical carcinogenesis. We evaluated the radiosensitizing effect of PI3K inhibition by LY294002 on clonogenic survival, growth characteristics, and gene expression in cervical cancer cell lines (HeLa and CaSki). EXPERIMENTAL DESIGN: Cervical cancer cells were treated separately and concurrently with the PI3K inhibitor LY294002 (10 micromol/L) and radiation (2 Gy) with serial analysis of cell count, apoptosis, and flow cytometry. PI3K inhibition was assessed by protein analysis of phosphorylated Akt. Clonogenic assays were done with varying doses of radiation and LY294002 and varied time points of administration of LY294002 proximate to the radiation dose. Surviving fractions and dose modification factors (DMF) were calculated. Each experiment was done in triplicate and analyzed using ANOVA regression analysis and Dunnett's t Test. Microarray gene expression analysis was done on the HeLa cell line. PI3K inhibition with LY294002 alone did not decrease cell survival. However, treatment with LY294002 significantly radiosensitized HeLa and CaSki cell lines with DMFs (1 log cell kill) of 1.95 and 1.37, respectively. Compared with post-irradiation, pretreatment produced more radiosensitization ($P < 0.0001$). DMFs were 2.2, 2.0, 2.0, and 1.2 for LY294002 added at 6, 2, and 0.5 hours before irradiation and 6 hours after irradiation, respectively. LY294002 pretreatment in irradiated HeLa cells led to altered gene expression. CONCLUSIONS: Although LY294002 alone did not produce cytotoxic effects, PI3K inhibition with LY294002 produced significant radiosensitization, showed significant time-dependent effects, increased apoptosis, and altered gene expression. These findings support future investigation of PI3K inhibitors in combination with radiation therapy for carcinoma of the cervix.

Lee, E. J., M. Jo, et al. (2006). "Alternative splicing variants of IRF-1 lacking exons 7, 8, and 9 in cervical cancer." Biochem Biophys Res Commun **347**(4): 882-8.

The two previously identified major splice variants of interferon regulatory factor 1 (IRF-1) do not appear to affect IRF-1-mediated gene activation. We searched for additional splice variants and examined their effect on wild-type IRF-1. RT-PCR experiments using normal and malignant human cervical tissue samples revealed five variants lacking some combination of exons 7, 8, and 9; their expression levels were higher in the malignant samples. These variants had predicted deletions of the functional domain or truncated protein isoforms, had different transcriptional activities, and attenuated transcriptional activity of IRF-1. Unlike the cell cycle-dependent IRF-1 transcript, the splice variant mRNA levels remained consistent throughout the cell cycle. The variant proteins were more stable than the IRF-1 protein, which may explain the strong inhibition of IRF-1 transcription in the presence of relatively small quantities of the alternative transcripts. In conclusion, alternative splicing in exons 7, 8, and 9 is an important mechanism for negatively regulating IRF-1 in cervical cancer.

Lee, E. J., M. Jo, et al. (2009). "Dkk3, downregulated in cervical cancer, functions as a negative regulator of beta-catenin." Int J Cancer **124**(2): 287-97.

The Wnt/beta-catenin signaling pathway is activated during the malignant transformation of keratinocytes that originate from the human uterine cervix. Dkk1, 2 and 4 have been shown to modulate the Wnt-induced stabilization of the beta-catenin signaling pathway. However, the function of Dkk3 in this pathway is unknown. Comparison of the Dkk3 gene expression profiles in cervical cancer and normal cervical tissue by cDNA microarray and subsequent real-time PCR revealed that the Dkk3 gene is frequently downregulated in the cancer. Methylation studies showed that the promoter of Dkk3 was methylated in cervical cancer cell lines and 22 (31.4%) of 70 cervical cancer tissue specimens. This promoter methylation was associated with reduced expression of Dkk3 mRNA in the paired normal and tumor tissue samples. Further, the reintroduction of Dkk3 into HeLa cervical cancer cells resulted in reduced colony formation and retarded cell growth. The forced expression of Dkk3 markedly attenuated beta-catenin-responsive luciferase activity in a dose-dependent manner and decreased the beta-catenin levels. By utilizing a yeast two-hybrid screen,

betaTrCP, a negative regulator of beta-catenin was identified as a novel Dkk3-interacting partner. Coexpression with betaTrCP synergistically enhanced the inhibitory function of Dkk3 on beta-catenin. The stable expression of Dkk3 blocks the nuclear translocation of beta-catenin, resulting in downregulation of its downstream targets (VEGF and cylcin D), whereas knockdown of Dkk3 abrogates this blocking. We conclude from our finding that Dkk3 is a negative regulator of beta-catenin and its downregulation contribute to an activation of the beta-catenin signaling pathway.

Lee, J. J., S. Kim, et al. (2006). "Enhanced specificity of the p53 family proteins-based adenoviral gene therapy in uterine cervical cancer cells with E2F1-responsive promoters." Cancer Biol Ther **5**(11): 1502-10.

p63 and p73, the p53 family proteins, are similar to p53 in many aspects: structural homology, transactivation of p53-downstream genes, and induction of apoptosis. Interestingly, they also differ from p53; in particular, they are not inhibited by viral oncoproteins such as HPV E6. This feature would be an advantage over p53 in HPV-associated cancers and therefore, we evaluated the therapeutic potentials of various p53 family proteins (p73alpha, p73beta, p63alpha and p63gamma) against HPV-infected cervical cancers. In clonogenic assay, exogenous expression of p73alpha, p73beta and p63gamma inhibited the colony formation of HPV-positive cervical cancer cells under G418- selection while p53 could not. Recombinant adenoviruses Ad/CMVp73alpha, Ad/CMVp73beta and Ad/CMVp63gamma induced potent apoptosis in all infected cervical cancers (CasKi, SiHa, HeLa, C33A, SNU682, SNU17, SNU1005, SNU703), irrespective of their HPV-infection status. Unfortunately however, Ad/CMVp73alpha, Ad/CMVp73beta, and Ad/CMVp63gamma inhibited also normal cells such as endothelial cells, fibroblasts, and keratinocytes thus, tumorspecific promoter was indispensable to the p53 family proteins-based therapy. Here we report a stringent tumor killing by p73beta in combination with ESM6, a synthetic promoter targeting the DNA tumor virusassociated cancers. Recombinant adenoviruses encoding p73beta by ESM6 (Ad/ESM6p73beta and Ad/ESM6p73bENH) expressed p73beta and induced apoptosis only in the cancer cells but not in normal cells. Collectively, we suggest that the p53 family proteins are potent therapeutic agents for HPV-associated uterine cervical cancers and ESM6 mediated expression of the p53 family proteins would be a safe and strong tumor targeting strategy.

Lee, S. H., J. W. Kim, et al. (2005). "IFN-gamma/IRF-1-induced p27kip1 down-regulates telomerase activity and human telomerase reverse transcriptase expression in human cervical cancer." FEBS Lett **579**(5): 1027-33.

Telomerase activation is regulated by the expression of human telomerase reverse transcriptase (hTERT) and is a key step in the development of human cancers. Interferon-gamma (IFN-gamma) signaling induces growth arrest in many tumors through multiple regulatory mechanisms. The p27 tumor suppressor protein inhibits the formation of tumors through the induction of cell cycle arrest and/or apoptosis. We demonstrate here that p27Kip1 inhibits hTERT mRNA expression and telomerase activity through post-transcriptional up-regulation by IFN-gamma/IRF-1 signaling. The ectopic expression of p27 suppressed hTERT expression and telomerase activity in human cervical cancer cell lines, HeLa and HT3. Furthermore, hTERT promoter activity of mouse embryonic fibroblasts (MEFs) deficient in p27 (p27-/- MEFs) was significantly higher than that of wild-type MEFs. Overexpression of p27 suppressed hTERT promoter activity and telomerase activity of p27-/- MEFs. In addition p27 down-regulated E7 protein expression and in transiently transfected HeLa cells, E7 increased hTERT promoter activity. In conclusion, we propose that inhibition of the hTERT expression and telomerase activity may be a novel tumor suppressor function of p27.

Lee, Y. S., S. M. Bae, et al. (2006). "Cell cycle regulatory protein expression profiles by adenovirus p53 infection in human papilloma virus-associated cervical cancer cells." Cancer Res Treat **38**(3): 168-77.

The tumor suppressor gene, p53, has been established as an essential component for the suppression of tumor cell growth. In this study, we investigated the time-course anticancer effects of adenoviral p53 (Adp53) infection on human ovarian cancer cells to provide insight into the molecular-level understanding of the growth suppression mechanisms involved in Adp53-mediated apoptosis and cell cycle arrest. Three human cervical cancer cell lines (SiHa, CaSki, HeLa and HT3) were used. The effect of Adp53 infection was studied via cell count assay, cell cycle analysis, FACS, Western blot and macroarray assay. Adp53 exerts a significant role in suppressing cervical cancer cell growth. Adp53 also showed growth inhibitory effects in each cell line, and it induced apoptosis and cell cycle arrest. Adp53 differentially regulated the expression of genes and proteins, and the gene expression profiles in the SiHa cells revealed that the p21, p53 and mdm2 expressions were significantly up-regulated at 24 and 48 hr.

Western blot shows that the p21 and p53 expression-levels were significantly increased after Adp53 infection. In addition, in all cell lines, both the CDK4 and PCNA protein expression levels were decreased 48 h after Adp53 infection. Cell cycle arrest at the G1 phase was induced only in the SiHa and HeLa cells, suggesting that exogenous infection of Adp53 in cancer cells was significantly different from the other HPV-associated cervical cancer cells. CONCLUSION: Adp53 can inhibit cervical cancer cell growth through induction of apoptosis and cell cycle arrest, as well as through the regulation of the cell cycle-related proteins. The Adp53-mediated apoptosis can be employed as an advanced strategy for developing preferential tumor cell-specific delivery.

Li, H. and X. Wu (2004). "Histone deacetylase inhibitor, Trichostatin A, activates p21WAF1/CIP1 expression through downregulation of c-myc and release of the repression of c-myc from the promoter in human cervical cancer cells." Biochem Biophys Res Commun **324**(2): 860-7.

Histone deacetylase (HDAC) inhibitors have shown promise in clinical cancer therapy and to consistently induce p21WAF1/CIP1 expression in a p53-independent manner and via increased acetylation of the chromatin at the Sp1 sites in the p21WAF1/CIP1 promoter region. However, the exact mechanism by which HDAC inhibitors induce p21WAF1/CIP1 remains unclear. In this study, we observed that Trichostatin A (TSA), a HDAC inhibitor, induced strikingly p21WAF1/CIP1 expression in human cervical cancer (HeLa) cells, and this induction correlated with downregulation of c-myc expression. Coincident with this observation, knock down of c-myc with a c-myc specific small interfering RNA dramatically induced expression of p21WAF1/CIP1 in these cancer cells. These data suggest that c-myc may play a critical role in repression of p21WAF1/CIP1 expression in HeLa cells. More importantly, using chromatin immunoprecipitation assay, we observed for the first time that c-myc bound to the endogenous p21WAF1/CIP1 promoter in untreated HeLa cells, but not in TSA-treated cells. Taken together, TSA induced c-myc downregulation and release from the endogenous p21WAF1/CIP1 promoter contributes, at least partially, to transcriptional activation of the p21WAF1/CIP1 in HeLa cells.

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. 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. 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, X. L., Q. H. Meng, et al. (2009). "Adenovirus-mediated expression of UHRF1 reduces the radiosensitivity of cervical cancer HeLa cells to gamma-irradiation." Acta Pharmacol Sin **30**(4): 458-66.

AIM: An in vitro study was carried out to determine the effect of UHRF1 overexpression on radiosensitivity in human cervical cancer HeLa cells using adenovirus-mediated UHRF1 gene transfer (Ad5-UHRF1). Cell survival was evaluated using the clonogenic survival assay and the MTT assay; apoptosis and cell cycle distribution were monitored by flow cytometry. Protein levels were measured by Western blotting. Silencing XRCC4 expression was performed by transfection of small interfering RNA (siRNA). Increased expression of UHRF1 by Ad5-UHRF1 significantly reduced the radiosensitivity of HeLa cells. The UHRF1-mediated radioresistance was correlated with increased DNA repair capability and increased expression of the DNA damage repair protein, XRCC4. Knocking down XRCC4 expression in the cells using XRCC4 siRNA markedly reduced the UHRF1-mediated radioresistance. CONCLUSION: These results provide the first evidence for revealing a functional role of UHRF1 in human cervical cancer cells as a negative regulator of radiosensitivity.

Li, Y., H. Li, et al. (2007). "Inhibition of telomerase RNA (hTR) in cervical cancer by adenovirus-delivered siRNA." Cancer Gene Ther **14**(8): 748-55.

Small interfering RNA (siRNA) has become a powerful tool for selectively silencing gene expression in cultured mammalian cells. In this study, a 67-bp oligonucleotide encoding human telomerase RNA (hTR) was introduced into pSIREN, a shuttle vector for construction of recombinant adenovirus. Then the U6-RNA promoter and siRNA-encoding insert were cut out from the pSIREN and subcloned into pAdeno-X to construct the plasmid pAd-hTR. After the pAd-hTR was transfected into a mammalian cell line HEK-293, adenovirus carrying the hTR-targeting siRNA (Ad-hTR-siRNA) was obtained. We performed a series of experiments to demonstrate silencing of the telomerase mediated by Ad-hTR-siRNA in HeLa cells. Compared with control virus (Ad-NT-siRNA), Ad-hTR-siRNA significantly reduced both hTR mRNA level (by 70.21%) and telomerase activity (by 58.87%) in HeLa cells. Moreover, it induced apoptosis in HeLa cells. Treatment of subcutaneous tumor xenografted with Ad-hTR-siRNA could slow down tumor growth, at least partially due to the induction of apoptosis ($P<0.05$) in vivo. Taken together, our results demonstrated efficient and specific knockdown of telomerase in HeLa cell line by the hTR siRNA, and indicated the prospect of applying this siRNA expressing recombinant adenovirus system in cancer gene therapy.

Lim, H. Y., M. Ahn, et al. (2004). "Tumor-specific gene therapy for uterine cervical cancer using MN/CA9-directed replication-competent adenovirus." Cancer Gene Ther **11**(8): 532-8.

Although gene therapies using tissue-specific promoters have been reported to be a promising tool for treating cancers, few studies have explored this possibility for uterine cervical cancer. MN/CA9 is a transmembrane glycoprotein that was first identified in the human cervical carcinoma cell line, HeLa. Since MN/CA9 protein is highly expressed in uterine cervical cancer tissues, but not in normal cervix, we constructed a tumor-specific replication-competent adenoviral vector utilizing MN/CA9 promoter (Ad-MN/CA9-E1a), which can replicate only in MN/CA9-expressing cells. Infection of Ad-MN/CA9-E1a to MN/CA9-positive uterine cervical cancer cells (HeLa, C-33 A and SiHa) resulted in much stronger Ad5 E1a protein expressions compared with MN/CA9-negative cells (SK-RC-29), suggesting a tissue-specific replication of this recombinant adenovirus. In vitro cytotoxicity assay revealed that the growth of MN/CA9-positive cells was significantly inhibited with 0.01-1 MOI of Ad-MN/CA9-E1a, but the growth of MN/CA9-negative cells (SK-RC-29) could only be inhibited by as many as 100 MOI. Intratumoral injection of Ad-MN/CA9-E1a effectively induced growth delay of HeLa tumors in nude mice. These results suggest that a novel replication-competent adenoviral vector mediated by MN/CA9 promoter, Ad-MN/CA9-E1a, can selectively replicate in MN/CA9-expressing tumors with cytotoxic effects and may be utilized for the treatment of uterine cervical cancer.

Lindel, K., P. Burri, et al. (2005). "Human papillomavirus status in advanced cervical cancer: predictive and prognostic significance for curative radiation treatment." Int J Gynecol Cancer **15**(2): 278-84.

Human papillomavirus (HPV) infection plays a major role in oncogenesis of squamous cell carcinoma of the cervix. This study was performed to investigate if HPV status and E2 gene integrity are prognostic parameters for clinical outcome and predictive for radiation response. Forty women with locally advanced cervical cancer treated with curative radiotherapy were analyzed for HPV infection and E2 gene integrity by multiplex polymerase chain reaction. Statistical analyses were performed for overall survival, disease-free survival (DFS), local progression-free survival, and treatment response (clinical complete remission). Twenty-eight (70%) of 40 carcinomas were HPV positive. The only significant factor for a better overall survival, DFS, and local progression-free survival was HPV positivity ($P < 0.02$, $P= 0.02$, and $P < 0.05$, log-rank, respectively). HPV-positive tumors had a significantly better clinical complete remission (67% vs 33%, $P= 0.04$, Fisher's exact test). An intact E2 gene region showed a trend for a better DFS ($P= 0.1$, log-rank). This study reveals HPV as an independent prognostic parameter for outcome and radiation response. Integration of the virus genome into host cell DNA might be a molecular target to determine the treatment response of HPV-positive cancers.

Liu, C. Y., T. K. Chao, et al. (2009). "Characterization of LMX-1A as a metastasis suppressor in cervical cancer." J Pathol **219**(2): 222-31.

DNA methylation is important in cancer development and is a promising biomarker for cancer detection. An epigenomic approach used in our previous work showed that LMX-1A is methylation-silenced in cervical cancer. LMX-1A, a LIM-homeobox gene, is known to participate in developmental events; however, there are at present no data on the role of LMX-1A in cancers. In this study, we characterized the function of this transcription factor by examining cell lines, animal

models and human cervical neoplastic tissues, and found that over-expression of LMX-1A does not affect cell proliferation or the cell cycle of cervical cancer cell lines but significantly inhibits colony formation and invasion in vitro. Analysis of changes in epithelial-mesenchymal transition (EMT) markers, such as CDH1, CDH2, VIMENTIN, SNAIL, SLUG and TWIST, revealed involvement of the EMT in LMX-1A-mediated cancer invasion; this result was validated in a stable transfectant over-expressing LMX-1A with RNA interference. Xenograft studies using immunocompromised mice confirmed the suppressor effects of LMX-1A on tumour formation and distant metastasis in cervical cancer cell lines. LMX-1A immunohistochemical staining of tissue arrays containing the full spectrum of cervical neoplasms, including normal cervix, low-grade cervical intra-epithelial neoplasia (CIN), high-grade CIN, locally invasive and distant metastatic cancers, demonstrated the critical role of LMX-1A in invasion and metastasis. Furthermore, we found by analysing TGFbeta-BMP signalling that BMP4 and BMP6 are down-regulated by LMX-1A. The results of this study suggest that LMX-1A suppresses cancer invasion and metastasis in cervical cancer through an incomplete EMT.

Liu, S. S., K. Y. Chan, et al. (2006). "Enhancement of the radiosensitivity of cervical cancer cells by overexpressing p73alpha." Mol Cancer Ther **5**(5): 1209-15.

Radiation therapy is the most effective therapy for cervical cancer in advanced stages. p53 plays a critical role in the cellular response to radiation-induced DNA damage. However, p53 function is often impaired in the presence of the oncoprotein E6 from human papillomavirus, which is often associated with the development of cervical cancer. p73, a p53 family member, is highly similar to p53, but is resistant to the degradation by human papillomavirus E6. In this study, we investigated the role of p73alpha in relation to cellular radiosensitivity in the p53-impaired cervical cancer cells. Radiosensitivity and irradiation-induced apoptotic cell death were examined in the exogenous overexpressed p73alpha- and p53-impaired cells. Our results showed that the endogenous p73alpha expressed only in the radiosensitive cervical cancer C4-1 cells, but not in the radioresistant SiHa, Caski, and HeLa cells. Overexpression of exogenous p73alpha by transfection in the radioresistant cells resulted in a significant increase of cellular sensitivity to radiation. Enhanced radiosensitivity in p73alpha-transfected cells was attributed by increase of cellular apoptosis. Coactivation of p21 was also observed in the p73alpha-transfected cells upon radiation treatment. In summary, our findings suggested that p73alpha is an important determinant of cellular radiosensitivity in the p53-impaired cervical cancer cells.

Liu, S. S., R. C. Leung, et al. (2004). "p73 expression is associated with the cellular radiosensitivity in cervical cancer after radiotherapy." Clin Cancer Res **10**(10): 3309-16.

Apoptosis is one of the causes of cell death in cervical cancer following radiotherapy. By studying the gene expression profile with cDNA apoptotic array, the p73 gene was found overexpressed in radiosensitive cervical cancers when compared with radioresistant ones. To investigate the role of the p73 gene in relation to clinical assessment of radiosensitivity in cervical cancer based on the findings of residual tumor cells in cervical biopsies after completion of radiotherapy, we studied the protein expression of p73 in 59 cervical cancers after radiotherapy and 68 normal cervices using immunohistochemistry. The expression of p73 was found to be significantly increased in cancer samples and, more importantly, in those samples sensitive to radiotherapy ($P < 0.001$). The overexpression of p73 actually predicted a better prognosis in cervical cancer patients ($P < 0.001$). To investigate the possible involvement of p73 downstream genes, the protein expressions of p21 and Bax were studied. The expression of p21, but not Bax, was found to be positively correlated with the expression of p73 ($P = 0.001$). Furthermore, the epigenetic regulation of p73 expression via DNA methylation was also investigated in 103 cervical cancers and 124 normals. Hypermethylation of p73 gene was observed in 38.8% of cervical cancers, and it was significantly associated with reduced or absent p73 expression ($P < 0.001$). Reactivation of p73 expression in two cervical cancer cell lines by demethylation treatment supported the role of methylation in the regulation of p73 expression. Our findings suggested that p73 expression was related to the radiosensitivity of cervical cancer cells and may play an important role in the regulation of cellular radiosensitivity.

Lou, P. J., W. F. Cheng, et al. (2009). "PMMA particle-mediated DNA vaccine for cervical cancer." J Biomed Mater Res A **88**(4): 849-57.

DNA vaccination is a novel immunization strategy that possesses many potential advantages over other vaccine strategies. One of the major difficulties hindering the clinical application of DNA vaccination is the relative poor immunogenicity of DNA vaccines. Poly(methyl methacrylate) (PMMA) is a synthetic polymer approved by the Food and Drug Administration for certain human clinical applications such as the bone cement. In vivo, PMMA particles are

phagocytosable and have the potential to initiate strong immune responses by stimulating the production of inflammatory cytokines. In this study, we synthesized a series of PMMA particles (PMMA 1-5) with different particle sizes and surface charges to test the feasibility of implementing such polymer particles for DNA vaccination. To our knowledge, this is the first report to show that the gene gun can deliver DNA vaccine by propelling PMMA particles mixed with plasmid DNA for cervical cancer. It was found that PMMA 4 particles (particle size: 460 +/- 160 nm, surface charge: +11.5 +/- 1.8 mV) stimulated the highest level of TNF-alpha production by macrophages in vitro and yielded the best result of antitumor protection in vivo. Therefore, our results possess the potential for translation and implementation of polymer particles in gene gun delivering DNA vaccination.

Mahdavi, A. and B. J. Monk (2005). "Vaccines against human papillomavirus and cervical cancer: promises and challenges." Oncologist **10**(7): 528-38.

Cervical cancer and precancerous lesions of the genital tract are major threats to the health of women worldwide. The introduction of screening tests to detect cervical cancer precursor lesions has reduced cervical cancer rates in the developed world, but not in developing countries. Human papillomavirus (HPV) is the primary etiologic agent of cervical cancer and dysplasia. Thus, cervical cancer and other HPV-associated malignancies might be prevented or treated by HPV vaccines. Two vaccine strategies have been developed. First, prevention of HPV infection through induction of capsid-specific neutralizing antibodies has been studied in clinical trials. However, because the capsid proteins are not expressed at detectable levels by infected basal keratinocytes or in HPV-transformed cells, a second approach of developing therapeutic vaccines by targeting nonstructural early viral antigens has also been developed. Because two HPV oncogenic proteins, E6 and E7, are critical to the induction and maintenance of cellular transformation and are coexpressed in the majority of HPV-containing carcinomas, most therapeutic vaccines target one or both of these gene products. A variety of approaches is being tested in therapeutic vaccine clinical trials, whereby E6 and/or E7 are administered in live vectors, as peptides or protein, in nucleic acid form, or in cell-based vaccines. The paradigm of preventing HPV infection through vaccination has been tested, and two vaccines are currently in phase III clinical trials. However, current therapeutic vaccine trials are less mature with respect to disease clearance. A number of approaches have shown significant therapeutic benefit in preclinical papillomavirus models and await testing in patient populations to determine the most effective curative strategy.

Martin, C. M., K. Astbury, et al. (2009). "Gene expression profiling in cervical cancer: identification of novel markers for disease diagnosis and therapy." Methods Mol Biol **511**: 333-59.

Cervical cancer, a potentially preventable disease, remains the second most common malignancy in women worldwide. Human papillomavirus is the single most important etiological agent in cervical cancer. HPV contributes to neoplastic progression through the action of two viral oncoproteins E6 and E7, which interfere with critical cell cycle pathways, p53, and retinoblastoma. However, evidence suggests that HPV infection alone is insufficient to induce malignant changes and other host genetic variations are important in the development of cervical cancer. Advances in molecular biology and high throughput gene expression profiling technologies have heralded a new era in biomarker discovery and identification of molecular targets related to carcinogenesis. These advancements have improved our understanding of carcinogenesis and will facilitate screening, early detection, management, and personalised targeted therapy. In this chapter, we have described the use of high density microarrays to assess gene expression profiles in cervical cancer. Using this approach we have identified a number of novel genes which are differentially expressed in cervical cancer, including several genes involved in cell cycle regulation. These include p16ink4a, MCM 3 and 5, CDC6, Geminin, Cyclins A-D, TOPO2A, CDCA1, and BIRC5. We have validated expression of mRNA using real-time PCR and protein by immunohistochemistry.

Martin, C. M., L. Kehoe, et al. (2007). "Gene discovery in cervical cancer : towards diagnostic and therapeutic biomarkers." Mol Diagn Ther **11**(5): 277-90.

Cervical cancer is a potentially preventable disease; however, it remains the second most common malignancy in women worldwide. The human papillomavirus (HPV) is the single most important etiological agent in cervical cancer. HPV contributes to neoplastic progression through the action of two viral oncoproteins E6 and E7, which interfere with critical cell cycle pathways, tumor protein p53, and retinoblastoma protein. However, evidence suggests that HPV infection alone is insufficient to induce malignant changes, and other host genetic variations are important in the development of cervical cancer. Advances in molecular biology and high throughput technologies have heralded a new era in biomarker discovery and identification of molecular targets related to carcinogenesis. These advancements have

improved our understanding of carcinogenesis and will facilitate screening, early detection, management, and personalized targeted therapy. A number of these developments and molecular targets associated with cervical cancer will be addressed in this review.

Mukherjee, S., S. Dey, et al. (2009). "Isothiocyanates sensitize the effect of chemotherapeutic drugs via modulation of protein kinase C and telomerase in cervical cancer cells." Mol Cell Biochem **330**(1-2): 9-22.

Isothiocyanates have potential chemopreventive and antitumor effects. In the present study, we examined the actions of PEITC and sulphoraphane in modulating the activity of protein kinase C (PKC) and telomerase in cervical cancer cell line HeLa. These tumor markers are highly activated in human cancers. These compound efficiently downregulated the antiapoptotic isoforms (PKC-alpha, -betaII, -epsilon, and -zeta) as well as telomerase, whereas the proapoptotic form (PKC-delta) was upregulated. Studies were performed to measure the degree of apoptotic cell death induced by either isothiocyanates alone, or in combination with adriamycin or etoposide. Apoptosis was evident from mitochondrial cytochrome c release, apoptotic index and caspases 3 and 8 activation. Results showed that pretreatment exhibited better efficacy in sensitizing HeLa cells toward apoptosis by modulating PKCs, telomerase. This effect of isothiocyanates might prove to be of considerable value in synergistic therapy of cancer such that the drug dose level could be minimized.

Nakamura, M., S. Kyo, et al. (2004). "hTERT-promoter-based tumor-specific expression of MCP-1 effectively sensitizes cervical cancer cells to a low dose of cisplatin." Cancer Gene Ther **11**(1): 1-7.

Cervical cancers at advanced stages or with recurrent status are mainly treated by platinum-based chemotherapy, such as cisplatin. However, a novel strategy to reduce the minimally effective dose is required to prevent severe adverse effects that limit the effectiveness of the treatment. Monocyte chemoattractant protein-1 (MCP-1) is a subtype of chemokines that can promote monocyte/macrophage infiltration and enhance their phagocytosis at not only sites of inflammatory lesions but also of tumors. The present study applies MCP-1-based gene therapy to treat cervical cancers. To achieve tumor-specific expression of MCP-1, retroviral expression vector was constructed using the human telomerase reverse transcriptase gene (hTERT) promoter. Retroviral expression of MCP-1 into cervical cancer ME180 cells did not affect their proliferation either in vitro or in vivo. However, when combined with a suboptimal low dose of cisplatin, tumor formation was obviously reduced in clones transduced with MCP-1, but not in control clones. Histological examination revealed that a substantial number of macrophages infiltrated the tumor sites of MCP-1-transduced cells, but not of controls. These findings suggest that MCP-1 expression sensitizes cervical cancer cells to an otherwise ineffective low dose of cisplatin, possibly by inducing the migration of macrophages to eradicate tumor cells. This system may be a novel strategy for chemotherapy combined with immunogene therapy against otherwise intractable cervical cancers.

Narayan, G., C. Goparaju, et al. (2006). "Promoter hypermethylation-mediated inactivation of multiple Slit-Robo pathway genes in cervical cancer progression." Mol Cancer **5**: 16.

BACKGROUND: Cervical Cancer (CC) exhibits highly complex genomic alterations. These include hemizygous deletions at 4p15.3, 10q24, 5q35, 3p12.3, and 11q24, the chromosomal sites of Slit-Robo pathway genes. However, no candidate tumor suppressor genes at these regions have been identified so far. Slit family of secreted proteins modulates chemokine-induced cell migration of distinct somatic cell types. Slit genes mediate their effect by binding to its receptor Roundabout (Robo). These genes have shown to be inactivated by promoter hypermethylation in a number of human cancers. To test whether Slit-Robo pathway genes are targets of inactivation at these sites of deletion, we examined promoter hypermethylation of SLIT1, SLIT2, SLIT3, ROBO1, and ROBO3 genes in invasive CC and its precursor lesions. We identified a high frequency of promoter hypermethylation in all the Slit-Robo genes resulting in down regulated gene expression in invasive CC, but the inhibitors of DNA methylation and histone deacetylases (HDACs) in CC cell lines failed to effectively reactivate the down-regulated expression. These results suggest a complex mechanism of inactivation in the Slit-Robo pathway in CC. By analysis of cervical precancerous lesions, we further show that promoter hypermethylation of Slit-Robo pathway occurs early in tumor progression. CONCLUSION: Taken together, these findings suggest that epigenetic alterations of Slit-Robo pathway genes (i) play a role in CC development, (ii) further delineation of molecular basis of promoter methylation-mediated gene regulation provides a potential basis for epigenetic-based therapy in advanced stage CC, and (iii) form epigenetic signatures to identify precancerous lesions at risk to progression.

Ohlschlager, P., M. Quetting, et al. (2009). "Enhancement of immunogenicity of a therapeutic

cervical cancer DNA-based vaccine by co-application of sequence-optimized genetic adjuvants." Int J Cancer **125**(1): 189-98.

Treatment of patients with cervical cancer by conventional methods (mainly surgery, but also radiotherapy and chemotherapy) results in a significant loss in quality of life. A therapeutic DNA vaccine directed to tumor-specific antigens of the human papilloma virus (HPV) could be an attractive treatment option. We have developed a nontransforming HPV-16 E7-based DNA vaccine containing all putative T cell epitopes (HPV-16 E7SH). DNA vaccines, however, are less immunogenic than protein- or peptide-based vaccines in larger animals and humans. In this study, we have investigated an adjuvant gene support of the HPV-16 E7SH therapeutic cervical cancer vaccine. DNA encoded cytokines (IL-2, IL-12, GM-CSF, IFN-gamma) and the chemokine MIP1-alpha were co-applied either simultaneously or at different time points pre- or post-E7SH vaccination. In addition, sequence-optimized adjuvant genes were compared to wild type genes. Three combinations investigated lead to an enhanced IFN-gamma response of the induced T cells in mice. Interestingly, IFN-gamma secretion of splenocytes did not strictly correlate with tumor response in tumor regression experiments. Gene-encoded MIP-1alpha applied 5 days prior to E7SH-immunization combined with IFN-gamma or IL-12 (3 days) or IL-2 (5 days) postimmunization lead to a significantly enhanced tumor response that was clearly associated with granzyme B secretion and target cells lysis. Our results suggest that a conditioning application and combination with adjuvant genes may be a promising strategy to enhance synergistically immune responses by DNA immunization for the treatment of cervical cancer.

Okamoto, E., T. Sumi, et al. (2005). "Expression of apoptosis-related proteins in advanced uterine cervical cancer after balloon-occluded arterial infusion chemotherapy as an indicator of the efficiency of this therapy." Int J Mol Med **15**(1): 41-7.

We previously reported satisfactory therapeutic results of cisplatin-based cyclic balloon-occluded arterial infusion chemotherapy (BOAI) as neoadjuvant chemotherapy, which enabled treatment by hysterectomy in patients with advanced cervical cancer. We also reported expression of apoptosis among these patients and determined that the bax gene is related to this apoptosis. In the present study, we investigated the relationship between the effectiveness of BOAI therapy and expression of apoptosis regulatory genes and proteins in these cases. The subjects were 27 women with advanced cervical cancer classified as FIGO (International Federation of Gynecology and Obstetrics) stage III or higher who were admitted to the Department of Gynecology, Osaka City University Medical School Hospital between 2000 and 2003. All patients were treated by BOAI, and expression of cancer cell apoptosis was examined by the TUNEL method, expression of bax, bcl-2 and bcl-xL proteins were examined by immunohistochemistry, and expression of bax, bcl-2 and bcl-xL mRNA was examined by quantitative RT-PCR before and 3 days after BOAI. The effectiveness of BOAI therapy was thus determined. The 20 patients in whom BOAI was effective showed significantly higher expression of the bax protein and gene after BOAI, and cancer cell apoptosis was accelerated. On the other hand, the 7 patients in whom BOAI was ineffective showed significantly higher expression of the bcl-xL protein and gene after BOAI. These results suggest that bax/bcl-xL expression can be used as an indicator of the effectiveness of BOAI therapy.

Park, D. C., S. G. Yeo, et al. (2006). "Clusterin confers paclitaxel resistance in cervical cancer." Gynecol Oncol **103**(3): 996-1000.

To measure clusterin expression in cervical cancer tissues and cell lines and to evaluate whether clusterin confers resistance to paclitaxel in cervical cancer cells. Immunohistochemical staining for clusterin was performed on 15 normal cervical tissues and 32 primary cervical cancer tissues, and clusterin expression in cervical cancer cell lines was quantified by Western blotting. The correlation between clusterin expression level and paclitaxel IC50 in cervical cancer cell lines was evaluated. The effect of clusterin siRNA on paclitaxel resistance was evaluated by XTT assays. Cervical cancer tissues expressed significantly higher levels of clusterin than did normal cervical tissues (4.08 vs. 1.35, $P<0.05$). Clusterin expression levels were correlated with paclitaxel resistance in cervical cancer cell lines, and transfection of clusterin siRNA into HeLaS3 cells significantly decreased their resistance to paclitaxel ($P<0.05$). CONCLUSION: Our finding that clusterin expression was significantly higher in cervical cancer than in normal cervical tissues suggests that clusterin may confer paclitaxel resistance in cervical cancer cells.

Psyrri, A. and D. DiMaio (2008). "Human papillomavirus in cervical and head-and-neck cancer." Nat Clin Pract Oncol **5**(1): 24-31.

Cervical cancer is a major cause of cancer mortality in women worldwide and is initiated by infection with high-risk human papillomaviruses (HPVs). High-risk HPVs, especially HPV-16, are associated with other anogenital cancers and a subgroup of head-and-neck cancers. Indeed, HPV infection could account for the development of head-

and-neck cancer in certain individuals that lack the classical risk factors for this disease (tobacco and alcohol abuse). This Review summarizes the main events of the HPV life cycle, the functions of the viral proteins, and the implications of HPV infection on their hosts, with an emphasis on carcinogenic mechanisms and disease outcomes in head-and-neck cancer. The demonstration that HPVs have a role in human carcinogenesis has allowed the development of preventive and therapeutic strategies aimed at reducing the incidence and mortality of HPV-associated cancers.

Qi, M., A. E. Anderson, et al. (2005). "Indole-3-carbinol prevents PTEN loss in cervical cancer in vivo." Mol Med **11**(1-12): 59-63.

Indole-3-carbinol (I3C) is a phytochemical (derived from broccoli, cabbage, and other cruciferous vegetables) with proven anticancer efficacy including the reduction of cervical intraepithelial neoplasia (CIN) and its progression to cervical cancer. In a breast cancer cell line, I3C inhibited cell adhesion, spreading, and invasion associated with an upregulation of the tumor suppressor gene PTEN, suggesting that PTEN is important in inhibition of late stages in the development of cancer. The goal of this study was to determine the expression of PTEN during the development of cervical cancer and whether I3C affected expression of PTEN in vivo. We show diminished PTEN expression during the progression from low-grade to high-grade cervical dysplasia in humans and in a mouse model for cervical cancer, the K14HPV16 transgenic mice promoted with estrogen. The implication is that loss of PTEN function is required for this transition. Additionally, dietary I3C increased PTEN expression in the cervical epithelium of the transgenic mouse, an observation that suggests PTEN upregulation by I3C is one mechanism by which I3C inhibits development of cervical cancer.

Qiao, Y., J. Cao, et al. (2009). "Cell growth inhibition and gene expression regulation by (-)-epigallocatechin-3-gallate in human cervical cancer cells." Arch Pharm Res **32**(9): 1309-15.

EGCG [(-)-epigallocatechin-3-gallate] has shown its antitumor ability and perhaps a potential regimen for cancer patients. The goal of this study was to investigate the effect of EGCG on human papilloma virus (HPV) positive cervical cancer cell lines. EGCG inhibited the growth of CaSki (HPV16 positive) and HeLa (HPV18 positive) cells in a time- and concentration-dependent manner. Cell cycle arrest and apoptosis were observed in two cell lines after EGCG exposure. More importantly, we focused on EGCG regulation ability on pivotal genes involved in cervical cancer: viral oncogenes E6/E7, estrogen receptor (ER) and aromatase. Our results suggested that EGCG may be suitable for prevention and treatment of cervical cancer.

Rein, D. T., M. Breidenbach, et al. (2004). "Gene transfer to cervical cancer with fiber-modified adenoviruses." Int J Cancer **111**(5): 698-704.

Successful adenoviral (Ad) vector-mediated strategies for cancer gene therapy mandate gene-delivery systems that are capable of achieving efficient gene delivery in vivo. In many cancer types, in vivo gene-transfer efficiency remains limited due to the low or highly variable expression of the primary Ad receptor, the coxsackie Ad receptor (CAR). In this study, we evaluated the expression of CAR on cervical cancer cells as well as CAR-independent targeting strategies to integrins (Ad5.RGD), heparan sulfate proteoglycans (Ad5.pK7) or both (Ad5.RGD.pK7). We used a panel of established cervical cancer cell lines and primary cervical cancer cells isolated from patients to quantify the expression of CAR mRNA and to evaluate the gene-transfer efficiency of fiber-modified Ads. Of the fiber-modified vectors, Ad5.pK7 and Ad5.RGD.pK7 displayed significantly enhanced gene-transfer efficiency in vitro. Gene-delivery efficiency in vivo was evaluated using an s.c. cervical cancer mouse model. Ad5.RGD.pK7 significantly improves tumor targeting in vivo, resulting in a significantly improved tumor/liver ratio in mice. Our results suggest that the double-modified Ad5.RGD.pk7 vector enhances gene transfer to clinically relevant cervical cancer substrates, while the infectivity of nontarget cells in the mouse is not increased and comparable to Ad5. The fiber-modified virus described here can help achieve higher clinical efficacy of cervical cancer gene therapy.

Reshmi, G. and M. R. Pillai (2008). "Beyond HPV: oncomirs as new players in cervical cancer." FEBS Lett **582**(30): 4113-6.

MicroRNAs (miRNAs) are a recently discovered family of 18-24 nucleotide non-coding RNAs that can negatively regulate target mRNAs. All studied multicellular eukaryotes utilize miRNAs to regulate basic cellular functions including proliferation, differentiation, and death. It is now apparent that abnormal miRNA expression is a common feature of human malignancies. This review discusses the various cancer-relevant miRNAs (oncomirs) especially in cervical tumorigenesis and the potential role of oncomirs as therapeutic agents and targets for the treatment of cervical cancer.

Rho, S. B., Y. G. Park, et al. (2006). "A novel cervical cancer suppressor 3 (CCS-3) interacts with the BTB

domain of PLZF and inhibits the cell growth by inducing apoptosis." FEBS Lett **580**(17): 4073-80.

Promyelocytic leukemia zinc finger protein (PLZF) is a sequence-specific, DNA binding, transcriptional repressor differentially expressed during embryogenesis and in adult tissues. PLZF is known to be a negative regulator of cell cycle progression. We used PLZF as bait in a yeast two-hybrid screen with a cDNA library from the human ovary tissue. A novel cervical cancer suppressor 3 (CCS-3) was identified as a PLZF interacting partner. Further characterization revealed the BTB domain as an interacting domain of PLZF. Interaction of CCS-3 with PLZF in mammalian cells was also confirmed by co-immunoprecipitation and in vitro binding assays. It was found that, although CCS-3 shares similar homology with eEF1A, the study determined CCS-3 to be an isoform. CCS-3 was observed to be downregulated in human cervical cell lines as well as in cervical tumors when compared to those from normal tissues. Overexpression of CCS-3 in human cervical cell lines inhibits cell growth by inducing apoptosis and suppressing human cyclin A2 promoter activity. These combined results suggest that the potential tumor suppressor activity of CCS-3 may be mediated by its interaction with PLZF.

Riezebos-Brilman, A., M. Walczak, et al. (2007). "A comparative study on the immunotherapeutic efficacy of recombinant Semliki Forest virus and adenovirus vector systems in a murine model for cervical cancer." Gene Ther **14**(24): 1695-704.

Currently, various therapeutic strategies are being explored as a potential means to immunize against metastatic malignant cells or even primary tumours. Using recombinant viral vectors systems or protein-based immunization approaches, we are developing immunotherapeutic strategies against cervical cancer or premalignant cervical disease, as induced by high-risk type human papillomaviruses (HPVs). We previously demonstrated that immunization of mice with recombinant replication-defective Semliki Forest virus (rSFV) encoding a fusion protein of HPV16 E6 and -E7 (SFV-eE6,7) induces strong cytotoxic T-lymphocyte (CTL) activity and eradication of established HPV-transformed tumours. In this study, we compared the antitumour efficacy of SFV-eE6,7 with that of a recombinant adenovirus (rAd) type 5 vector, expressing the same antigen construct (Ad-eE6,7). Prime-boosting with SFV-eE6,7 resulted in higher precursor CTL frequencies and CTL activity compared to prime-boosting with Ad-eE6,7 and also in murine tumour treatment experiments SFV-eE6,7 was more effective than Ad-eE6,7. To elicit a therapeutic effect with Ad-eE6,7, 100/1000-fold higher doses were needed compared to SFV-eE6,7. In vivo T-cell depletion experiments demonstrated that these differences could not be explained by the induction of a different type of effector cells, since CD8+ T cells were the main effector cells involved in the protection against tumour growth in both rSFV- and rAd-immunized mice. Also comparable amounts of in vivo transgene expression were found upon immunization with rSFV and rAd encoding the reportor gene luciferase. However, anti-vector responses induced by a single injection with rAd resulted in a more than 3-log decrease in luciferase expression after a second injection of rAd. With rSFV, transgene expression was inhibited by only one to two orders of magnitude in preinjected mice. As an antigen-specific booster immunization strongly increases the level of the CTL response and is essential for efficient induction of immunological memory, it is likely that (part of) the difference in efficacy between rSFV and rAd type 5 can be ascribed to a diminished efficacy of the booster immunization in the case of rAd due to anti-vector antibody responses.

Rossi, A., S. Ciafre, et al. (2006). "Targeting the heat shock factor 1 by RNA interference: a potent tool to enhance hyperthermochemotherapy efficacy in cervical cancer." Cancer Res **66**(15): 7678-85.

Carcinoma of the uterine cervix is one of the highest causes of mortality in female cancer patients worldwide, and improved treatment options for this type of malignancy are highly needed. Local hyperthermia has been successfully used in combination with systemic administration of cisplatin-based chemotherapy in phase I/II clinical studies. Heat-induced expression of cytoprotective and antiapoptotic heat shock proteins (HSP) is a known complication of hyperthermia, resulting in thermotolerance and chemoresistance and hindering the efficacy of the combination therapy. Heat shock transcription factor 1 (HSF1) is the master regulator of heat-induced HSP expression. In the present report, we used small interfering RNA (siRNA) to silence HSF1 and to examine the effect of HSF1 loss of function on the response to hyperthermia and cisplatin-based chemotherapy in HeLa cervical carcinoma. We have identified the 322-nucleotide to 340-nucleotide HSF1 sequence as an ideal target for siRNA-mediated HSF1 silencing, have created a pSUPER-HSF1 vector able to potently suppress the HSF1 gene, and have generated for the first time human cancer cell lines with stable loss of HSF1 function. We report that, although it surprisingly does not affect cancer cell sensitivity to cisplatin or elevated temperatures up to 43 degrees C when administered separately, loss of HSF1 function causes a dramatic increase in sensitivity to

hyperthermochemotherapy, leading to massive (>95%) apoptosis of cancer cells. These findings indicate that disruption of HSF1-induced cytoprotection during hyperthermochemotherapy may represent a powerful strategy to selectively amplify the damage in cancer cells and identify HSF1 as a promising therapeutic target in cervical carcinoma.

Sami, S., N. Hoti, et al. (2008). "Valproic acid inhibits the growth of cervical cancer both in vitro and in vivo." J Biochem **144**(3): 357-62.

Valproic acid (VPA), a well-known anti-convulsant, is currently under extensive evaluation as an anti-cancer agent. It is known to exert its anti-cancer effect mainly by inhibiting the enzyme histone deacetylase I. In our study, we investigated the effects of VPA on cervical cancer both in vitro and in vivo cancer models. We examined the effects of acute VPA (0, 1.2, 2.4, 5.0 mM) treatment on cell proliferation in cervical cancer cell lines HeLa, SiHa and Ca Ski and histone acetylation, p21 and p53 gene expression in HeLa cell line. We also investigated the effect of chronic VPA administration in tumour xenograft growth studies. Our results show that with acute treatment, VPA can increase the expression of net histone H3 acetylation and up-regulate p21 expression with no effect on p53 expression. Chronic administration of VPA had a net cytostatic effect that resulted in a statistically significant reduction of tumour growth and improved survival advantages in tumour xenografts studies. Furthermore, we also demonstrated that VPA has a direct anti-angiogenic effect in tumour studies and could potentially be a promising candidate for further cervical cancer trails.

Santin, A. D., F. Zhan, et al. (2005). "Gene expression profiles of primary HPV16- and HPV18-infected early stage cervical cancers and normal cervical epithelium: identification of novel candidate molecular markers for cervical cancer diagnosis and therapy." Virology **331**(2): 269-91.

With the goal of identifying genes with a differential pattern of expression between invasive cervical carcinomas (CVX) and normal cervical keratinocytes (NCK), we used oligonucleotide microarrays to interrogate the expression of 14,500 known genes in 11 primary HPV16 and HPV18-infected stage IB-IIA cervical cancers and four primary normal cervical keratinocyte cultures. Hierarchical cluster analysis of gene expression data identified 240 and 265 genes that exhibited greater than twofold up-regulation and down-regulation, respectively, in primary CVX when compared to NCK. Cyclin-dependent kinase inhibitor 2A (CDKN2A/p16), mesoderm-specific transcript, forkhead box M1, v-myb myeloblastosis viral oncogene homolog (avian)-like2 (v-Myb), minichromosome maintenance proteins 2, 4, and 5, cyclin B1, prostaglandin E synthase (PTGES), topoisomerase II alpha (TOP2A), ubiquitin-conjugating enzyme E2C, CD97 antigen, E2F transcription factor 1, and dUTP pyrophosphatase were among the most highly overexpressed genes in CVX when compared to NCK. Down-regulated genes in CVX included transforming growth factor beta 1, transforming growth factor alpha, CFLAR, serine proteinase inhibitors (SERPING1 and SERPINF1), cadherin 13, protease inhibitor 3, keratin 16, and tissue factor pathway inhibitor-2 (TFPI-2). Differential expression of some of these genes including CDKN2A/p16, v-Myb, PTGES, and TOP2A was validated by quantitative real-time PCR. Flow cytometry on primary CVX and NCK and immunohistochemical staining of formalin fixed paraffin-embedded tumor specimens from which primary CVX cultures were derived as well as from a separate set of invasive cervical cancers confirmed differential expression of the CDKN2A/p16 and PTGES markers on CVX versus NCK. These results identify several genes that are coordinately disregulated in cervical cancer, likely representing common signaling pathways triggered by HPV transformation. Moreover, these data obtained with highly purified primary tumor cultures highlight novel molecular features of human cervical cancer and provide a foundation for the development of new type-specific diagnostic and therapeutic strategies for this disease.

Scholten, K. B., M. W. Schreurs, et al. (2005). "Preservation and redirection of HPV16E7-specific T cell receptors for immunotherapy of cervical cancer." Clin Immunol **114**(2): 119-29.

Human papilloma virus (HPV) type 16 infections of the genital tract are associated with the development of cervical cancer (CxCa) in women. HPV16-derived oncoproteins E6 and E7 are expressed constitutively in these lesions and might therefore be attractive candidates for T-cell-mediated adoptive immunotherapy. However, the low precursor frequency of HPV16E7-specific T cells in patients and healthy donors hampers routine isolation of these cells for adoptive transfer. To overcome this problem, we have isolated T cell receptor (TCR) genes from four different HPV16E7-specific healthy donor and patient-derived human cytotoxic T lymphocyte (CTL) clones. We examined whether genetic engineering of peripheral blood-derived CD8+ T cells in order to express HPV16E711-20-specific TCRs is feasible for adoptive transfer purposes. Reporter cells (Jurkat/MA) carrying a transgenic TCR were shown to bind relevant but not irrelevant tetramers. Moreover, these

TCR-transgenic Jurkat/MA cells showed reactivity towards relevant target cells, indicating proper functional activity of the TCRs isolated from already available T cell clones. We next introduced an HPV16E711-20-specific TCR into blood-derived, CD8+ recipient T cells. Transgenic CTL clones stained positive for tetramers presenting the relevant HPV16E711-20 epitope and biological activity of the TCR in transduced CTL was confirmed by lytic activity and by interferon (IFN)-gamma secretion upon antigen-specific stimulation. Importantly, we show recognition of the endogenously processed and HLA-A2 presented HPV16E711-20 CTL epitope by A9-TCR-transgenic T cells. Collectively, our data indicate that HPV16E7 TCR gene transfer is feasible as an alternative strategy to generate human HPV16E7-specific T cells for the treatment of patients suffering from cervical cancer and other HPV16-induced malignancies.

Shehata, M., M. Shehata, et al. (2004). "Dual apoptotic effect of Xrel3 c-Rel/NF-kappaB homolog in human cervical cancer cells." Cell Biol Int **28**(12): 895-904.

Cervical cancer is one of the most common cancers affecting a woman's reproductive organs. Despite its frequency and recurrence, the death rate has been declining over the past 40 years, due to early detection and treatment. In a previous report [Shehata Marlene, Shehata Marian, Shehata Fady, Pater Alan. Apoptosis effects of Xrel3 c-Rel/Nuclear factor-kappa B homolog in human cervical cancer cells. Cell Biology International, in press], we studied the role of the NF-kappaB gene family in HeLa human cervical cancer cells, using the Xrel3 c-Rel homologue of Xenopus laevis. These results showed that the expression of Xrel3/c-Rel slowed cell growth, consistent with an upregulated expression of the cell cycle inhibitor p21 and the activated poly(ADP-ribose) polymerase (PARP) apoptosis effector. However, in this report, we examined more apoptotic and anti-apoptotic factors acting upstream and downstream in apoptosis pathways after cisplatin treatment of HeLa cervical cancer cells. After 1 microM cisplatin treatment, Xrel3 had an anti-apoptotic effect, based on significantly lower levels of apoptotic proteins, including caspase-8, caspase-3 and p21. Anti-apoptotic BAG-1 isoforms were upregulated. After 5 microM cisplatin treatment, expression of HeLa Xrel3 had an apoptotic effect, based on significantly increased expression of the cell cycle inhibitor p21 and apoptotic proteins, including cleaved PARP, caspase-8, and caspase-3. However, anti-apoptotic Bcl-2 and Bcl-X(L) were elevated and the cell cycle regulator cyclin D1 was slightly upregulated with both 1 and 5 microM cisplatin treatment. The HPV E6 oncoprotein showed no significant changes. These results support previous conclusions on the potential anti-apoptotic effects of c-Rel/NF-kappaB in mild stress environments, as opposed to the apoptotic effects associated with high stress conditions [Lake BB, Ford R, Kao KR. Xrel3 is required for head development in Xenopus laevis. Development 2001; 128(2), 263-73.]. Thus, c-Rel/NF-kappaB may potentially be of clinical significance in chemotherapy.

Shen, L., S. Zeng, et al. (2008). "E1A inhibits the proliferation of human cervical cancer cells (HeLa cells) by apoptosis induction through activation of HER-2/Neu/Caspase-3 pathway." Med Oncol **25**(2): 222-8.

This study is to investigate the inhibitory effect of E1A gene on the cell proliferation of HeLa cells and its mechanism related to apoptosis. MTT assay and soft agar colony formation assay were employed to justify the inhibition activity of E1A on the proliferation of HeLa cells transfected with E1A gene. Western Blot, RT-PCR and Real-time quantitative RT-PCR were used to detect the gene expression of E1A, HER-2/Neu and Caspase-3 in HeLa cells, respectively. The Caspase-3 activity was monitored by ApoAlert Caspase-3 Assay. The redistribution of cell cycles and apoptosis of HeLa cells regulated by E1A expression were evaluated by flow cytometry. E1A expression significantly inhibits the cell proliferation and anchorage-independent cell growth of HeLa, with the respective highest inhibition rate of 40.7% and 43.4% ($P < 0.01$). HER-2/Neu expression in HeLa was significantly down-regulated by E1A, while the protein expression and activity of Caspase-3 was up-regulated by E1A expression. Flow cytometry revealed that E1A transfection in HeLa increased the cell number at G1 stage and simultaneously decreased the cell number at S stage. E1A transfection induced 8.71% of HeLa cells at apoptosis status. CONCLUSIONS: E1A significantly inhibits the cell proliferation of HeLa by the apoptosis induction through HER-2/Neu/Caspase-3 pathway. These results encourage us to continue an in-vivo study and preclinical development of LPD-E1A as a novel gene therapeutic agent for human cervical cancer.

Shi, H., L. L. Wei, et al. (2007). "Melanoma differentiation-associated gene-7/interleukin 24 inhibits invasion and migration of human cervical cancer cells in vitro." Saudi Med J **28**(11): 1671-5.

In this study, we used an adenoviral vector - melanoma differentiation-associated gene-7 (Ad-mda7) to examine the effect of the ectopic production of MDA-7/IL-24 on cell migration and invasion by

human cervical cancer cells. The study took place in the Department of Biochemistry and Molecular Biology, Chongqing Medical University, Chongqing, China, between April 2006 and November 2006. The change of metastasis of cervical cancer cells (CaSki) cells were detected by Cell Migration Assay and Cell Invasion Assay after treated with Ad-mda7. The production of proteins associated with cell migration and invasion were detected by western blot. Cervical cancer cells treated in vitro with Ad-mda7 migrated and invaded less than cells treated with phosphate-buffered saline (PBS) or Ad-Luc (vector control). Melanoma differentiation-associated gene-7 /IL-24 inhibited migration and invasion by down-regulating the production of matrix metalloproteinase-2 (MMP-2) and by up-regulating the production of p38 mitogen-activated protein kinase. relative to PBS and Ad-Luc. CONCLUSION: These results show that MDA-7/IL-24 inhibits invasion and migration by cervical cancer cells by down- or up- regulating proteins associated with these processes, resulting in reduced metastasis. Thus, Ad-mda7 should be considered a therapeutic agent that can inhibit primary tumor growth and prevent metastasis.

Shin, J. I., J. H. Shim, et al. (2008). "Sensitization of the apoptotic effect of gamma-irradiation in genistein-pretreated CaSki cervical cancer cells." J Microbiol Biotechnol **18**(3): 523-31.

Radiotherapy is currently applied in the treatment of human cancers. We studied whether genistein would enhance the radiosensitivity and explored its precise molecular mechanism in cervical cancer cells. After co-treatment with genistein and irradiation, the viability, cell cycle analysis, and apoptosis signaling cascades were elucidated in CaSki cells. The viability was decreased by co-treatment with genistein and irradiation compared with irradiation treatment alone. Treatment with only gamma-irradiation led to cell cycle arrest at the G1 phase. On the other hand, co-treatment with genistein and gamma-irradiation caused a decrease in the G1 phase and a concomitant increase up to 56% in the number of G2 phase. In addition, cotreatment increased the expression of p53 and p21, and Cdc2-tyr-15-p, supporting the occurrence of G2/M arrest. In general, apoptosis signaling cascades were activated by the following events: release of cytochrome c, upregulation of Bax, downregulation of Bcl-2, and activation of caspase-3 and -8 in the treatment of genistein and irradiation. Apparently, co-treatment downregulated the transcripts of E6*I, E6*II, and E7. Genistein also stimulated irradiation-induced intracellular reactive oxygene, species (ROS) production, and co-treatment-induced apoptosis was inhibited by the antioxidant N-acetylcysteine, suggesting that apoptosis has occurred through the increase in ROS by genistein and gamma-irradiation in cervical cancer cells. Gamma-irradiation increased cyclooxygenase-1 (COX-2) expression, whereas the combination with genistein and gamma-irradiation almost completely prevented irradiation-induced COX-2 expression and PGE2 production. Co-treatment with genistein and gamma-irradiation inhibited proliferation through G2/M arrest and induced apoptosis via ROS modulation in the CaSki cancer cells.

Sima, N., W. Wang, et al. (2008). "RNA interference against HPV16 E7 oncogene leads to viral E6 and E7 suppression in cervical cancer cells and apoptosis via upregulation of Rb and p53." Apoptosis **13**(2): 273-81.

The simultaneous expression of human papillomavirus type 16 (HPV16) E6 and E7 oncogenes is pivotal for malignant transformation and maintenance of malignant phenotypes. Silencing these oncogenes is considered to be applicable in molecular therapies of human cervical cancer. However, it remains to be determined whether HPV16 E6 and E7 could be both silenced to obtain most efficient antitumor activity by using RNA interference (RNAi) technology. Herein, we designed a small interfering RNA (siRNA) targeting HPV16-E7 region to degrade either E6, or truncated E6 (E6*) and E7 mRNAs and to simultaneously knockdown both E6 and E7 expression. Firstly, the sequence targeting HPV16-E7 region was inserted into the shRNA packing vector pSIREN-DNR, yielding pSIREN-16E7 to stably express corresponding shRNA. HPV16-transformed SiHa and CaSki cells were used as a model system; RT-PCR, Western Blotting, MTT assay, TUNEL staining, Annexin V apoptosis assay and flow cytometry were applied to examine the effects of pSIREN-16E7. Our results indicated that HPV16-E7 specific shRNA (16E7-shRNA) induced selective degradation of E6 and E7 mRNAs and proteins. E6 silencing induced accumulation of cellular p53 and p21. In contrast, E7 silencing induced hypophosphorylation of retinoblastoma (Rb) protein. The loss of E6 and E7 reduced cell growth and ultimately resulted in massive apoptotic cell death selectively in HPV-positive cancer cells, compared with the HPV-negative ones. We demonstrated that 16E7-shRNA can induce simultaneous E6 and E7 suppression and lead to striking apoptosis in HPV16-related cancer cells by activating cellular p53, p21 and Rb. Therefore, RNAi using E7 shRNA may have the gene-specific therapy potential for HPV16-related cancers.

Song, Y. and C. Zhang (2009). "Hydralazine inhibits human cervical cancer cell growth in vitro in association with APC demethylation and re-expression." Cancer Chemother Pharmacol **63**(4): 605-13.

The tumor suppressor adenomatous polyposis coli (APC) is frequently silenced by promoter hypermethylation in human cervical cancer. Clinically, it has been approved that DNA methylation inhibitors, such as 5-aza-2'-deoxycytidine (5-Aza-dC), can reverse APC promoter methylation, but widespread clinical use of these inhibitors is limited by their toxicity and instability in aqueous solution. Hydralazine is a stable DNA methylation inhibitor that has minimal toxicity in vitro and in vivo. The purpose of this study was to evaluate the effects of hydralazine on APC reactivation and the inhibition of human cervical cancer cells in vitro. Expression of APC gene, and methylation status were analyzed by RT-PCR, quantitative real time RT-PCR, and methylation-specific PCR methods. beta-Catenin protein that correlates closely with APC was detected by immunohistochemistry method after treatment with hydralazine. MTT and FCM assays were used to observe the changes of proliferation activity, cell cycle, and apoptosis of the cells. Methylated APC was not expressed in HeLa cell, hemimethylated APC was expressed in CaSki cells, and unmethylated APC was expressed normally in SiHa cells. Hydralazine induces APC expression and promotes demethylation in HeLa and CaSki cells. After treatment with 40 mumol/L hydralazine for 72 h, growth inhibitive rates (%) of HeLa, CaSki, and SiHa cell lines were 52.12 +/- 3.78, 44.31 +/- 2.59, and 47.73 +/- 4.73, respectively. On the contrary, the normal cell ECV304 growth inhibitory rate was only 27.18 +/- 0.79. The expression of APC mRNA in HeLa, CaSki, and SiHa cell lines increased 10.35-, 11.40-, and 0.73-fold, respectively. HeLa and CaSki cells were arrested in S phase of the cell cycle by hydralazine, and the percentage of apoptotic cells in the two cell lines treated with hydralazine was increased significantly compared to the untreated cells ($P < 0.01$). The expression of beta-catenin protein in the cell membrane was observed after the treatment with hydralazine. CONCLUSIONS: Hydralazine, an effective inhibitor of APC methylation and promoter of APC re-expression, can inhibit cell growth in human cervical cancer in vitro and be potentially used for the clinical treatment of human cervical cancer.

Sopov, I., T. Sorensen, et al. (2004). "Detection of cancer-related gene expression profiles in severe cervical neoplasia." Int J Cancer **112**(1): 33-43.

The molecular signatures of 20 severe cervical intraepithelial neoplasia (CIN3) cases and 10 cervical squamous cell cancers were determined to define cancer-related gene expression profiles. RNAs extracted from microdissected tissues were amplified by SMART technology and used as probes for hybridization of commercially available cDNA array filters comprising 1,176 cancer-related genes. Ninety-two differentially expressed genes were identified by comparison of pooled cDNA from CIN3 vs. cervical cancer. Heterogeneity in expression of this subset of genes was then analyzed for each biopsy using an algorithm for self-organizing maps. For several gene clusters, the expression pattern for CIN3 differed significantly from that of cancer. Moreover, hierarchical clustering revealed significant differences in distribution of CIN and cancer. Several CIN cases were more strongly related to cancer, suggesting that gene expression profiling may be useful for subdividing pathologically indistinguishable precancers into different biologic entities. This approach also provides a basis for the identification of putative prognostic markers and for targeted molecular therapy.

Sultana, H., J. Kigawa, et al. (2003). "Chemosensitivity and p53-Bax pathway-mediated apoptosis in patients with uterine cervical cancer." Ann Oncol **14**(2): 214-9.

OBJECTIVES: To determine whether and how apoptosis through the p53-Bax pathway affects sensitivity to chemotherapy in cervical cancer. Thirty patients with cervical squamous cell carcinoma, who had human papilloma virus (HPV) and underwent neoadjuvant chemotherapy, were entered in the present study. Tumor specimens were obtained before and after chemotherapy. HPV was detected by polymerase chain reaction. The expression of Ki-67, p53, Bax and Bcl-2 proteins was determined by immunohistochemical staining. Apoptotic cells were identified by the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate biotin nick-end labeling method. Of 30 patients, 18 responded to chemotherapy and 12 did not. The apoptotic index in tumors of responders was significantly higher than in non-responders after chemotherapy. The Ki-67 labeling index (LI) in responders was significantly higher than in non-responders before chemotherapy. Patients with tumors >33% of the LI, which was determined by a receiver operating characteristic curve, had a better survival rate. The incidence of p53 protein expression did not differ between responders and non-responders. After chemotherapy, the expression of Bax protein in responders was more frequent and Bcl-2 protein expression was less frequent than in non-responders. CONCLUSIONS: Chemosensitivity in cervical cancer

may be associated with apoptosis via the p53-Bax pathway.

Sun de, J., Y. Liu, et al. (2007). "Endothelin-3 growth factor levels decreased in cervical cancer compared with normal cervical epithelial cells." Hum Pathol **38**(7): 1047-56.

We used cDNA microarray analysis of RNA extracted from normal, dysplastic, and cancerous cervical tissues to identify the changes in gene expression during the procession from normal to cancerous cervical epithelial cells. We found the expression of 5 genes in cancerous cervical epithelial cells that were not found in normal cervical epithelial cells, among which were lymphoid-restricted membrane protein, protease serine 2, WD repeat domain 59, thyrotropin-releasing hormone degrading enzyme, and the endothelin-3 growth factor. We then analyzed the expression levels of endothelin growth factors 1, 2, and 3 (ET-1, ET-2, and ET-3) and their receptors A and B (ETR-A and ETR-B) by reverse transcriptase-polymerase chain reaction in 3 cervical cancer cell lines and by immunohistochemical staining in cervical normal, dysplastic, and cancer tissues. ET-1, ET-2, and ET-3 growth factor levels were detectable in the maturing layer of cervical epithelium but not in the germinal layer. All 3 growth factors (ET-1, ET-2, and ET-3) were detected in the cytoplasm of the maturing normal cervical epithelial cells. In addition, there were decreased levels of ET-3 and increased levels of ET-1, ET-2, ETR-A, and ETR-B in cancerous cervical epithelial cells compared with normal cervical epithelial cells. These results suggest that the reduction of ET-3 growth factor levels may be important in the transition from normal to cancerous cervical epithelium.

Tang, B., L. Li, et al. (2005). "Characterization of the mechanisms of electrochemotherapy in an in vitro model for human cervical cancer." Int J Oncol **26**(3): 703-11.

Electrochemical treatment is among the most effective therapies in the management of cervical malignancy. However, the mechanism of action of this treatment remains largely unknown. Therefore, the purpose of the current project was to establish a suitable eletrochemotherapy regimen for cervical cancer and to investigate the mechanism of the therapy in an in vitro model for human cervical carcinoma. HeLa cells were used as a model for cervical cancer in this study, and the effect of electrochemical treatment on these cells was examined in four different dosage groups (5 V + 5 C, 10 V + 5 C, 5 V + 10 C and 10 V + 10 C). Our results showed that the combinations of lower voltage and higher current (5 V/10 V + 10 C) had a greater anticancer effect in this model as compared to other groups. In addition, we compared the cytotoxic effect between electrochemical treatment and different pH condition treatments in this system, and found that the efficacy of electrochemical treatment in cell killing was better than that of acidic or basic medium treatment. Moreover, we demonstrated that the efficacy of electrochemical treatment was correlated with the degree of ionization and alteration in pH scale. The electrodes were basic on the cathode side which elevated the cations K+, Ca2+ and Mg2+, while the electrodes were acidic on the anode side which reduced the anion Cl-. We also assessed the effect of electrochemical treatment on cell cycle distribution in HeLa cells and showed that the percentage of cells in the G1 phase of the cell cycle was increased (G1 arrest), while the cell population in the S phase was decreased. Furthermore, we demonstrated that the levels of the cell cycle regulator cyclin D1 expression were dramatically reduced when 5 V/10 V + 10 C treatments were applied to these cells, as determined by RT-PCR analysis. By contrast, no significant changes in the levels of cyclin B1, CDK1 or CDK4 were detected. Based on these observations, we conclude that the combination of lower voltage and higher current may be a potentially effective eletrochemotherapy regimen for cervical cancer in the clinic, and that the antitumor effect of electrochemical treatment on cervical carcinoma cells is mediated partly via regulating ionization degree, pH state and cell cycle control.

Tillmanns, T. D., S. A. Kamelle, et al. (2005). "Sensitization of cervical cancer cell lines to low-dose radiation by retinoic acid does not require functional p53." Gynecol Oncol **97**(1): 142-50.

Current therapy for cervical cancer includes radiation therapy. Retinoic acid (RA) can increase the sensitivity of cervical cancer cell lines to radiation. The mechanism of this sensitization may not involve the p53 protein because the human papillomavirus (HPV) E6 protein, which is present in the majority of cervical cancers, promotes p53 degradation. The objective of this study was to determine if p53 is involved in the mechanism of RA radiosensitization. METHOD: The effects of radiation on cervical (SiHa, CC-1, and C33a) and vulvar (SW962) cancer cell lines under various experimental conditions were evaluated using clonogenic, Coulter Counter, electrophoretic mobility shift (EMSA) and a multi-probe RNase protection assay of p53-inducible genes. RA (5 microM 9-cis-RA) radiosensitized the SiHa and CC-1 cell lines that contain HPV-degraded p53, but did not radiosensitize the SW962 cell line, which is HPV negative and contains wild-type p53, nor the C33a cell line, which contains mutant p53 (R273C). Expression

of mutant p53 (R273H) in SiHa cells increased the growth rate, but did not prevent RA-induced differentiation or radiosensitization at clinically relevant doses. Inhibition of p53 transactivation with pifithirin alpha did not prevent RA radiosensitization of SiHa at 5 Gy. RA repressed c-fos mRNA expression in control and irradiated SiHa cultures, but did not repress bcl-x(L), p53, GADD45, p21, bax, bcl-2, or mcl-1 mRNA expression. CONCLUSIONS: The mechanism of RA radiosensitization does not require functional p53 and may involve c-fos in cervical cancer cell lines.

Wakatsuki, M., T. Ohno, et al. (2008). "p73 protein expression correlates with radiation-induced apoptosis in the lack of p53 response to radiation therapy for cervical cancer." Int J Radiat Oncol Biol Phys **70**(4): 1189-94.

p73 belongs to the p53 tumor suppressor family of genes and can inhibit cell growth in a p53-like manner by inducing apoptosis or cell cycle arrest. Here, we investigated whether p73 could compensate for impaired p53 function in apoptosis induced by radiation therapy (RT) for cervical cancer. Sixty-eight patients with squamous cell carcinoma of the cervix who received definitive RT combined with (n=37) or without (n=31) cisplatin were investigated. Biopsy specimens were excised from the cervical tumor before RT and after 9 Gy. Mean apoptosis index (AI) was 0.93% before RT and 1.97% after 9 Gy with a significant increase ($p<0.001$). For all patients, there was a significant correlation between p73 expression positivity after 9 Gy and AI ratio (AI after 9 Gy/AI before RT) ($p=0.021$). Forty-one patients were regarded as the p53-responding group according to the expression of p53 after 9 Gy, whereas the remaining 27 patients were regarded as the p53-nonresponding group. A significant correlation between p73 expression after 9 Gy and AI ratio was observed in the p53-non-responding group ($p<0.001$) but not in the p53-responding group ($p=0.940$). CONCLUSION: Our results suggest that p73 plays an important role in compensating for the lack of p53 function in radiation-induced apoptosis of cervical cancer.

Watari, H., Y. Ohta, et al. (2008). "Clusterin expression predicts survival of invasive cervical cancer patients treated with radical hysterectomy and systematic lymphadenectomy." Gynecol Oncol **108**(3): 527-32.

OBJECTIVES: The aim of this study was to evaluate the prognostic significance of clusterin expression in invasive cervical cancer patients treated with radical hysterectomy and systematic lymphadenectomy. Invasive cervical cancer specimens were obtained from 52 patients who underwent radical hysterectomy and systematic lymphadenectomy at Hokkaido University Hospital from 1997 to 2004. The expression of clusterin protein was analyzed by immunohistochemical staining. Findings were evaluated in relation to several clinicopathological factors. Survival analyses were performed by the Kaplan-Meier curves and the log-rank test. Independent prognostic factors were determined by multivariate Cox regression analysis. Clusterin protein was present in the cytoplasm of cervical cancer cells. The expression of clusterin protein in invasive cervical cancer tissues was not related to any clinicopathologic factors analyzed. Patients with positive clusterin expression showed significantly worse prognosis than those with negative clusterin expression ($p=0.017$). Multivariate analysis including clusterin expression revealed that clusterin expression ($p=0.006$) and the number of positive node groups ($p=0.002$) were independent prognostic factors for survival. Survival of patients with invasive cervical cancer could be stratified into three groups by combination of clusterin expression and number of positive node groups with an estimated 5-year survival rate of 100.0% for no or one positive node group irrespective of clusterin expression (group A), 78.7% for multiple node groups with negative clusterin expression (group B), and 14.3% for multiple node groups with positive clusterin expression (group C) ($p=0.03$ for group A vs. group B, $p=0.004$ for group B vs. group C, and $p<0.0001$ for group A vs. group C). CONCLUSIONS: Clusterin expression and the number of positive node groups were independent prognostic factors for invasive cervical cancer patients treated with radical hysterectomy and systematic lymphadenectomy. Clusterin might be a new molecular marker to predict the survival of cervical cancer patients with multiple positive node groups.

Weidhaas, J. B., S. X. Li, et al. (2009). "Changes in gene expression predicting local control in cervical cancer: results from Radiation Therapy Oncology Group 0128." Clin Cancer Res **15**(12): 4199-206.

To evaluate the potential of gene expression signatures to predict response to treatment in locally advanced cervical cancer treated with definitive chemotherapy and radiation. EXPERIMENTAL DESIGN: Tissue biopsies were collected from patients participating in Radiation Therapy Oncology Group (RTOG) 0128, a phase II trial evaluating the benefit of celecoxib in addition to cisplatin chemotherapy and radiation for locally advanced cervical cancer. Gene expression profiling was done and signatures of pretreatment, mid-treatment (before the first implant), and "changed" gene expression patterns between pre- and mid-treatment samples were determined. The ability of the gene signatures to predict local control

versus local failure was evaluated. Two-group t test was done to identify the initial gene set separating these end points. Supervised classification methods were used to enrich the gene sets. The results were further validated by leave-one-out and 2-fold cross-validation. Twenty-two patients had suitable material from pretreatment samples for analysis, and 13 paired pre- and mid-treatment samples were obtained. The changed gene expression signatures between the pre- and mid-treatment biopsies predicted response to treatment, separating patients with local failures from those who achieved local control with a seven-gene signature. The in-sample prediction rate, leave-one-out prediction rate, and 2-fold prediction rate are 100% for this seven-gene signature. This signature was enriched for cell cycle genes. CONCLUSIONS: Changed gene expression signatures during therapy in cervical cancer can predict outcome as measured by local control. After further validation, such findings could be applied to direct additional therapy for cervical cancer patients treated with chemotherapy and radiation.

Weismann, P., E. Weismanova, et al. (2009). "The detection of circulating tumor cells expressing E6/E7 HR-HPV oncogenes in peripheral blood in cervical cancer patients after radical hysterectomy." Neoplasma **56**(3): 230-8.

The aim of this study was to establish the sensitive, specific and clinically acceptable method for detection of tumor cells (TCs) circulating in peripheral blood (PB) of cervical cancer patients without the clinically detectable risk of disease progression. The 7.5 ml of PB of healthy donor was spiked with 5 to 100 cells from SiHa or HeLa cell lines. The spiked tumor cells were collected without gradient centrifugation, by standard gradient centrifugation or by modified gradient centrifugation combined with immunomagnetic separation using EpCAM antibody with affinity for epithelial cell adhesion molecule. The number of collected TCs was determined by EpCAM-FITC-staining and their viability was detected by nested RT-PCR amplifying E6/E7 HR-HPV 16 or HR-HPV 18 oncogenes. For the technical validation of this approach the TCs separation and RT-PCRs were repeated several times. The recovery of viable TCs was reproducibly higher using modified gradient centrifugation combined with immunomagnetic separation in comparison with standard approach. The recovery of TCs in low number of spiked TCs (range from 5 - 20 TCs in 7.5 ml of PB) using modified gradient centrifugation was not reproducible. The recovery of TCs in higher number of spiked TCs (25 TCs and more in 7.5 ml of PB) was reproducible with average recovery about 50 %. The sensitivity of nested RT-PCR amplifying E6/E7 oncogenes was decisively influenced by the number of recovered TCs and the amount of cDNA introduced to RT-PCR, as well. Using this approach we were allowed to detect circulating TCs (CTCs) in cervical cancer patients without metastases, thus this procedure might become a tool to early estimation of disease progression. According to our knowledge, this is the first report describing the use of EpCAM antibody for CTCs detection in cervical cancer patients.

Wells, S. I., B. J. Aronow, et al. (2003). "Transcriptome signature of irreversible senescence in human papillomavirus-positive cervical cancer cells." Proc Natl Acad Sci U S A **100**(12): 7093-8.

A frequent characteristic of human papillomavirus (HPV)-positive cervical cancers is the loss of viral E2 gene expression in HPV-infected cervical epithelial cells as a consequence of viral DNA integration into the cellular genome. The expression of E2 in HPV-positive cancer cells results in the repression of the viral E6/E7 oncogenes, activation of the p53 and pRB pathways, and a G1 cell cycle arrest, followed by induction of cellular senescence. The transcriptional consequences of E2-mediated cell cycle arrest that lead to senescence currently are unknown. Using conditional senescence induction in HeLa cells and microarray analysis, we describe here the expression profile of cells irreversibly committed to senescence. Our results provide insight into the molecular anatomy of senescence pathways and its regulation by HPV on-coproteins. These include the induction of the RAB vesicular transport machinery and a general down-regulation of chromatin regulatory molecules. The repression of tumor-specific G antigens during E2 senescence supports a reversal of the tumorigenic phenotype by E2 and the potential approach of tumor-specific G antigen-specific immunotherapy for cervical cancer.

Widschwendter, A., L. Ivarsson, et al. (2004). "CDH1 and CDH13 methylation in serum is an independent prognostic marker in cervical cancer patients." Int J Cancer **109**(2): 163-6.

Cervical cancer is the principal cause of death due to cancer in women. Five-year survival rate ranges from 15-80%, depending on the extent of the disease. New predictive markers for relapse may increase survival rates by improving treatment of patients at high risk for relapse. The gene products of CDH1 and CDH13, namely E-cadherin and H-cadherin, play a key role in cell-cell adhesion. Inactivation of the cadherin-mediated cell adhesion system, caused by aberrant methylation, is a common finding in human cancers. To test the hypothesis that CDH1/CDH13 methylation is a prognostic marker in cervical cancer we determined the methylation status

of CDH1/CDH13 in serum samples from 93 cervical cancer patients. Methylation analysis was carried out using MethyLight. Aberrant methylation of the 5'-region of CDH1 or CDH13 was observed in 43% (40 of 93) of the patients. Cervical cancer patients with unmethylated CDH1/CDH13 in serum samples showed significantly better disease-free survival in univariate and multivariate analysis. Median disease-free survival for CDH1/CDH13 methylation negative and positive patients was 4.3 years and 1.2 years, respectively. Our results suggest that detection of aberrant methylation of CDH1/CDH13 may be of potential use as a marker for selecting cervical cancer patients at high risk for relapse who could benefit from additional systemic therapy.

Wolf, J. K., E. L. Franco, et al. (2003). "Innovations in understanding the biology of cervical cancer." Cancer **98**(9 Suppl): 2064-9.

Revelation of the connection between the human papillomavirus (HPV) and cervical neoplasia and invasive cervical cancer is prompting new investigations to expand that understanding and promote vaccines, gene therapy, and other interventions. At the Second International Conference on Cervical Cancer (Houston, TX, April 11-14, 2002), laboratory and clinical researchers reported advances in new studies meant to increase understanding of the natural history of HPV and cervical intraepithelial neoplasia, to evaluate new cervical cancer screening techniques, and to promote new therapies. Using K14-HPV type 16 transgenic mice, researchers are investigating the effects of estrogen on cervical cancer carcinogenesis, and results are lending support to epidemiological theories showing a difference in HPV infection rates and the development of cervical lesions in women using oral contraceptives. Other work involves investigating genes that are up-regulated by HPV infection and the role of the p53 homologue, p63, in cervical neoplasia evolution. Telomerase also is under investigation as a biomarker in high-risk populations. Gene therapy that replaced p53 in cervical cancer cell lines in vitro and a nude mouse model inhibited cell and tumor growth, confirming previous findings in squamous epithelial carcinomas of the head and neck. Furthermore, research in intracellular targeting of antigens to subcellular locations shows promise for treating cervical cancer preclinically. Identification of molecular changes in cervical cancer and knowledge about the importance of HPV infection in cervical cancer can lead to new therapies to treat existing cervical cancer and, in the long term, prevent the disease.

Wong, Y. F., T. H. Cheung, et al. (2006). "Genome-wide gene expression profiling of cervical cancer in Hong Kong women by oligonucleotide microarray." Int J Cancer **118**(10): 2461-9.

An analysis of gene expression profiles obtained from cervical cancers was performed to find those genes most aberrantly expressed. Total RNA was prepared from 29 samples of cervical squamous cell carcinoma and 18 control samples, and hybridized to Affymetrix oligonucleotide microarrays with probe sets complementary to over 20,000 transcripts. Unsupervised hierarchical clustering of the expression data readily distinguished normal cervix from cancer. Supervised analysis of gene expression data identified 98 and 139 genes that exhibited >2-fold upregulation and >2-fold downregulation, respectively, in cervical cancer compared to normal cervix. Several of the genes that were differentially regulated included SPP1 (Osteopontin), CDKN2A (p16), RPL39L, Clorf1, MAL, p11, ARS and NICE-1. These were validated by quantitative RT-PCR on an independent set of cancer and control specimens. Gene Ontology analysis showed that the list of differentially expressed genes included ones that were involved in multiple biological processes, including cell proliferation, cell cycle and protein catabolism. Immunohistochemical staining of cancer specimens further confirmed differential expression of SPP1 in cervical cancer cells vs. nontumor cells. In addition, 2 genes, CTGF and RGS1 were found to be upregulated in late stage cancer compared to early stage cancer, suggesting that they might be involved in cancer progression. The pathway analysis of expression data showed that the SPP1, VEGF, CDC2 and CKS2 genes were coordinately differentially regulated between cancer and normal. The present study is promising and provides potential new insights into the extent of expression differences underlying the development and progression of cervical squamous cell cancer. This study has also revealed several genes that may be highly attractive candidate molecular markers/targets for cervical cancer diagnosis, prognosis and therapy.

Wong, Y. F., D. S. Sahota, et al. (2006). "Gene expression pattern associated with radiotherapy sensitivity in cervical cancer." Cancer J **12**(3): 189-93.

The objective of the present preliminary study was to determine if a difference in the pattern of gene expression exists between tumors that were subsequently found to be sensitive to radiotherapy and tumors found to be resistant to radiotherapy. A total of 16 patients with invasive squamous cell carcinoma of the uterine cervix were included in this study. All patients were treated with standardized radiotherapy alone. Ten of the tumors were clinically radiosensitive and six were radioresistant. Total RNA, extracted from tumor specimens obtained prior to treatment, was hybridized onto an oligonucleotide microarray

with probe sets complementary to over 20,000 transcripts. The genes were first subjected to a statistical filter to identify genes with statistically significant differential expression levels between those that were radiosensitive and those that were radioresistant. A back-propagation neural network was then constructed to model the differences so that patterns could be easily identified. Although a number of genes were found to express differentially between radiosensitive and radioresistant tumors; the 10 most discriminating genes were used to construct the model. Using the expressions from these 10 genes, we found that neural networks constructed from random subsets of the whole data were capable of predicting radiotherapy responses in the remaining subset, which appears stable within the dataset. DISCUSSION: This study shows that such an approach has the potential to differentiate tumor radiosensitivity, although confirmation of such a pattern using other larger independent datasets is necessary before firm conclusions can be drawn.

Yamato, K., J. Fen, et al. (2006). "Induction of cell death in human papillomavirus 18-positive cervical cancer cells by E6 siRNA." Cancer Gene Ther **13**(3): 234-41.

Human cervical cancer is caused by high-risk types of human papillomavirus (HPV) such as HPV16 and HPV18, which possess the E6 and E7 oncogenes, whose concurrent expression is a prerequisite for cancer development and maintaining malignant phenotypes. Silencing these oncogenes is considered to be applicable in molecular therapies of human cervical cancer. However, it remains to be determined whether E6, E7, or both should be silenced to obtain most efficient antitumor activity by an HPV small-interfering RNA (siRNA). Herein, we report two types of siRNAs targeting HPV18 E6, that exerted a negative growth effect on HPV18-positive cervical cancer cells (HeLa and SW756), in part, inducing cell death. One siRNA (Ex-18E6), designed to target both E6-E7 mRNA and its splicing variant, E6*I-E7 mRNA, efficiently knocked down both E6 and E7 expression. The other (Sp-18E6), designed to specifically target E6-E7 mRNA but not E6*I-E7 mRNA, suppressed E6 to a similar level as Ex-18E6; however, it less efficiently inhibited E7 as compared to Ex-18E6. Although both siRNAs induced cell death, Sp-18E6 siRNA induced more prominent cell death than Ex-18E6. Our results suggest that E6-specific suppression may induce more potent anticancer activity than simultaneous E6 and E7 suppression, and that E6-specific targeting is a promising strategy for siRNA-based therapy for HPV-positive cervical cancer.

Yamato, K., T. Yamada, et al. (2008). "New highly potent and specific E6 and E7 siRNAs for treatment of HPV16 positive cervical cancer." Cancer Gene Ther **15**(3): 140-53.

Persistent infection by high-risk types of human papillomaviruses (HPV) is a necessary cause of cervical cancer, with HPV16 the most prevalent, accounting for more than 50% of reported cases. The virus encodes the E6 and E7 oncoproteins, whose expression is essential for maintenance of the malignant phenotype. To select efficacious siRNAs applicable to RNAi therapy for patients with HPV16+ cervical cancer, E6 and E7 siRNAs were designed using siDirect computer software, after which 10 compatible with all HPV16 variants were selected, and then extensively examined for RNAi activity and specificity using HPV16+ and HPV16-cells. Three siRNAs with the highest RNAi activities toward E6 and E7 expression, as well as specific and potent growth suppression of HPV16+ cancer cells as low as 1 nM were chosen. Growth suppression was accompanied by accumulation of p53 and p21(WAF1/CIP1), as well as morphological and cytochemical changes characteristic of cellular senescence. Antitumor activity of one of the selected siRNAs was confirmed by retarded tumor growth of HPV16+ cells in NOD/SCID mice when locally injected in a complex with atelocollagen. Our results demonstrate that these E6 and E7 siRNAs are promising therapeutic agents for treatment of virus-related cancer.

Yao, Z. and Z. Shulan (2008). "Inhibition effect of Guizhi-Fuling-decoction on the invasion of human cervical cancer." J Ethnopharmacol **120**(1): 25-35.

AIM OF THE STUDY: Guizhi-Fuling-decoction (GZFLD), a traditional Chinese medical formulation, exerts an anti-tumor effect, but the mechanisms of its action on invasive tumor inhibition have not been documented. The aims of this study were to identify the inhibitory effect of GZFLD on the invasive of cervical cancer and to elucidate the extensional mechanisms of its action. MATERIALS AND METHOD: The invasive ability of HeLa cells was tested with Transwell chamber. The expressions and activities of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) were measured by zymography/reverse zymography, RT-PCR and Western blot analysis. Establish tumor-bearing mice model to assess the ability of GZFLD to inhibit tumor growth and angiopoiesis in vivo. We have found that GZFLD suppressed the invasive ability of HeLa cells, inhibited MMPs expressions and activities, increased TIMPs expressions and activities, and furthermore restored the MMPs-TIMPs balance in HeLa cells in a concentration-dependent manner. Meanwhile in vivo,

GZFLD had significantly inhibited tumor growth and angiopoiesis. CONCLUSION: In general, our results showed that GZFLD had inhibited the invasion of cervical cancer both in vitro and vivo. The inhibitory effects may be associated with restoring the MMPs-TIMPs balance, and then suppressing the degradation of extracellular matrix.

Yashar, C. M., W. J. Spanos, et al. (2005). "Potentiation of the radiation effect with genistein in cervical cancer cells." Gynecol Oncol **99**(1): 199-205.

Early stage cervical cancer is treated with surgery or radiation with equivalent results. Radiation is used for curative therapy of locally advanced disease and is combined with additional anti-tumor agents to improve control. We determined the potential role of genistein as a radiosensitizer for cervical cancer cells. Human cervical cell lines (CaSki and ME180) were used. Sensitivity of cells to genistein, radiation and the combination was determined by colony assays. Western blotting was used to study the expression of cell-response-related gene products. Genistein results in the dose-dependent inhibition of all cell lines (2.5-40.0 microM). Effect of genistein on the radiosensitivity of the two cervical tumor cells was variable. Me180 cells were more sensitive at 20 and 40 microM of genistein. At 40 microM, less than 5% of Me180 cells survived the radiation (200-800 cGy). Potentiation of the radiation effect in CaSki cells was seen (500-800 cGy). The most significant enhancement of radiosensitivity was seen at 20 and 40 microM genistein at 500 and 800 cGy. G(2)M arrest was demonstrated only in ME180 cells with genistein. There was significant inhibition of Mcl-1 by genistein that correlated with increase in radiosensitivity in Me180 cells. Activated pAKT (Thr 308) was inhibited with genistein and radiation in CaSki cells. CONCLUSIONS.: Genistein inhibits growth of cervical cancer cells. Genistein results in variable and significant enhancement of the radiation effect that may be partially mediated by G(2)M arrest, Mcl-1 and activation of the AKT gene.

Yatabe, N., S. Kyo, et al. (2004). "HIF-1-mediated activation of telomerase in cervical cancer cells." Oncogene **23**(20): 3708-15.

Hypoxia-inducible factor 1 (HIF-1) is a key regulator of O(2) homeostasis, which regulates the expression of several genes linked to angiogenesis and energy metabolism. Tumor hypoxia has been shown to be associated with poor prognosis in a variety of tumors, and HIF-1 induced by hypoxia plays pivotal roles in tumor progression. The presence of putative HIF-1-binding sites on the promoter of human telomerase reverse transcriptase gene (hTERT) prompted us to examine the involvement of HIF-1 in the regulation of hTERT and telomerase in tumor hypoxia. The telomeric repeat amplification protocol (TRAP) assay revealed that hypoxia activated telomerase in cervical cancer ME180 cells, with peak induction at 24-48 h of hypoxia. Notably, hTERT mRNA expression was upregulated at 6-12 h of hypoxia, concordant with the elevation of HIF-1 protein levels at 6 h. hTERT protein levels were subsequently upregulated at 24 h and later. Luciferase assays using reporter plasmids containing hTERT core promoter revealed that hTERT transcription was significantly activated in hypoxia and by HIF-1 overexpression, and that the two putative binding sites within the core promoter are responsible for this activation. Chromatin immunoprecipitation assay identified the specific binding of HIF-1 to these sites (competing with c-Myc), which was enhanced in hypoxia. The present findings suggest that hypoxia activates telomerase via transcriptional activation of hTERT, and that HIF-1 plays a critical role as a transcription factor. They also suggest the existence of novel mechanisms of telomerase activation in cancers, and have implications for the molecular basis of hypoxia-induced tumor progression and HIF-1-based cancer gene therapy.

Yoon, J. S., J. C. Seo, et al. (2006). "Pinelliae Rhizoma herbal-acupuncture solution induced apoptosis in human cervical cancer cells, SNU-17." Am J Chin Med **34**(3): 401-8.

Pinelliae Rhizoma has been used traditionally in Korea to promote the liver Qi activity and the function of the digestive system. We investigated whether the Pinelliae Rhizoma herbal-acupuncture solution (PRHS) would induce cell-death on SNU-17, human cervical cancer cells. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to investigate the cytotoxicity of PRHS. The cell death was identified as apoptosis with 4, 6-diamidineo-2-phenylindole (DAPI) staining, and terminal deoxy-nucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) assay. PRHS could induce apoptosis of SNU-17 via Bax-related caspase-3 activation. The expressions of both Bax, a pro-apoptotic gene, and caspase-3, an apoptotic gene, were increased. The results might provide the experimental data for the clinical use of Pinelliae Rhizoma on cervical cancer.

Yoshinouchi, M., T. Yamada, et al. (2003). "In vitro and in vivo growth suppression of human papillomavirus 16-positive cervical cancer cells by E6 siRNA." Mol Ther **8**(5): 762-8.

Human papillomavirus type 16 (HPV16), a causative agent of cervical cancers, encodes the E6 and E7 oncogenes, whose simultaneous expression is

pivotal for malignant transformation and maintenance of malignant phenotypes. In the hope of developing a gene-specific therapy for HPV-related cancer, we examined the effects of E6 short-interfering RNA (siRNA) on the expression of these oncogenes and on the cell growth of HPV16-related cervical cancer cells. Using SiHa cervical cancer cells, we demonstrated that E6 siRNA decreased the levels of mRNA encoding E6 as well as that encoding E7 protein and also induced nuclear accumulation of p53, the most important target of E6. E6 siRNA suppressed monolayer and anchorage-independent growth of SiHa cells, which was associated with p21(CIP1/WAF1) induction and hypophosphorylation of retinoblastoma protein. Further, SiHa cells treated with E6 siRNA formed tumors in NOD/SCID mice that were significantly smaller than in those treated with control siRNA. Our results show HPV E6 siRNA as a candidate for gene-specific therapy for HPV-related cervical cancer.

Yoshitake, H., M. Takahashi, et al. (2007). "Aldo-keto reductase family 1, member B10 in uterine carcinomas: a potential risk factor of recurrence after surgical therapy in cervical cancer." Int J Gynecol Cancer **17**(6): 1300-6.

Aldo-keto reductase family 1, member B10 (AKR1B10), an enzyme that converts retinals into retinols is known to detect in non-small cell lung carcinoma (squamous cell- and adeno-carcinomas), but is barely expressed in normal tissues. Since these types of carcinoma occur frequently in the uterus (like in the lung), AKR1B10 may also be overexpressed in two major types of uterine cancer, cervical cancer (CC), and endometrial cancer (EMC). The objective of this study is to investigate AKR1B10 expression in uterine cancer and to analyze its clinical significance. In samples from uterine cancer patients, AKR1B10 was detected in 6 out of 30 (20.0%) CC cases and 6 out of 38 (15.8%) EMC cases. Statistical analysis indicated that AKR1B10 expression was associated with tumor recurrence after surgery and keratinization of squamous cell carcinoma only in CC. Although retinol (a metabolic product by AKR1B10) was observed in the normal epithelium, the molecule was not observed in cancer cells of AKR1B10-positive CC samples suggesting that the recurrence in CC may not depend on the convert of retinals into retinols via AKR1B10, a potential indicator in the management of patients with CC.

Yu, J., H. Qian, et al. (2007). "Arsenic trioxide (As2O3) reduces the invasive and metastatic properties of cervical cancer cells in vitro and in vivo." Gynecol Oncol **106**(2): 400-6.

OBJECTIVES: Arsenic trioxide (As(2)O(3)) was found to induce apoptosis in certain types of cancer cells including acute promyelocytic leukemia, and recently in solid tumors. We have previously demonstrated that As(2)O(3) has a therapeutic effect on cervical cancer by apoptosis promotion in vitro and in vivo. Here we further our study on the role of arsenic trioxide in regulating invasive activity of cervical cancer cells in vitro and in vivo. The effects of As(2)O(3) on human cervical cancer cell lines (HeLa, SiHa, Caski) adhesion, migration and invasion were observed by means of cell adhesion test, cell migration test and cell invasion test. The effects of As(2)O(3) on p-IkappaB, MMP-2, E-cadherin, caveolin-1 and beta-catenin protein expressions of tumor cells were determined by Western blot. In addition, the effects of As(2)O(3) on NF-kappaB activity of tumor cells were analyzed by immunoblot in whole lysates, cytosol and nucleus, respectively. In animal experiments, cervical cancer cells TC-1 were injected into tail veins of C57BL/6 mice and then the mice were treated by intraperitoneal injection of different doses As(2)O(3). Lung weights and the foci on the surface of lungs were measured. As(2)O(3) inhibited attachment of tumor cells to Fibronectin and Matrigel, reduced cell motility and inhibited tumor invasion potential. As(2)O(3) treatment also resulted in a positive regulation of caveolin-1, upregulation of E-cadherin and decreased activity of beta-catenin, NF-kappaB and NF-kappaB-regulated gene MMP-2. In animal experiments, lung weights in PBS group (0.31+/-0.07 g) were significantly elevated compared with those in As(2)O(3)-treated groups (0.21+/-0.03 g and 0.17+/-0.03 g) also As(2)O(3) reduced number of metastatic lesions of lungs (15.4+/-3.5 vs. 8.3+/-2.0 and 6.3+/-2.3) in a dose-dependent manner. CONCLUSIONS: This study is the first to report the effectiveness of As(2)O(3) as an inhibitor of cervical cancer invasion both in vitro and in vivo, suggesting a potential clinical application of As(2)O(3) in cervical cancer therapies combining apoptosis induction and metastasis inhibition.

Zheng, L. D., Z. F. Xiong, et al. (2005). "Effects of Smac gene over-expression on the radiotherapeutic sensitivities of cervical cancer cell line HeLa." Chin Med J (Engl) **118**(3): 226-30.

BACKGROUND: The second mitochondria-derived activator of caspases (Smac) is a novel proapoptotic gene, which plays an important role in the apoptosis-inducing effects of irradiation on tumor cells. The purpose of this study was to investigate the effects of extrinsic Smac gene transfer and its over-expression in radiotherapeutic sensitivities of cervical cancer cells. After the Smac gene was transferred into the cervical cancer cell line HeLa, subcloned cells

were obtained by persistent G418 selection. Cellular Smac gene expression was detected by RT-PCR and Western blot, while in vitro cell viabilities were detected by trypan blue staining assay. After treatment with X-ray irradiation, cellular radiotherapeutic sensitivities were investigated by tetrazolium bromide colorimetry. Cellular apoptosis and its rate were determined by electronic microscopy, annexin V-FITC and propidium iodide staining flow cytometry. The expression and activities of cellular caspase-3 were assayed by Western blot and colorimetry. Smac mRNA and protein levels in HeLa/Smac cells and the selected subclone cell line of cervical cancer were significantly higher than those of HeLa ($P < 0.01$). There was no significant difference in cellular viabilities between them ($P > 0.05$). However, after irradiation with 8 Gy X-ray, growth activities of HeLa/Smac were reduced by 22.42% ($P < 0.01$). When compared with those of HeLa, partial HeLa/Smac cells presented characteristic morphological changes of apoptosis under electronic microscope, with higher apoptosis rates (16.4% vs. 6.2%, $P < 0.01$); the caspase-3 expression levels in HeLa/Smac cells were improved significantly ($P < 0.01$), while its activities were increased by 3.42 times ($P < 0.01$). CONCLUSIONS: Stable transfer of the extrinsic Smac gene and its over-expression in cervical cancer cell line could significantly enhance the expression and activities of cellular caspase-3 and ameliorate apoptosis-inducing effects of irradiation on cancer cells, which was a novel strategy to improve radiotherapeutic effects on cervical cancer.

Zheng, Y., Y. Zhang, et al. (2008). "Enhancement of immunotherapeutic effects of HPV16E7 on cervical cancer by fusion with CTLA4 extracellular region." J Microbiol **46**(6): 728-36.

Cervical cancer is caused by infection by high-risk human papillomavirus (HPV), especially HPV16. Limitations in current treatments of cervical cancers call for the development of new and improved immunotherapies. This study aims at investigating the efficacy of a novel vaccine consisting of modified HPV 16E7 fused with human cytotoxic T-lymphocyte antigen 4 (CTLA4). The regions in HPV16 E7 gene associated with its transformation and CTL-enhanced response were modified; the resultant HPV16mE7 was fused with extracellular region of CTLA4 to generate HPVml6E7-eCTLA4 fusion protein. Binding of this fusion protein to B7 molecules expressed on antigen presenting-cells (APCs) was demonstrated. C57BL/6 (H-2b) mice immunized with low dose of the fusion protein (10 microg) produced higher titer antibody and stronger specific CTL response, and expressed higher levels of IFN-gamma and IL-12, compared with those immunized with HPVml6E7 only or admixture of HPVml6E7 and CTLA4, or PBS; and were protected from lethal dose tumor challenge. Tumor growth was retarded and survival prolonged in mouse models with the fusion protein treatment. Our results demonstrate that fusion of HPV16 E7 with eCTLA4 targeting APCs resulted in enhanced immunity, and that this fusion protein may be useful for improving the efficacy of immunotherapeutic treatments of cervical cancer and other HPV16 infection-associated tumors.

Zijlmans, H. J., G. J. Fleuren, et al. (2009). "Expression of endoglin (CD105) in cervical cancer." Br J Cancer **100**(10): 1617-26.

In this study, we have investigated the role of endoglin (CD105), a regulator of transforming growth factor (TGF)-beta(1) signalling on endothelial cells, basic fibroblast growth factor (bFGF) and vascular endothelial growth factor-A (VEGF-A) in cervical cancer. We have measured the number and determined the location of both newly formed (CD105-positive) and the overall number of (CD31-positive) blood vessels, and bFGF and VEGF-A expression using immunohistochemistry in 30 cervical carcinoma specimens. Vascular endothelial growth factor-A mRNA expression was determined using RNA-in situ hybridisation. CD105- and CD31-positive vessels and bFGF- and VEGF-A-positive cells were predominantly present in the stroma. The presence of CD105- and CD31-positive vessels in the stroma did neither correlate with the number of VEGF-A-positive cells nor the number of bFGF-positive cells. However, the number of CD105- and CD31-positive vessels was associated with the expression of VEGF-A mRNA in the epithelial cell clusters ($P=0.013$ and $P=0.005$, respectively). The presence of CD105-positive and CD31-positive vessels was associated with the expression of alphavbeta6 (a TGF-beta(1) activator; $P=0.013$ and $P=0.006$, respectively). Clinically, the number of CD105-positive vessels associated with the number of lymph node metastasis ($P<0.001$). Furthermore, the presence of CD105-positive vessels within the epithelial cell clusters associated with poor disease-free survival ($P=0.007$).

References

1. Abu, J., M. Batuwangala, et al. (2005). "Retinoic acid and retinoid receptors: potential chemopreventive and therapeutic role in cervical cancer." Lancet Oncol **6**(9): 712-20.
2. Ahn, W. S., S. M. Bae, et al. (2004). "Anti-cancer effect of adenovirus p53 on human cervical cancer cell growth in vitro and in vivo." Int J Gynecol Cancer **14**(2): 322-32.
3. Ahn, W. S., S. M. Bae, et al. (2004). "Recombinant adenovirus-p53 gene transfer and cell-specific growth suppression of human cervical cancer cells in vitro and in vivo." Gynecol Oncol **92**(2): 611-21.

4. Bae, S. M., H. J. Min, et al. (2006). "Protein expression profile using two-dimensional gel analysis in squamous cervical cancer patients." Cancer Res Treat **38**(2): 99-107.
5. Banno, K., M. Yanokura, et al. (2007). "Epigenetic inactivation of the CHFR gene in cervical cancer contributes to sensitivity to taxanes." Int J Oncol **31**(4): 713-20.
6. Bauerschmitz, G. J., A. Kanerva, et al. (2004). "Evaluation of a selectively oncolytic adenovirus for local and systemic treatment of cervical cancer." Int J Cancer **111**(2): 303-9.
7. Belcastro, M., M. R. Miller, et al. (2004). "C/EBPbeta activity and HPV-16 E6/E7 mRNA expression are not altered by imiquimod (ALDARA) in human cervical cancer cells in vitro." Gynecol Oncol **92**(2): 660-8.
8. Borkamo, E. D., B. C. Schem, et al. (2009). "cDNA microarray analysis of serially sampled cervical cancer specimens from patients treated with thermochemoradiotherapy." Int J Radiat Oncol Biol Phys **75**(5): 1562-9.
9. Branca, M., C. Giorgi, et al. (2006). "Over-expression of topoisomerase IIalpha is related to the grade of cervical intraepithelial neoplasia (CIN) and high-risk human papillomavirus (HPV), but does not predict prognosis in cervical cancer or HPV clearance after cone treatment." Int J Gynecol Pathol **25**(4): 383-92.
10. Cane, S., E. Bignotti, et al. (2004). "The novel serine protease tumor-associated differentially expressed gene-14 (KLK8/Neuropsin/Ovasin) is highly overexpressed in cervical cancer." Am J Obstet Gynecol **190**(1): 60-6.
11. Daniel, D., C. Chiu, et al. (2005). "CD4+ T cell-mediated antigen-specific immunotherapy in a mouse model of cervical cancer." Cancer Res **65**(5): 2018-25.
12. Das, S. and K. Somasundaram (2006). "Therapeutic potential of an adenovirus expressing p73 beta, a p53 homologue, against human papilloma virus positive cervical cancer in vitro and in vivo." Cancer Biol Ther **5**(2): 210-7.
13. de la Cruz-Hernandez, E., E. Perez-Cardenas, et al. (2007). "The effects of DNA methylation and histone deacetylase inhibitors on human papillomavirus early gene expression in cervical cancer, an in vitro and clinical study." Virol J **4**: 18.
14. Ferrandina, G., F. O. Ranelletti, et al. (2006). "Celecoxib up-regulates the expression of the zeta chain of T cell receptor complex in tumor-infiltrating lymphocytes in human cervical cancer." Clin Cancer Res **12**(7 Pt 1): 2055-60.
15. Fujii, T., M. Saito, et al. (2006). "Intratumor injection of small interfering RNA-targeting human papillomavirus 18 E6 and E7 successfully inhibits the growth of cervical cancer." Int J Oncol **29**(3): 541-8.
16. Green, K. L., T. D. Southgate, et al. (2006). "Diffusible VP22-E2 protein kills bystander cells and offers a route for cervical cancer gene therapy." Hum Gene Ther **17**(2): 147-57.
17. Gu, W., L. N. Putral, et al. (2007). "The development and future of oligonucleotide-based therapies for cervical cancer." Curr Opin Mol Ther **9**(2): 126-31.
18. Gu, W., L. Putral, et al. (2006). "Inhibition of cervical cancer cell growth in vitro and in vivo with lentiviral-vector delivered short hairpin RNA targeting human papillomavirus E6 and E7 oncogenes." Cancer Gene Ther **13**(11): 1023-32.
19. Gu, W., L. Putral, et al. (2008). "siRNA and shRNA as anticancer agents in a cervical cancer model." Methods Mol Biol **442**: 159-72.
20. Gupta, N., P. M. Martin, et al. (2006). "Down-regulation of BCRP/ABCG2 in colorectal and cervical cancer." Biochem Biophys Res Commun **343**(2): 571-7.
21. Gurska, S., T. Farkasova, et al. (2007). "Radiosensitivity of cervical cancer cell lines: the impact of polymorphisms in DNA repair genes." Neoplasma **54**(3): 195-201.
22. Hamada, K., N. Ueda, et al. (2005). "The nude rat as an orthotopic model for cervical cancer." Gynecol Oncol **99**(3 Suppl 1): S159-65.
23. Hamada, K., T. Shirakawa, et al. (2006). "Adenovirus-mediated transfer of human papillomavirus 16 E6/E7 antisense RNA and induction of apoptosis in cervical cancer." Gynecol Oncol **103**(3): 820-30.
24. Harima, Y., A. Togashi, et al. (2004). "Prediction of outcome of advanced cervical cancer to thermoradiotherapy according to expression profiles of 35 genes selected by cDNA microarray analysis." Int J Radiat Oncol Biol Phys **60**(1): 237-48.
25. Hasina, R., A. L. Pontier, et al. (2006). "NOL7 is a nucleolar candidate tumor suppressor gene in cervical cancer that modulates the angiogenic phenotype." Oncogene **25**(4): 588-98.
26. He, J., C. Huang, et al. (2008). "Proteomic analysis of cervical cancer cells treated with suberonylanilide hydroxamic acid." J Biosci **33**(5): 715-21.
27. Heideman, D. A., R. D. Steenbergen, et al. (2005). "Oncolytic adenovirus expressing a p53 variant resistant to degradation by HPV E6 protein exhibits potent and selective replication in cervical cancer." Mol Ther **12**(6): 1083-90.
28. Heo, M. Y., S. A. Salama, et al. (2008). "Abrogation of estrogen receptor signaling augments cytotoxicity of anticancer drugs on CaSki cervical cancer cells." Anticancer Res **28**(4B): 2181-7.
29. Kanerva, A., S. Lavilla-Alonso, et al. (2008). "Systemic therapy for cervical cancer with potentially regulatable oncolytic adenoviruses." PLoS One **3**(8): e2917.
30. Kang, B. Y., H. You, et al. (2009). "Cervical cancer isolate PT3, super-permissive for adeno-associated virus replication, over-expresses DNA polymerase delta, PCNA, RFC and RPA." BMC Microbiol **9**: 79.
31. Kawanaka, T., A. Kubo, et al. (2008). "Prognostic significance of HIF-2alpha expression on tumor infiltrating macrophages in patients with uterine cervical cancer undergoing radiotherapy." J Med Invest **55**(1-2): 78-86.
32. Kim, S. H., C. I. Hwang, et al. (2006). "GADD153 mediates celecoxib-induced apoptosis in cervical cancer cells." Carcinogenesis **27**(10): 1961-9.

33. Kim, S. H., C. I. Hwang, et al. (2007). "GADD153 mediates celecoxib-induced apoptosis in cervical cancer cells." Carcinogenesis **28**(1): 223-31.
34. Kim, Y. W., S. M. Bae, et al. (2004). "Comparison of As(2)O(3) and As(4)O(6) in the detection of SiHa cervical cancer cell growth inhibition pathway." Cancer Res Treat **36**(4): 255-62.
35. Koivusalo, R., A. Mialon, et al. (2006). "Activation of p53 in cervical cancer cells by human papillomavirus E6 RNA interference is transient, but can be sustained by inhibiting endogenous nuclear export-dependent p53 antagonists." Cancer Res **66**(24): 11817-24.
36. Kubota, H., T. Suzuki, et al. (2005). "Increased expression of GRP94 protein is associated with decreased sensitivity to X-rays in cervical cancer cell lines." Int J Radiat Biol **81**(9): 701-9.
37. Lea, J. S., N. Sunaga, et al. (2007). "Silencing of HPV 18 oncoproteins With RNA interference causes growth inhibition of cervical cancer cells." Reprod Sci **14**(1): 20-8.
38. Lee, C. M., C. B. Fuhrman, et al. (2006). "Phosphatidylinositol 3-kinase inhibition by LY294002 radiosensitizes human cervical cancer cell lines." Clin Cancer Res **12**(1): 250-6.
39. Lee, E. J., M. Jo, et al. (2006). "Alternative splicing variants of IRF-1 lacking exons 7, 8, and 9 in cervical cancer." Biochem Biophys Res Commun **347**(4): 882-8.
40. Lee, E. J., M. Jo, et al. (2009). "Dkk3, downregulated in cervical cancer, functions as a negative regulator of beta-catenin." Int J Cancer **124**(2): 287-97.
41. Lee, J. J., S. Kim, et al. (2006). "Enhanced specificity of the p53 family proteins-based adenoviral gene therapy in uterine cervical cancer cells with E2F1-responsive promoters." Cancer Biol Ther **5**(11): 1502-10.
42. Lee, S. H., J. W. Kim, et al. (2005). "IFN-gamma/IRF-1-induced p27kip1 down-regulates telomerase activity and human telomerase reverse transcriptase expression in human cervical cancer." FEBS Lett **579**(5): 1027-33.
43. Lee, Y. S., S. M. Bae, et al. (2006). "Cell cycle regulatory protein expression profiles by adenovirus p53 infection in human papilloma virus-associated cervical cancer cells." Cancer Res Treat **38**(3): 168-77.
44. Li, H. and X. Wu (2004). "Histone deacetylase inhibitor, Trichostatin A, activates p21WAF1/CIP1 expression through downregulation of c-myc and release of the repression of c-myc from the promoter in human cervical cancer cells." Biochem Biophys Res Commun **324**(2): 860-7.
45. 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.
46. Li, X. L., Q. H. Meng, et al. (2009). "Adenovirus-mediated expression of UHRF1 reduces the radiosensitivity of cervical cancer HeLa cells to gamma-irradiation." Acta Pharmacol Sin **30**(4): 458-66.
47. Li, Y., H. Li, et al. (2007). "Inhibition of telomerase RNA (hTR) in cervical cancer by adenovirus-delivered siRNA." Cancer Gene Ther **14**(8): 748-55.
48. Lim, H. Y., M. Ahn, et al. (2004). "Tumor-specific gene therapy for uterine cervical cancer using MN/CA9-directed replication-competent adenovirus." Cancer Gene Ther **11**(8): 532-8.
49. Lindel, K., P. Burri, et al. (2005). "Human papillomavirus status in advanced cervical cancer: predictive and prognostic significance for curative radiation treatment." Int J Gynecol Cancer **15**(2): 278-84.
50. Liu, C. Y., T. K. Chao, et al. (2009). "Characterization of LMX-1A as a metastasis suppressor in cervical cancer." J Pathol **219**(2): 222-31.
51. Liu, S. S., K. Y. Chan, et al. (2006). "Enhancement of the radiosensitivity of cervical cancer cells by overexpressing p73alpha." Mol Cancer Ther **5**(5): 1209-15.
52. Liu, S. S., R. C. Leung, et al. (2004). "p73 expression is associated with the cellular radiosensitivity in cervical cancer after radiotherapy." Clin Cancer Res **10**(10): 3309-16.
53. Lou, P. J., W. F. Cheng, et al. (2009). "PMMA particle-mediated DNA vaccine for cervical cancer." J Biomed Mater Res A **88**(4): 849-57.
54. Mahdavi, A. and B. J. Monk (2005). "Vaccines against human papillomavirus and cervical cancer: promises and challenges." Oncologist **10**(7): 528-38.
55. Martin, C. M., K. Astbury, et al. (2009). "Gene expression profiling in cervical cancer: identification of novel markers for disease diagnosis and therapy." Methods Mol Biol **511**: 333-59.
56. Martin, C. M., L. Kehoe, et al. (2007). "Gene discovery in cervical cancer : towards diagnostic and therapeutic biomarkers." Mol Diagn Ther **11**(5): 277-90.
57. Psyrri, A. and D. DiMaio (2008). "Human papillomavirus in cervical and head-and-neck cancer." Nat Clin Pract Oncol **5**(1): 24-31.
58. Qi, M., A. E. Anderson, et al. (2005). "Indole-3-carbinol prevents PTEN loss in cervical cancer in vivo." Mol Med **11**(1-12): 59-63.
59. Qiao, Y., J. Cao, et al. (2009). "Cell growth inhibition and gene expression regulation by (-)-epigallocatechin-3-gallate in human cervical cancer cells." Arch Pharm Res **32**(9): 1309-15.
60. Rho, S. B., Y. G. Park, et al. (2006). "A novel cervical cancer suppressor 3 (CCS-3) interacts with the BTB domain of PLZF and inhibits the cell growth by inducing apoptosis." FEBS Lett **580**(17): 4073-80.
61. Riezebos-Brilman, A., M. Walczak, et al. (2007). "A comparative study on the immunotherapeutic efficacy of recombinant Semliki Forest virus and adenovirus vector systems in a murine model for cervical cancer." Gene Ther **14**(24): 1695-704.
62. Rossi, A., S. Ciafre, et al. (2006). "Targeting the heat shock factor 1 by RNA interference: a potent tool to enhance hyperthermochemotherapy efficacy in cervical cancer." Cancer Res **66**(15): 7678-85.
63. Sami, S., N. Hoti, et al. (2008). "Valproic acid inhibits the growth of cervical cancer both in vitro and in vivo." J Biochem **144**(3): 357-62.
64. Santin, A. D., F. Zhan, et al. (2005). "Gene expression profiles of primary HPV16- and HPV18-infected early stage cervical cancers and normal cervical epithelium:

identification of novel candidate molecular markers for cervical cancer diagnosis and therapy." Virology **331**(2): 269-91.
65. Scholten, K. B., M. W. Schreurs, et al. (2005). "Preservation and redirection of HPV16E7-specific T cell receptors for immunotherapy of cervical cancer." Clin Immunol **114**(2): 119-29.
66. Sultana, H., J. Kigawa, et al. (2003). "Chemosensitivity and p53-Bax pathway-mediated apoptosis in patients with uterine cervical cancer." Ann Oncol **14**(2): 214-9.
67. Sun de, J., Y. Liu, et al. (2007). "Endothelin-3 growth factor levels decreased in cervical cancer compared with normal cervical epithelial cells." Hum Pathol **38**(7): 1047-56.
68. Tang, B., L. Li, et al. (2005). "Characterization of the mechanisms of electrochemotherapy in an in vitro model for human cervical cancer." Int J Oncol **26**(3): 703-11.
69. Wakatsuki, M., T. Ohno, et al. (2008). "p73 protein expression correlates with radiation-induced apoptosis in the lack of p53 response to radiation therapy for cervical cancer." Int J Radiat Oncol Biol Phys **70**(4): 1189-94.
70. Watari, H., Y. Ohta, et al. (2008). "Clusterin expression predicts survival of invasive cervical cancer patients treated with radical hysterectomy and systematic lymphadenectomy." Gynecol Oncol **108**(3): 527-32.
71. Wolf, J. K., E. L. Franco, et al. (2003). "Innovations in understanding the biology of cervical cancer." Cancer **98**(9 Suppl): 2064-9.
72. Wong, Y. F., D. S. Sahota, et al. (2006). "Gene expression pattern associated with radiotherapy sensitivity in cervical cancer." Cancer J **12**(3): 189-93.
73. Wong, Y. F., T. H. Cheung, et al. (2006). "Genome-wide gene expression profiling of cervical cancer in Hong Kong women by oligonucleotide microarray." Int J Cancer **118**(10): 2461-9.
74. Yamato, K., J. Fen, et al. (2006). "Induction of cell death in human papillomavirus 18-positive cervical cancer cells by E6 siRNA." Cancer Gene Ther **13**(3): 234-41.
75. Yamato, K., T. Yamada, et al. (2008). "New highly potent and specific E6 and E7 siRNAs for treatment of HPV16 positive cervical cancer." Cancer Gene Ther **15**(3): 140-53.
76. Yao, Z. and Z. Shulan (2008). "Inhibition effect of Guizhi-Fuling-decoction on the invasion of human cervical cancer." J Ethnopharmacol **120**(1): 25-35.
77. Yashar, C. M., W. J. Spanos, et al. (2005). "Potentiation of the radiation effect with genistein in cervical cancer cells." Gynecol Oncol **99**(1): 199-205.
78. Yatabe, N., S. Kyo, et al. (2004). "HIF-1-mediated activation of telomerase in cervical cancer cells." Oncogene **23**(20): 3708-15.
79. Yoon, J. S., J. C. Seo, et al. (2006). "Pinelliae Rhizoma herbal-acupuncture solution induced apoptosis in human cervical cancer cells, SNU-17." Am J Chin Med **34**(3): 401-8.
80. Yoshinouchi, M., T. Yamada, et al. (2003). "In vitro and in vivo growth suppression of human papillomavirus 16-positive cervical cancer cells by E6 siRNA." Mol Ther **8**(5): 762-8.
81. Yoshitake, H., M. Takahashi, et al. (2007). "Aldo-keto reductase family 1, member B10 in uterine carcinomas: a potential risk factor of recurrence after surgical therapy in cervical cancer." Int J Gynecol Cancer **17**(6): 1300-6.
82. Yu, J., H. Qian, et al. (2007). "Arsenic trioxide (As2O3) reduces the invasive and metastatic properties of cervical cancer cells in vitro and in vivo." Gynecol Oncol **106**(2): 400-6.
83. Zheng, L. D., Z. F. Xiong, et al. (2005). "Effects of Smac gene over-expression on the radiotherapeutic sensitivities of cervical cancer cell line HeLa." Chin Med J (Engl) **118**(3): 226-30.
84. Zheng, Y., Y. Zhang, et al. (2008). "Enhancement of immunotherapeutic effects of HPV16E7 on cervical cancer by fusion with CTLA4 extracellular region." J Microbiol **46**(6): 728-36.
85. Zijlmans, H. J., G. J. Fleuren, et al. (2009). "Expression of endoglin (CD105) in cervical cancer." Br J Cancer **100**(10): 1617-26.
86. PubMed (2011). http://www.ncbi.nlm.nih.gov/pubmed.
87. Cancer. Wikipedia. (2011) http://en.wikipedia.org/wiki/Cancer.

2/3/2012