

Bladder Cancer Literatures

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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 cancer and the bladder.

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1. Introduction

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

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

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

The objective of this article is to introduce the reader to general references cancers and bladder.

Literatures

Adam, L., P. C. Black, et al. (2007). "Adenoviral mediated interferon-alpha 2b gene therapy suppresses the pro-angiogenic effect of vascular endothelial growth factor in superficial bladder cancer." *J Urol* 177(5): 1900-6.

Intravesical adenovirus mediated interferon-alpha gene transfer has a potent therapeutic effect against superficial human bladder carcinoma xenografts growing in the bladder of athymic nude mice. Adam et al determined whether the inhibition of angiogenesis might contribute to the antitumor effect. Adam et al treated several human urothelial carcinoma cells with adenovirus mediated interferon-alpha 2b and monitored its effects on the production of angiogenic factors using real-time reverse-transcription polymerase chain reaction, Western blotting, and immunohistochemical analysis and a gel shift based transcription factor array. To assess the role of adenovirus mediated interferon 2b in angiogenic activity we used in vitro invasion assays and evaluated the anti-angiogenic effects of adenovirus mediated interferon gene therapy in an orthotopic murine model of human superficial bladder cancer. In adenovirus mediated interferon-alpha infected 253J B-V cells vascular endothelial growth factor was decreased and anti-angiogenic interferon-gamma inducible protein 10 was up-regulated. In contrast, the addition of as much as 100,000 IU recombinant interferon had no apparent effect on vascular endothelial growth factor production. Conditioned medium derived from adenovirus mediated interferon 2b infected 253J B-V cells greatly decreased the invasive potential of human endothelial cells and down-regulated their matrix metalloproteinase 2 expression compared to controls. Furthermore, adenovirus mediated interferon 2b blocked pro-angiogenic nuclear signals, such as the transcription factors activating protein-1 and 2, stimulating protein-1, nuclear factor kappaB and c-myc. In vivo experiments revealed significant vascular endothelial growth factor down-regulation and decreased tumor vessel density in the adenovirus mediated interferon 2b treated group compared to controls. Treatment with adenovirus mediated interferon 2b increases the angiostatic activity of the

bladder cancer microenvironment. This inhibition may prove beneficial for treating superficial bladder cancer with adenovirus mediated interferon-alpha and hopefully contribute to a decreased recurrence rate of this neoplasm.

Adam, L., M. Zhong, et al. (2009). "miR-200 expression regulates epithelial-to-mesenchymal transition in bladder cancer cells and reverses resistance to epidermal growth factor receptor therapy." *Clin Cancer Res* **15**(16): 5060-72.

The epithelial-to-mesenchymal transition (EMT) is a cell development-regulated process in which noncoding RNAs act as crucial modulators. Recent studies have implied that EMT may contribute to resistance to epidermal growth factor receptor (EGFR)-directed therapy. The changes in EGFR sensitivity by silencing or forced expression of ERRF1-1 or by miR-200 expression have also been validated in additional cell lines, UMUC5 and T24. Finally, luciferase assays using 3'-untranslated region/ERRF1-1/luciferase and miR-200 cotransfections showed that the direct down-regulation of ERRF1-1 was miR-200-dependent because mutations in the two putative miR-200-binding sites have rescued the inhibitory effect. Members of the miR-200 family appear to control the EMT process and sensitivity to EGFR therapy in bladder cancer cells and the expression of miR-200 is sufficient to restore EGFR dependency at least in some of the mesenchymal bladder cancer cells. The targets of miR-200 include ERRF1-1, which is a novel regulator of EGFR-independent growth.

Ahirwar, D., P. Kesarwani, et al. (2008). "Anti- and proinflammatory cytokine gene polymorphism and genetic predisposition: association with smoking, tumor stage and grade, and bacillus Calmette-Guerin immunotherapy in bladder cancer." *Cancer Genet Cytogenet* **184**(1): 1-8.

Cytokines mediate many immune and inflammatory responses contributing to tumorigenesis. The present study evaluated polymorphisms of IL4, IL6, and TNF (previously TNFA) genes influencing risk in development of transitional cell carcinoma of bladder and recurrence after bacillus Calmette-Guerin (BCG) immunotherapy. The study included 136 unrelated histopathologically confirmed cases and 200 population-based controls. The low-producing variant C/C of IL6 may be a risk factor for bladder cancer, whereas high-producing genotypes of IL4 (B1/B2+B2/B2) may predispose to higher risk in patients with high-grade or late-stage tumor and smoking habits. The low-producing C/C IL6 genotype, which favors Th1 response, may be a

beneficial prognostic indicator for treatment and survival of BCG-treated patients.

Aleman, A., V. Cebrian, et al. (2008). "Identification of PMF1 methylation in association with bladder cancer progression." *Clin Cancer Res* **14**(24): 8236-43.

Polyamines are important regulators of cell growth and death. The polyamine modulated factor-1 (PMF-1) is involved in polyamine homeostasis. After identifying an enriched CpG island encompassing the PMF1 promoter, we aimed at evaluating the clinical relevance of PMF1 methylation in bladder cancer. The association of PMF1 methylation with tumor progression and its diagnostic ability using urinary specimens support including PMF1 assessment for the clinical management of bladder cancer patients.

Alonzi, R. and P. Hoskin (2005). "Novel therapies in bladder cancer." *Clin Oncol (R Coll Radiol)* **17**(7): 524-38.

The most effective non-surgical treatment for bladder cancer remains radiotherapy. The dramatic technical developments in radiotherapy have enabled greater accuracy and reliability based on three-dimensional imaging for both planning and verification. Particle therapy, in particular using protons, provides further opportunities for optimising radiation delivery and dose escalation. Novel fractionation schedules with both hyperfractionation and hypofractionation may have added benefits. Chemoradiation has been shown in one randomised-controlled trial to improve the results of radiotherapy alone, and requires further investigation. Hypoxia modification using carbogen and nicotinamide has also shown promising results in a phase II trial, and is now in phase III evaluation. Novel drug agents for bladder cancer are few, but the anti-EGFR agents and anti-angiogenic agents may have promise; the development of anti-apoptotic agents and antisense gene therapy may also become a component of the future multimodality management of this tumour.

Als, A. B., L. Dyrskjot, et al. (2007). "Emmprin and survivin predict response and survival following cisplatin-containing chemotherapy in patients with advanced bladder cancer." *Clin Cancer Res* **13**(15 Pt 1): 4407-14.

Cisplatin-containing chemotherapy is the standard of care for patients with locally advanced and metastatic transitional cell carcinoma of the urothelium. The response rate is approximately 50% and tumor-derived molecular prognostic markers are desirable for improved estimation of response and survival. Affymetrix GeneChip expression profiling was carried out using tumor material from 30 patients.

Emmprin and survivin proteins were identified as strong independent prognostic factors for response and survival after cisplatin-containing chemotherapy in patients with advanced bladder cancer.

Atlasi, Y., S. J. Mowla, et al. (2009). "Differential expression of survivin and its splice variants, survivin-DeltaEx3 and survivin-2B, in bladder cancer." *Cancer Detect Prev* **32**(4): 308-13.

Survivin, an inhibitor of apoptosis protein (IAP), has been regarded as a valuable tumor marker for diagnosis and prognosis of bladder cancer. The data revealed for the first time a differential expression pattern of survivin splice variants in bladder tissues, which potentially could have a practical usefulness in diagnosis and/or therapy of the tumor.

Baffa, R., J. Letko, et al. (2006). "Molecular genetics of bladder cancer: targets for diagnosis and therapy." *J Exp Clin Cancer Res* **25**(2): 145-60.

Transitional cell carcinoma of the bladder is a common tumor. While most patients presenting superficial disease can be expected to do well following treatment, still many patients will return to our office with muscle invasive and metastatic disease. Survival in advanced bladder cancer is less than 50%. Tumors of similar histologic grade and stage have variable behavior, suggesting that genetic alterations must be present to explain the diverse behavior of bladder cancer. It is hoped that through the study of the subtle genetic alterations in bladder cancer, important prognostic and therapeutic targets can be exploited. Many new diagnostic tests and gene therapy approaches rely on the identification and targeting of these unique genetic alterations. A review of literature published on the molecular genetics of bladder cancer from 1970 to the present was conducted. The UroVysion bladder cancer assay relies on FISH to detect genetic alterations in this disease. Continuing identification of the molecular genetic alterations in bladder cancer will enhance future diagnostic and therapeutic approaches to bladder cancer. Capitalizing on these alterations will allow early detection, providing important prognostic information and unique targets for gene therapy and other therapeutic approaches.

Beecken, W. D., T. Engl, et al. (2006). "Clinical relevance of maspin expression in bladder cancer." *World J Urol* **24**(3): 338-44.

Transitional cell carcinoma (TCC) of the bladder is a solid tumor that induces angiogenesis to maintain nutrition and oxygenation of tumor cells. Maspin, a serpin with tumor suppressing activity, has recently been identified as an inhibitor of

angiogenesis. This study examined the impact of maspin expression in the growth pattern of TCC of the bladder. Maspin was identified in a panel of normal tissues, in several bladder carcinoma cell lines, and 51 patient samples of TCC of the bladder. Expression was detected by RT-PCR and immunohistochemistry. Furthermore, the level of maspin was correlated to the growth rate of bladder tumor cell lines in vitro and in vivo. Maspin expression was found in high quantities in normal urothelium. Maspin expression was preserved in superficial bladder cancers but was significantly diminished in invasive carcinomas. Within the group of invasive TCCs, maspin expression was inversely correlated to the patient prognosis. Furthermore, low maspin expression level was coupled to an increased tumor cell growth in vivo. Down-regulation of maspin expression seems to be a specific event in the progression of invasive bladder carcinoma. Maspin might be a useful marker to determine the prognosis of invasive TCC. Furthermore, maspin re-expression might become a therapeutic option in the treatment of invasive, metastatic TCC.

Benedict, W. F., Z. Tao, et al. (2004). "Intravesical Ad-IFNalpha causes marked regression of human bladder cancer growing orthotopically in nude mice and overcomes resistance to IFN-alpha protein." *Mol Ther* **10**(3): 525-32.

Benedict et al produced prolonged, high local concentrations of interferon in vivo by intravesical instillation of adenoviruses encoding interferon-alpha (Ad-IFNalpha) together with the gene transfer-enhancing agent Syn3. Benedict et al found sustained interferon protein levels for days, both in normal mouse urothelium and in human bladder cancer cells growing as superficial bladder tumors in nude mice using an orthotopic bladder model developed by us. Tumor burden in the bladder was determined utilizing cancer cells containing the green fluorescent protein. Marked tumor regression was observed following two 1-h exposures of Ad-IFNalpha/Syn3 and little or no cytotoxicity was detected in normal cells. Similar intravesical instillation of clinically relevant concentrations of IFN protein alone or Ad-IFNalpha without Syn3 was ineffective. Surprisingly, in vitro, Ad-IFNalpha also caused caspase-dependent death of bladder cancer cell lines that were resistant to high concentrations of IFN-alpha protein, including the cell line used in vivo. These findings demonstrate that Ad-IFNalpha can overcome resistance to IFN-alpha protein both in vitro and in vivo and support evaluation of intravesical Ad-IFNalpha/Syn3 for the treatment of superficial bladder cancer.

Bevers, R. F., K. H. Kurth, et al. (2004). "Role of urothelial cells in BCG immunotherapy for superficial bladder cancer." *Br J Cancer* **91**(4): 607-12.

Intravesical instillation of Bacillus Calmette-Guerin (BCG) is used for the treatment of superficial bladder cancer, both to reduce the recurrence rate of bladder tumour and to diminish the risk of progression. Since its first therapeutic application in 1976, major research efforts have been directed to decipher the exact mechanism of action of the BCG-associated antitumour effect. Bacillus Calmette-Guerin causes an extensive local inflammatory reaction in the bladder wall. Of this, the massive appearance of cytokines in the urine of BCG-treated patients stands out. Activated lymphocytes and macrophages are the most likely sources of these cytokines, but at present other cellular sources such as urothelial tumour cells cannot be ruled out. Bacillus Calmette-Guerin is internalised and processed both by professional antigen-presenting cells and urothelial tumour cells, resulting in an altered gene expression of these cells that accumulates in the presentation of BCG antigens and secretion of particular cytokines.

Blaveri, E., J. L. Brewer, et al. (2005). "Bladder cancer stage and outcome by array-based comparative genomic hybridization." *Clin Cancer Res* **11**(19 Pt 1): 7012-22.

Bladder carcinogenesis is believed to follow alternative pathways of disease progression driven by an accumulation of genetic alterations. The purpose of this study was to evaluate associations between measures of genomic instability and bladder cancer clinical phenotype. Genome-wide copy number profiles were obtained for 98 bladder tumors of diverse stages (29 pT(a), 14 pT1, 55 pT(2-4)) and grades (21 low-grade and 8 high-grade superficial tumors) by array-based comparative genomic hybridization (CGH). Supervised tumor classification (prediction analysis for microarrays) had a 71% classification success rate based on 102 unique clones. Array-based CGH identified quantitative and qualitative differences in DNA copy number alterations at high resolution according to tumor stage and grade. Fraction genome altered was associated with worse outcome in muscle-invasive tumors, independent of other clinicopathologic parameters. Measures of genomic instability add independent power to outcome prediction of bladder tumors.

Blehm, K. N., P. E. Spiess, et al. (2006). "Mutations within the kinase domain and truncations of the epidermal growth factor receptor are rare events in bladder cancer: implications for therapy." *Clin Cancer Res* **12**(15): 4671-7.

It has previously been reported that the patient response to gefitinib depends on the presence of mutations within the kinase domain of epidermal growth factor receptor (EGFR) or the expression of its truncated form, EGFR variant III (EGFRvIII). The focus of this study was to determine if these alterations are present within the tyrosine kinase and ligand-binding domain of EGFR in urothelial carcinoma. The analysis failed to detect mutations within the tyrosine kinase domain of EGFR in the 11 cell lines and 75 patients tested. The initial analysis of EGFRvIII expression by immunohistochemistry revealed that at least 50% of the patient tumors expressed EGFRvIII in a urothelial carcinoma tissue microarray. Conflicting reports exist, however, regarding the extent of EGFRvIII expression in tissues owing to the specificity of the antibodies and the methodologies used. Therefore, we sought to validate this observation by reverse transcription PCR, real-time PCR, and Western blot analysis. In these assays, none of the samples were positive for EGFRvIII except for control transfectants and glioblastomas. When the results are taken together, we conclude that alterations within the tyrosine kinase domain and expression of EGFRvIII are rare events in bladder cancer. The present study has clinical implications in selecting tyrosine kinase inhibitors for the therapy of urothelial carcinoma.

Boorjian, S., D. S. Scherr, et al. (2005). "Retinoid receptor mRNA expression profiles in human bladder cancer specimens." *Int J Oncol* **26**(4): 1041-8.

Retinoids, which include vitamin A (retinol) and its derivatives, have previously been investigated as potential chemopreventive and chemotherapeutic agents in bladder cancer. We examined mRNA expression of the retinoid receptors RARalpha, RARbeta2, RARGamma and RXRalpha, as well as two putative RARbeta2 target genes, DAB2 and Midkine, in normal and malignant bladder tissue specimens from human patients. We evaluated 24 normal and malignant bladder specimens for retinoid receptor, DAB2 and Midkine mRNA expression using RT-PCR. We also examined the effects of retinoic acid and retinol on the expression of these genes in five human bladder cancer cell lines. Expression of RARalpha, RARbeta2, RARGamma and RXRalpha mRNA was detected in all of the non-neoplastic patient bladder specimens. RARbeta2 mRNA expression was undetectable in 7/13 tumors, RARalpha in 3/13, RARGamma in 1/13 and RXRalpha in 2/13. DAB2 mRNA was expressed in all non-neoplastic and all tumor specimens, while Midkine mRNA was detected in 8/11 non-neoplastic specimens versus 11/13 tumors. Two of the five bladder cancer cell lines expressed RARbeta2

independent of retinoid exposure; in three cell lines RARbeta2 expression was induced by retinoids. RARalpha, RARgamma and RXRalpha mRNA expression was detected in 5/5 cell lines, independent of retinoid exposure.

Buckley, M. T., J. Yoon, et al. (2007). "The histone deacetylase inhibitor belinostat (PXD101) suppresses bladder cancer cell growth in vitro and in vivo." *J Transl Med* 5: 49.

Treatment options for patients with recurrent superficial bladder cancer are limited, necessitating aggressive exploration of new treatment strategies that effectively prevent recurrence and progression to invasive disease. Growth inhibition and cell cycle distribution effect of belinostat on 5637, T24, J82, and RT4 urothelial lines were assessed. Ha-ras transgenic mice with established superficial bladder cancer were randomized to receive either belinostat or vehicle alone, and assessed for bladder weight, hematuria, gene expression profiling, and immunohistochemistry (IHC). Belinostat had a significant linear dose-dependent growth inhibition on all cell lines (IC50 range of 1.0-10.0 microM). The 5637 cell line, which was derived from a superficial papillary tumor, was the most sensitive to treatment. Belinostat (100 mg/kg, intraperitoneal, 5 days each week for 3 weeks) treated mice had less bladder weight ($p < 0.05$), and no hematuria compared with 6/10 control mice that developed at least one episode. IHC of bladder tumors showed less cell proliferation and a higher expression of p21WAF1 in the belinostat-treated mice. Gene expression profile analysis revealed 56 genes significantly different in the treated group; these included the upregulation of p21WAF1, induction of core histone deacetylase (HDAC), and cell communication genes. The data demonstrate that belinostat inhibits bladder cancer and supports the clinical evaluation of belinostat for the treatment of patients with superficial bladder cancer.

Buytaert, E., J. Y. Matroule, et al. (2008). "Molecular effectors and modulators of hypericin-mediated cell death in bladder cancer cells." *Oncogene* 27(13): 1916-29.

Photodynamic therapy (PDT) is an anticancer approach utilizing a light-absorbing molecule and visible light irradiation to generate, in the presence of O(2), cytotoxic reactive oxygen species, which cause tumor ablation. Given that the photosensitizer hypericin is under consideration for PDT treatment of bladder cancer we used oligonucleotide microarrays in the T24 bladder cancer cell line to identify differentially expressed genes with therapeutic potential. This study reveals that the expression of several genes involved in various metabolic processes,

stress-induced cell death, autophagy, proliferation, inflammation and carcinogenesis is strongly affected by PDT and pinpoints the coordinated induction of a cluster of genes involved in the unfolded protein response pathway after endoplasmic reticulum stress and in antioxidant response. Analysis of PDT-treated cells after p38(MAPK) inhibition or silencing unraveled that the induction of an important subset of differentially expressed genes regulating growth and invasion, as well as adaptive mechanisms against oxidative stress, is governed by this stress-activated kinase. Moreover, p38(MAPK) inhibition blocked autonomous regrowth and migration of cancer cells escaping PDT-induced cell death. This analysis identifies new molecular effectors of the cancer cell response to PDT opening attractive avenues to improve the therapeutic efficacy of hypericin-based PDT of bladder cancer.

Catto, J. W., S. Miah, et al. (2009). "Distinct microRNA alterations characterize high- and low-grade bladder cancer." *Cancer Res* 69(21): 8472-81.

Urothelial carcinoma of the bladder (UCC) is a common disease that arises by at least two different molecular pathways. The biology of UCC is incompletely understood, making the management of this disease difficult. Recent evidence implicates a regulatory role for microRNA in cancer. Catto et al hypothesized that altered microRNA expression contributes to UCC carcinogenesis. To test this hypothesis, we examined the expression of 322 microRNAs and their processing machinery in 78 normal and malignant urothelial samples using real-time rtPCR. Genes targeted by differentially expressed microRNA were investigated using real-time quantification and microRNA knockdown. We also examined the role of aberrant DNA hypermethylation in microRNA downregulation. We found that altered microRNA expression is common in UCC and occurs early in tumorigenesis. In normal urothelium from patients with UCC, 11% of microRNAs had altered expression when compared with disease-free controls. High-grade UCC were characterized by microRNA upregulation, including microRNA-21 that suppresses p53 function. In low-grade UCC, there was downregulation of many microRNA molecules. In particular, loss of microRNAs-99a/100 leads to upregulation of FGFR3 before its mutation. Promoter hypermethylation is partly responsible for microRNA downregulation. In conclusion, distinct microRNA alterations characterize UCC and target genes in a pathway-specific manner. These data reveal new insights into the disease biology and have implications regarding tumor diagnosis, prognosis and therapy.

Cebrian, V., M. Alvarez, et al. (2008). "Discovery of myopodin methylation in bladder cancer." *J Pathol* **216**(1): 111-9.

Myopodin is an actin-binding protein that shuttles between the nucleus and the cytoplasm. After identifying an enriched CpG island encompassing the transcription site of myopodin, we aimed at evaluating the potential relevance of myopodin methylation in bladder cancer. The epigenetic silencing of myopodin by hypermethylation was tested in bladder cancer cells before and after azacytidine treatment. Myopodin hypermethylation was associated with gene expression, being increased *in vitro* by this demethylating agent. Myopodin methylation in 164 urinary specimens distinguished patients with bladder cancer from controls with a sensitivity of 65.0%, a specificity of 79.8%, and a global accuracy of 75.3%. Myopodin was identified to be epigenetically modified in bladder cancer. The association of myopodin methylation and nuclear expression patterns with cancer progression and clinical outcome, together with its ability to detect bladder cancer patients using urinary specimens, suggests the utility of incorporating myopodin methylation assessment in the clinical management of patients affected by uroepithelial neoplasias.

Chadalapaka, G., I. Jutooru, et al. (2008). "Curcumin decreases specificity protein expression in bladder cancer cells." *Cancer Res* **68**(13): 5345-54.

Curcumin is the active component of tumeric, and this polyphenolic compound has been extensively investigated as an anticancer drug that modulates multiple pathways and genes. In this study, 10 to 25 micromol/L curcumin inhibited 253JB-V and KU7 bladder cancer cell growth, and this was accompanied by induction of apoptosis and decreased expression of the proapoptotic protein survivin and the angiogenic proteins vascular endothelial growth factor (VEGF) and VEGF receptor 1 (VEGFR1). Because expression of survivin, VEGF, and VEGFR1 are dependent on specificity protein (Sp) transcription factors, we also investigated the effects of curcumin on Sp protein expression as an underlying mechanism for the apoptotic and antiangiogenic activity of this compound. The results show that curcumin induced proteasome-dependent down-regulation of Sp1, Sp3, and Sp4 in 253JB-V and KU7 cells. Moreover, using RNA interference with small inhibitory RNAs for Sp1, Sp3, and Sp4, we observed that curcumin-dependent inhibition of nuclear factor kappaB (NF-kappaB)-dependent genes, such as bcl-2, survivin, and cyclin D1, was also due, in part, to loss of Sp proteins. Curcumin also decreased bladder tumor growth in athymic nude mice bearing KU7 cells as xenografts and this was accompanied by decreased Sp1, Sp3, and

Sp4 protein levels in tumors. These results show for the first time that one of the underlying mechanisms of action of curcumin as a cancer chemotherapeutic agent is due, in part, to decreased expression of Sp transcription factors in bladder cancer cells.

Chakraborty, A. and S. Guha (2007). "Granulocyte colony-stimulating factor/granulocyte colony-stimulating factor receptor biological axis promotes survival and growth of bladder cancer cells." *Urology* **69**(6): 1210-5.

A significant fraction of invasive bladder carcinomas express both granulocyte colony-stimulating factor (G-CSF) and granulocyte colony-stimulating factor receptor (G-CSFR). Anti-G-CSF antibody significantly increased apoptosis in serum-starved 5637-GR cells, G-CSF abrogated apoptosis in serum-starved TCC-SUP-GR cells in a dose-dependent manner. STAT3-dominant-negative expression blocked G-CSF-mediated STAT3 phosphorylation and survivin expression in TCC-SUP-GR cells. Furthermore, 5637-GR cells produced a significantly larger tumor in the subcutaneous nude mice xenograft model. The G-CSF/G-CSFR autocrine/paracrine signaling loop significantly promotes survival and growth of bladder cancer cells.

Chan, M. W., L. W. Chan, et al. (2003). "Frequent hypermethylation of promoter region of RASSF1A in tumor tissues and voided urine of urinary bladder cancer patients." *Int J Cancer* **104**(5): 611-6.

High frequency loss of 3p21.3 region where RASSF1A located was demonstrated in several tumors. We aimed to investigate the methylation status of RASSF1A and the frequency of LOH in 3p21.3 region in bladder cancer. Three bladder cancer cell lines, 40 cases of bladder TCC and 14 cases of paired voided urine samples were subjected to methylation analysis. By methylation specific PCR, complete methylation of promoter region of RASSF1A gene were detected in cell lines T24 and UMUC3. Demethylation treatment re-expressed RASSF1A in these 2 cell lines. Methylation of RASSF1A was also detected in 47.5% (19/40) of the TCC cases but not in 6 carcinoma in situ (CIS) or 6 normal urothelium samples. For LOH study, loss of 3p21.3 region was detected in 57.9% (11/19) of our cases. Interestingly, methylation of RASSF1A was found in 72.7% (8/11) of the cases with LOH but only in 12.5% (1/8) of the cases without LOH. Methylation of RASSF1A was detected in 50% (7/14) of voided urine samples, but not in normal control. It showed a higher sensitivity than conventional urine cytology in detecting cancer cells, especially for low grade cases. In conclusion, our results demonstrated a high frequency of RASSF1A methylation with frequent

LOH in 3p21.3 region in bladder cancer. It suggested that it may be a potential tumor suppressor gene in this chromosomal region and can be silenced by promoter hypermethylation. Detection of aberrant gene methylation in routine voided urine was feasible and may provide a non-invasive and sensitive approach for cancer detection.

Chen, Z., R. F. Place, et al. (2008). "Antitumor effect of dsRNA-induced p21(WAF1/CIP1) gene activation in human bladder cancer cells." *Mol Cancer Ther* 7(3): 698-703.

We recently reported that synthetic dsRNAs targeting promoter regions can induce gene expression in a phenomenon referred to as dsRNA-induced gene activation/RNA activation (RNAa) [Li et al. *Proc Natl Acad Sci U S A* 2006;103:17337-42]. The present study investigates the in vitro antitumor activity RNAa can elicit through triggering the expression of cell cycle repressor protein p21(WAF1/CIP1) (p21) in human bladder cancer cells. Transfection of a 21-nucleotide dsRNA targeting the p21 promoter (dsP21) was used to induce p21 expression in T24 and J82 bladder cancer cell lines. Reverse transcription-PCR and Western blot analysis accessed the increase p21 mRNA and protein levels, respectively, in transfected cells. In association to p21 induction, dsP21 transfection significantly inhibited bladder cancer cell proliferation and clonogenicity.

Chen, Z., J. Yang, et al. (2007). "Attenuated expression of xeroderma pigmentosum group C is associated with critical events in human bladder cancer carcinogenesis and progression." *Cancer Res* 67(10): 4578-85.

Xeroderma pigmentosum group C (XPC) is an important DNA damage recognition protein that binds to damaged DNA at a very early stage during DNA repair. The XPC protein is also involved in DNA damage-induced cell cycle checkpoint regulation and apoptosis. XPC defects are associated with many types of solid tumors. The mechanism of the XPC protein in cancer progression, however, remains unclear. In this report, we showed the strong correlation between bladder cancer progression and attenuated XPC protein expression using tissues derived from patients with bladder cancer. The results obtained from our immunohistochemical studies further revealed a strong correlation of XPC deficiency, p53 mutation, and the degree of malignancy of bladder tumors. In addition, the results obtained from our studies have also shown that HT1197 bladder cancer cells, which carry a low-level XPC protein, exhibited a decreased DNA repair capability and were resistant to cisplatin treatment. When an XPC gene cDNA-expression vector was

stably transfected into the HT1197 cells, however, the cisplatin treatment-induced apoptotic cell death was increased. Increased p53 and p73 responses following cisplatin treatment were also observed in HT1197 cells stably transfected with XPC cDNA. Taken together, these results suggest that XPC deficiency is an important contributing factor in bladder tumor progression and bladder cancer cell drug resistance.

Cheng, J. C., D. J. Weisenberger, et al. (2004). "Continuous zebularine treatment effectively sustains demethylation in human bladder cancer cells." *Mol Cell Biol* 24(3): 1270-8.

During tumorigenesis, tumor suppressor and cancer-related genes are commonly silenced by aberrant DNA methylation in their promoter regions. Recently, we reported that zebularine [1-(beta-D-ribofuranosyl)-1,2-dihydropyrimidin-2-one] acts as an inhibitor of DNA methylation and exhibits chemical stability and minimal cytotoxicity both in vitro and in vivo. Here we show that continuous application of zebularine to T24 cells induces and maintains p16 gene expression and sustains demethylation of the 5' region for over 40 days, preventing remethylation. In addition, continuous zebularine treatment effectively and globally demethylated various hypermethylated regions, especially CpG-poor regions. The drug caused a complete depletion of extractable DNA methyltransferase 1 (DNMT1) and partial depletion of DNMT3a and DNMT3b3. Last, sequential treatment with 5-aza-2'-deoxycytidine followed by zebularine hindered the remethylation of the p16 5' region and gene resiliencing, suggesting the possible combination use of both drugs as a potential anticancer regimen.

Chester, J. D., W. Kennedy, et al. (2003). "Adenovirus-mediated gene therapy for bladder cancer: efficient gene delivery to normal and malignant human urothelial cells in vitro and ex vivo." *Gene Ther* 10(2): 172-9.

Existing local therapies for superficial transitional cell carcinoma (TCC) of the bladder have limited success in preventing progression to life-threatening, muscle-invasive disease, and novel therapies are needed. Recent studies have raised doubts concerning the feasibility of adenovirus-mediated gene therapy for bladder cancer. We have therefore investigated adenoviral transduction of normal and malignant human urothelial cells, both as primary cultures and in intact epithelium. All 15 primary normal human urothelial cell lines tested were transduced in vitro by Adv-cmv-beta-gal at high efficiency, and better than most human TCC cell lines. Eight primary human TCC explants were also successfully transduced. In contrast, in intact normal urothelium, transduction efficiency was lower, and

occurred only in superficial epithelial layers. Expression of the hCAR adenovirus receptor, however, occurred throughout the full thickness of urothelium. Transduction of human TCC biopsy specimens was at least as efficient as intact normal urothelium. We demonstrate for the first time that adenoviral transduction of both normal and malignant human urothelial cells is feasible. A physical barrier, rather than hCAR status, may be the main determinant of transduction of intact epithelium. Clinical trials of adenovirus-mediated gene therapy for superficial bladder cancer are warranted.

Cho, H. J., J. K. Kim, et al. (2006). "Upregulation of Bcl-2 is associated with cisplatin-resistance via inhibition of Bax translocation in human bladder cancer cells." *Cancer Lett* **237**(1): 56-66.

The efficacy of cisplatin in cancer chemotherapy is limited by the development of resistance. To elucidate the molecular basis of resistance to cisplatin, we compared cisplatin-induced apoptotic responses of the parental human bladder cancer cell line, T24 and its resistant subclone, T24R2. In T24 cells, cisplatin induce apoptosis and the activation of caspase-8, -9 and -3 and poly(ADP-ribose) polymerase cleavage. The expression levels of Fas, FasL, and FADD were not changed by the treatment with cisplatin. Furthermore, neither Fas-neutralizing antibody nor dominant negative mutant of FADD affected cisplatin-induced apoptosis. Western blot analysis of subcellular fractions showed that cisplatin induced redistribution of Bax and cytochrome c. Thus, cisplatin causes apoptosis in a death receptor-independent and mitochondria-dependent fashion in T24 cells. In contrast, overexpressed Bcl-2 protein inhibited cisplatin-induced Bax translocation and its downstream events in T24R2. Downregulation of Bcl-2 by RNAi potentiated the redistribution of Bax and cytochrome c and reversed cisplatin-resistance. Our results indicate that upregulation of Bcl-2 contributes to the development of cisplatin-resistance and usage of siRNA which targets the Bcl-2 gene may offer a potential tool to reverse the resistance to cisplatin in bladder cancer.

Davies, B. R. (2003). "Gene products involved in metastasis of bladder cancer." *Histol Histopathol* **18**(3): 969-80.

Metastasis is usually responsible for mortality in patients suffering from muscle invasive bladder cancer. Whilst expression of a great number of genes and their protein products have been associated with metastasis and/or poor prognosis in bladder cancer, evidence that they actively drive the metastatic process, and hence make potentially good

therapeutic targets, is often lacking. This is due to the limited number and application of effective animal models which reflect the pathogenesis of the human disease. In this review I will discuss the processes involved in metastasis, consider the established animal models of bladder cancer progression and metastasis, and review the evidence for a role of various gene products in this process. Consideration of clinical studies in conjunction with evidence from experimental animal models reveals that the tyrosine kinase receptor erbB1/EGFR, the calcium binding protein S100A4 and the the cell cycle arrest/apoptosis-inducing p53 protein are amongst the most promising targets for therapy against metastatic disease in patients with bladder cancer.

Ding, Y., G. Wang, et al. (2006). "Significance of Id-1 up-regulation and its association with EGFR in bladder cancer cell invasion." *Int J Oncol* **28**(4): 847-54.

Epidermal growth factor receptor (EGFR) is suggested to be one of the positive factors in the invasive progression of bladder cancer. Id-1 (inhibitor of differentiation or DNA binding), a helix-loop-helix (HLH) transcription factor, was recently identified as a key factor in the EGFR signalling pathway. The aim of this study was to investigate the role of Id-1 in bladder cancer progression and its relationship with EGFR. Using clinical specimens from different stages of bladder cancer, immunohistochemical staining was performed to determine if Id-1 expression was positively associated with tumour staging and EGFR expression. The direct role of Id-1 in cancer cell invasion was also investigated through ectopically expressing the Id-1 gene in a RT112 bladder cancer cell line by wound closure and collagen invasion assays. To explore the therapeutic potential of targeting the Id-1 gene in the treatment of invasive bladder cancer, we studied if inactivation of the Id-1 gene through small RNA interference could lead to the suppression of invasion in a MGHU1 bladder cancer cell line. The results showed that the up-regulation of Id-1 was associated with increased EGFR expression, clinical staging and the invasion ability of bladder cancer cells. Inactivation of Id-1 may be a potential therapeutic target to inhibit the invasion by bladder cancer cells.

Dozmorov, M. G., K. D. Kyker, et al. (2006). "Analysis of the interaction of extracellular matrix and phenotype of bladder cancer cells." *BMC Cancer* **6**: 12.

The extracellular matrix has a major effect upon the malignant properties of bladder cancer cells both in vitro in 3-dimensional culture and in vivo. Comparing gene expression of several bladder cancer

cells lines grown under permissive and suppressive conditions in 3-dimensional growth on cancer-derived and normal-derived basement membrane gels respectively and on plastic in conventional tissue culture provides a model system for investigating the interaction of malignancy and extracellular matrix. Understanding how the extracellular matrix affects the phenotype of bladder cancer cells may provide important clues to identify new markers or targets for therapy. Five bladder cancer cell lines and one immortalized, but non-tumorigenic, urothelial line were grown on Matrigel, a cancer-derived ECM, on SISgel, a normal-derived ECM, and on plastic, where the only ECM is derived from the cells themselves. The transcriptomes were analyzed on an array of 1186 well-annotated cancer derived cDNAs containing most of the major pathways for malignancy. Hypervariable genes expressing more variability across cell lines than a set expressing technical variability were analyzed further. Unique underlying regulatory networks were driving gene expression and could be identified by the approach outlined here.

Duggan, B. J., J. J. McKnight, et al. (2003). "The need to embrace molecular profiling of tumor cells in prostate and bladder cancer." *Clin Cancer Res* **9**(4): 1240-7.

Current treatment strategies for urological cancer are still based on empirical formulae as opposed to treatment tailored for each cancer patient. To individualize treatment, the multiple molecular abnormalities within tumor cell populations needs to be mapped out. The aim of this article is to explain molecular profiling (MP) and its associated techniques so that the process is not purely seen as a research tool but as a future adjunctive measure in patient diagnosis and treatment. A Medline search of publications relating to MP of prostate and bladder cancer was carried out. A review article was written combining the relevant published literature along with the clinical and scientific experience of both centers. The advent of MP now provides a strategy by which these molecular abnormalities can be assessed. As well as being of diagnostic and prognostic use, these molecular profiles will identify putative molecular abnormalities within tumor cells that may be appropriate for therapeutic modulation. In prostate and bladder cancer, mapping out the molecular abnormalities could be translated into a valuable tool to help solve difficult issues regarding patient management decisions.

Dyrskjot, L. (2003). "Classification of bladder cancer by microarray expression profiling: towards a general clinical use of microarrays in cancer diagnostics." *Expert Rev Mol Diagn* **3**(5): 635-47.

At present there are no clinically useful markers available for identifying bladder cancer patients with a high risk of disease recurrence or progression. Thus, identification and tailor-suited treatment, for example radical cystectomy and adjuvant therapy, of patients with a poor prognosis is not possible using current methods. The completion of the Human Genome Project and the simultaneous advances in microarray technology have paved the way for performing systematic, full genome screens for prognostic and diagnostic molecular cancer markers. Furthermore, utilization of microarray technology for identifying clinically relevant subclasses of cancer patients and for discovering new potential drug targets seems promising. This article summarizes some of the clinical aspects of bladder cancer and reviews the potential of using tumor expression profiling for the identification of new molecular cancer markers and drug targets, and for generating disease classifiers and outcome predictors using several key gene markers.

Ellinger, J., N. El Kassem, et al. (2008). "Hypermethylation of cell-free serum DNA indicates worse outcome in patients with bladder cancer." *J Urol* **179**(1): 346-52.

CpG island hypermethylation is a frequent event in bladder carcinogenesis and progression. We investigated the diagnostic and prognostic value of hypermethylation in cell-free serum DNA of patients with bladder cancer. The study cohort consisted of 45 patients with bladder cancer undergoing cystectomy and 45 with histologically confirmed benign prostatic hyperplasia serving as controls. Bladder cancer specific mortality was significantly increased in patients with APC hypermethylation. The detection of hypermethylation in cell-free serum DNA provides valuable diagnostic and prognostic information that can still be improved by combining the results of 3 gene sites (APC, GSTP1 and TIG1). The presence of hypermethylated DNA in the serum of patients with bladder cancer is associated with a worse outcome. The results suggest that measuring hypermethylation in the serum of patients with bladder cancer is a useful biomarker.

Eltze, E., P. J. Wild, et al. (2009). "Expression of the endothelin axis in noninvasive and superficially invasive bladder cancer: relation to clinicopathologic and molecular prognostic parameters." *Eur Urol* **56**(5): 837-45.

The endothelin (ET) axis plays a role in cancer biology and plays a potential role as a target for molecular therapy in urogenital tumours. Alterations of several proteins of the ET axis were detected in invasive bladder cancer. To examine the potential role

of the expression of ET axis proteins compared to other prognostic parameters (kinase inhibitor 67 [Ki-67], tumour protein 53 [TP53], and fibroblast growth factor receptor 3 gene [FGFR3] mutations) in noninvasive and invasive bladder cancer. DESIGN, SETTING, AND PARTICIPANTS: Tissue microarrays from 154 consecutive patients with pT_apT₂ urothelial bladder cancer were immunohistochemically stained for endothelin 1 (ET-1), endothelin A and B receptors (ET(A)R, ET(B)R), TP53, and Ki-67. FGFR3 mutations were detected by SNaPshot analysis.

El-Zawahry, A., P. Lu, et al. (2006). "In vitro efficacy of AdTRAIL gene therapy of bladder cancer is enhanced by trichostatin A-mediated restoration of CAR expression and downregulation of cFLIP and Bcl-XL." *Cancer Gene Ther* **13**(3): 281-9.

Current therapies for bladder cancer are suboptimal and adenoviral gene therapy has been explored as an alternative treatment. In this study, we evaluated the in vitro efficacy of an adenovirus expressing TNF-related apoptosis-inducing ligand (AdTRAIL). At low concentrations of virus, T24 cells were more resistant to AdTRAIL-induced apoptosis than 5637 bladder carcinoma cells. Resistance in T24 cells correlated with poor infectivity and lack of surface expression of coxsackie and adenovirus receptor (CAR). Pretreatment with low concentrations of the histone deacetylase inhibitor trichostatin A, restored CAR expression in T24 cells, which facilitated viral infection and resulted in apoptosis at low concentrations of AdTRAIL. In addition, trichostatin A reduced the expression of Bcl-X(L) and cFLIP resulting in increased sensitivity to recombinant TRAIL. Overexpression of cFLIP inhibited TRAIL-mediated killing in trichostatin A pretreated cells, indicating that downregulation of this antiapoptotic protein is required for sensitization. Therefore, trichostatin A can enhance the efficacy of AdTRAIL by restoring CAR expression and by generating a more pro-apoptotic phenotype that would facilitate bystander activity of TRAIL. Combination of histone deacetylase inhibitors with intravesical AdTRAIL gene therapy may be a novel treatment strategy for bladder cancer.

Fauconnet, S., S. Bernardini, et al. (2009). "Expression analysis of VEGF-A and VEGF-B: relationship with clinicopathological parameters in bladder cancer." *Oncol Rep* **21**(6): 1495-504.

The present investigation was conducted first to determine whether correlation exists between VEGF-A and -B mRNA levels and clinicopathological parameters and to assess their prognostic value in bladder cancer, then to clarify the expression level and

biological significance of VEGF-A isoforms. Total RNA was isolated from 37 specimens of bladder cancer. Northern blot analysis revealed that VEGF-B mRNA was not expressed either in normal urothelium or in bladder cancer and detected three VEGF-A transcripts of 5.2, 4.5 and 1.7 kb in length, respectively. The VEGF-A transcript levels were greater in cancer tissues than in normal urothelium. There was only a significant correlation between the increased expression level of VEGF121 and 165 and the histological grade of the lesion ($p < 0.05$). To conclude, VEGF-A mRNA level is a potential prognostic indicator of progression in bladder cancer as well as the expression level of the different VEGF-A splice variants.

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

Despite clinical use, the radiosensitizing effect of gemcitabine (2'-difluorodeoxycytidine) in human transitional cell carcinoma (TCC) has not been shown to date. Gemcitabine alone had a dose-dependent cytotoxic and apoptosis inducing effect on all TCC cell lines independent of p53 status. Assays combining radiation with gemcitabine in different dose and time schedules demonstrated no radiosensitizing effect in TCC cells. Gemcitabine is effective in TCC cell lines independent of p53 status. A radiosensitizing effect could not be demonstrated. Again, p53 status was not predictive of the radioresponse in the bladder cancer cell lines. Clinical studies with gemcitabine and radiotherapy might nevertheless yield different results but should be performed with utmost caution.

Flaig, T. W., L. J. Su, et al. (2009). "Dual epidermal growth factor receptor and vascular endothelial growth factor receptor inhibition with vandetanib sensitizes bladder cancer cells to cisplatin in a dose- and sequence-dependent manner." *BJU Int* **103**(12): 1729-37.

To investigate the activity of the combination of vandetanib and cytotoxic agents using in vitro models of bladder cancer, as modern chemotherapy regimens are built around cisplatin, with gemcitabine or a taxane such as docetaxel also commonly added in combination for the treatment of advanced bladder cancer. Human bladder cancer cells HTB3, HT1376, J82, RT4, CRL1749, T24, SUP and HTB9 were cultured. The activity of gefitinib (ZD1839) and vandetanib (ZD6474) was assessed in these eight bladder cancer cell lines with a tetrazolium-based assay of cell viability. RT4 bladder cancer cells, determined to have moderate cisplatin resistance and

also moderate sensitivity to vandetanib, were treated with vandetanib and cisplatin. RT4 and T24 cells were treated with six different regimens. The apoptosis and cell-cycle analysis were studied by flow cytometry. Expression of p21 and p27 was detected by Western blotting. Fluorescence in situ hybridization (FISH) analysis of epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 was performed for all cell lines. At equal concentrations, vandetanib was a more potent inhibitor of cell viability, compared to gefitinib. At vandetanib concentrations of ≤ 2 μM , the combination with cisplatin was synergistic, especially in the treatment sequence of cisplatin followed by vandetanib, and additive with vandetanib followed by cisplatin. An analysis of the cell-cycle distribution showed that vandetanib treatment induced G1 arrest at high concentrations, but not at lower concentrations. High-concentration treatment was associated with increased levels of the cyclin-dependent kinase p27. FISH analysis showed that there was a low level of genomic gain, and no gene amplification. Vandetanib has synergistic activity when given at low concentration with cytotoxic chemotherapy. The addition of vandetanib to cisplatin-based chemotherapy regimens merits further study.

Franekova, M., E. Halasova, et al. (2008). "Gene polymorphisms in bladder cancer." *Urol Oncol* **26**(1): 1-8.

In Europe, cancer of the bladder is the fourth most common cancer among men, accounting for 7% of total cancers. In the USA, bladder cancer is the fifth most common cancer in men and seventh in women. This disease is three times more common in men than in women. Several risk factors, such as cigarette smoking and occupational chemical exposure, contribute to bladder cancer development. The balance between activation and detoxification of carcinogens affects the amount of DNA damage that accumulates in cells. The entire process leading to DNA damage and subsequent repair of the damage involves a host of enzymes, many of which are polymorphic. Polymorphisms in metabolic enzyme genes and repair genes may cause alterations in protein product functions that can finally lead to genomic instability and carcinogenesis. In this article, we review the polymorphisms in a number of genes that have been found to be the modulators of bladder cancer risk. Improved understanding of the molecular biology of urothelial malignancies is helping to more clearly define the role of new prognostic indices and multidisciplinary treatment for this disease.

Freund, C. T., X. W. Tong, et al. (2003). "Combination of adenovirus-mediated thymidine

kinase gene therapy with cytotoxic chemotherapy in bladder cancer in vitro." *Urol Oncol* **21**(3): 197-205.

Freund et al evaluated efficacy, toxicity and potential synergism of adenoviral-mediated thymidine kinase (tk)- ganciclovir (GCV) gene therapy in combination with 4 cytotoxic chemotherapeutic agents (doxorubicin, cisplatin, mitomycin C, and methotrexate) in 3 human bladder cancer cell lines. Cell lines were exposed to (1) 10 different concentrations of adenovirus expressing tk plus GCV; (2) 8 different concentrations of either doxorubicin, methotrexate, mitomycin C or cisplatin; or (3) combination treatment consisting of either low-, medium- or high-dose tk-GCV gene therapy plus 8 different concentrations of a single chemotherapeutic agent. Cell survival was determined using a MTT-based cell proliferation-assay. For most combinations, adding chemotherapy to tk-GCV gene therapy did not result in any therapeutic benefit. In some scenarios, we observed modest improvement with combinations of high-dose tk-GCV gene therapy and high-dose standard chemotherapy over tk-GCV monotherapy. Low concentrations of methotrexate enhanced the antitumor effects of low- and medium-dose tk-GCV gene therapy. Low level negative interference between tk-GCV gene therapy and chemotherapy occurred in some combinations but was overall negligible. In general, adding chemotherapy to tk-GCV gene therapy did not demonstrate significant therapeutic benefit in vitro. High doses of chemotherapeutic agents should be used in combination with tk-GCV gene therapy in order to take advantage of the occasional instance where modest improvement occurred with combination therapy. Additional studies exploring the role of methotrexate in enhancing the tk-GCV system are required. Investigation of other, potentially more synergistic chemotherapeutic agents in combination with tk-GCV is warranted.

Frohlich, C., R. Albrechtsen, et al. (2006). "Molecular profiling of ADAM12 in human bladder cancer." *Clin Cancer Res* **12**(24): 7359-68.

The purpose of this study was to determine the gene and protein expression profiles of ADAM12 in different grades and stages of bladder cancer. ADAM12 gene expression was evaluated in tumors from 96 patients with bladder cancer using a customized Affymetrix GeneChip. Gene expression in bladder cancer was validated using reverse transcription-PCR, quantitative PCR, and in situ hybridization. Protein expression was evaluated by immunohistochemical staining on tissue arrays of bladder cancers. The presence and relative amount of ADAM12 in the urine of cancer patients were determined by Western blotting and densitometric measurements, respectively. ADAM12 mRNA

expression was significantly up-regulated in bladder cancer, as determined by microarray analysis, and the level of ADAM12 mRNA correlated with disease stage. Reverse transcription-PCR, quantitative PCR, and in situ hybridization validated the gene expression results. Using immunohistochemistry, Frohlich et al found ADAM12 protein expression correlated with tumor stage and grade. Finally, ADAM12 could be detected in the urine by Western blotting; ADAM12 was present in higher levels in the urine from patients with bladder cancer compared with urine from healthy individuals. Significantly, following removal of tumor by surgery, in most bladder cancer cases examined, the level of ADAM12 in the urine decreased and, upon recurrence of tumor, increased. ADAM12 is a promising biomarker of bladder cancer.

Fu, W. J., B. F. Hong, et al. (2005). "Expression of a recombinant vector of a mutant human telomerase reverse transcriptase gene in human bladder cancer cell line T24, and its clinical significance." *BJU Int* **96**(6): 890-4.

To construct a mutant enhanced green fluorescence protein (pEGFP) human telomerase reverse transcriptase (hTERT) gene expression vector (pEGFP-hTERT), to observe its expression in transfected human bladder cancer cell line T24 and its role in the molecular regulatory mechanisms of telomerase, and to provide a new target gene for bladder cancer therapy. Polymerase chain reaction (PCR) amplification was performed using primers based on the gene sequence of hTERT. The PCR product was cloned into plasmid pGEMT-T Easy and the sequence of mutant hTERT gene analysed. A recombinant mutant hTERT vector (pEGFP-hTERT) was constructed at the EcoR I and Sal I sites of the pEGFP-C1 vector. After transfecting the fusion gene into T24 cells by the method of calcium phosphate-DNA co-precipitation, we detected steady expression of the GFP-hTERT fusion protein by fluorescent-light microscopy. Changes in the proliferation of T24 cells were detected by light microscopy, and beta-galactosidase staining correlated with senescence. Identification of pEGFP-hTERT by enzyme digestion showed that the mutant hTERT fragment had been cloned into EcoR I and Sal I sites of the pEGFP-C1 vector. Steady expression of GFP-hTERT fusion protein was located in the nucleus of transfected cells. Positive expression senescence-associated beta-galactosidase staining in transfected cells increased gradually with extended cultured time, and their growth was suppressed. The recombinant mutant vector (pEGFP-hTERT) was successfully constructed and expressed steadily in T24 cells. The mutant-type hTERT gene suppresses the proliferation of T24 cells by a competitive effect on telomerase activity. This

suggests that the hTERT gene might be a suitable gene target for bladder cancer therapy.

Fuessel, S., B. Kueppers, et al. (2004). "Systematic in vitro evaluation of survivin directed antisense oligodeoxynucleotides in bladder cancer cells." *J Urol* **171**(6 Pt 1): 2471-6.

The rather poor responses to conventional treatment for bladder cancer (BCa) require novel, specific therapy approaches. The down-regulation of BCa associated genes may represent a new option to inhibit specifically BCa cell growth and induce cell death. Survivin, an apoptosis inhibitor that is up-regulated in the majority of malignancies, including BCa, provides an attractive target for molecular therapies, such as treatment with specific antisense oligodeoxynucleotides (AS-ODNs). Fuessel et al used mRNA secondary structure prediction to design survivin directed AS-ODNs. After lipid mediated transfection with 30 selected antisurvivin AS-ODN inhibitory effects on cell growth properties as well as on survivin expression were measured. Three of 30 tested constructs reproducibly impaired the growth characteristics of 4 BCa cell lines. Detailed analysis of the cell line EJ28 treated with the constructs SVV261, SVV264 and SVV286 revealed a clear decrease in viability (down to 35%) and long-term proliferation (down to 14%), which were caused by cell cycle arrest and an increase in apoptosis (from 19.5% to 51.3% maximum). The inhibition of tumor cell growth was associated with up to 60% to 80% survivin expression down-regulation. Interestingly all 3 evolved AS-ODNs were directed against the putative single strand survivin mRNA motif between 274 to 285 nucleotides, identified by secondary structure prediction. The reported accessibility of this motif to other nucleic acid based inhibitors such as ribozymes and small interfering RNAs emphasizes the rationale of a systematic selection of mRNA target sites. The survivin directed AS-ODNs shown to inhibit effectively the proliferation of BCa cells in the current study may provide suitable adjuvant therapeutic agents for the specific local treatment of BCa.

Fullerton, N. E., R. J. Mairs, et al. (2005). "Application of targeted radiotherapy/gene therapy to bladder cancer cell lines." *Eur Urol* **47**(2): 250-6.

A targeted radiotherapy/gene therapy strategy for transitional cell carcinoma of bladder is described, using [¹³¹I]meta-iodobenzylguanidine ([¹³¹I]MIBG), a radionuclide combined with a tumour-seeking drug. The aim is to decrease side effects from radiation toxicity, while increasing radiation dose to tumour. This tumour cell kill approach is augmented by radiological bystander effects. The bladder cancer cell line EJ138 was transfected with a gene encoding the

noradrenaline transporter (NAT) under the control of tumour-specific telomerase promoters. Resulting uptake of [131I]MIBG was assessed by gamma-counting of cell lysates, and NAT transgene expression by real-time RT-PCR. Cell kill of monolayers and disaggregated spheroids, dosed with [131I]MIBG, was assessed by clonogenic assay. NAT gene transfected cells exhibited a significantly increased active uptake of [131I]MIBG, leading to dose-dependent cell kill. Clonogenic assay of disaggregated spheroids, a three-dimensional model, suggested cell kill via bystander effects. Expression of a functional NAT after in vitro transfection of bladder cancer cells with the NAT gene under the control of telomerase promoters leads to active uptake of [131I]MIBG and dose-dependent cell kill. This strategy could produce a promising new treatment option for bladder cancer.

Gazzaniga, P., I. Silvestri, et al. (2007). "Gemcitabine-induced apoptosis in 5637 cell line: an in-vitro model for high-risk superficial bladder cancer." *Anticancer Drugs* **18**(2): 179-85.

Recent data suggest that new treatment options for superficial bladder cancer are necessary, owing to the high recurrence rate after conventional treatment, especially in T1G3 and Bacillus Calmette-Guerin-refractory patients. Phase I and II studies have demonstrated that gemcitabine may represent a candidate for intravesical therapy in superficial bladder cancer. Despite clinical trials, the in-vitro cytotoxic and proapoptotic effects of gemcitabine have been poorly investigated. In the present study, we investigated how gemcitabine affects apoptosis in bladder cancer cell line 5637, which has the same molecular features of high-risk superficial bladder cancer. Apoptosis was evaluated by DNA fragmentation, flow cytometry and caspase activation. bcl-2, bcl-X, bax, survivin and fas gene expression were also evaluated by reverse-transcriptase polymerase chain reaction. Nuclear factor-kappa B activation was assessed by immunofluorescence. Gemcitabine induced apoptosis in 5637 cells in a time-dependent manner, with activation of caspase-3, -8 and -9. Expression of bcl-2, bax, survivin and bcl-X was not affected by treatment, whereas fas strongly increased after 24 h of treatment. After treatment, we failed to find any nuclear localization of nuclear factor-kappa B. As gemcitabine-induced apoptosis involves fas upregulation, these results may encourage the investigation of intravesical gemcitabine in fas-negative bladder tumors. Furthermore, as nuclear factor-kappa B activation by cisplatin, doxorubicin and adriamycin may result in enhanced proliferation, migration, immortality and inhibition of apoptosis, the observation that gemcitabine does not activate nuclear

factor-kappa B may have implications in intravesical therapy of high-risk superficial bladder cancer.

George, B., R. H. Datar, et al. (2007). "p53 gene and protein status: the role of p53 alterations in predicting outcome in patients with bladder cancer." *J Clin Oncol* **25**(34): 5352-8.

The p53 gene status (mutation) and protein alterations (nuclear accumulation detectable by immunohistochemistry; p53 protein status) are associated with bladder cancer progression. Substantial discordance is documented between the p53 protein and gene status, yet no studies have examined the relationship between the gene-protein status and clinical outcome. This study evaluated the clinical relationship of the p53 gene and protein statuses. The complete coding region of the p53 gene was queried using DNA from paraffin-embedded tissues and employing a p53 gene-sequencing chip. George et al compared p53 gene status, mutation site, and protein status with time to recurrence. The p53 gene and protein statuses show significant concordance, yet 35% of cases showed discordance. Exon 5 mutations demonstrated a wild-type protein status in 18 of 22 samples. Both the p53 gene and protein statuses were significantly associated with stage and clinical outcome. Specific mutation sites were associated with clinical outcome; tumors with exon 5 mutations showed the same outcome as those with the wild-type gene. Combining the p53 gene and protein statuses stratifies patients into three distinct groups, based on recurrence-free intervals: patients showing the best outcome (wild-type gene and unaltered protein), an intermediate outcome (either a mutated gene or an altered protein) and the worst outcome (a mutated gene and an altered protein). **CONCLUSION:** We show that evaluation of both the p53 gene and protein statuses provides information in assessing the clinical recurrence risk in bladder cancer and that the specific mutation site may be important in assessing recurrence risk. These findings may substantially impact the assessment of p53 alterations and the management of bladder cancer.

Gildea, J. J., M. Herlevsen, et al. (2004). "PTEN can inhibit in vitro organotypic and in vivo orthotopic invasion of human bladder cancer cells even in the absence of its lipid phosphatase activity." *Oncogene* **23**(40): 6788-97.

A role of PTEN in bladder cancer invasion is further suggested by the fact that PTEN is a regulator of cell motility, a necessary component of tumor invasion. However, it is unknown whether PTEN is mechanistically involved in 'in vivo' tumor invasion or merely an epiphenomenon and, if the former is true, whether this process is dependent on its protein or

lipid phosphatase activities. To address these issues, we stably transfected several commonly used human bladder cancer cell lines with known invasive phenotypes with either wild-type PTEN constructs or those deficient in the lipid phosphatase (G129E) or both protein and lipid phosphatase (G129R) activities. Here we show that chemotaxis was inhibited by both the wild-type and G129E mutant of PTEN but not by G129R-transfected cells. Using a novel organotypic in vitro invasion assay, we evaluated the impact of wild-type and mutant PTEN transgene expression on the invasive ability of T24T, a human bladder cancer cell line with a functionally impaired PTEN. Results indicate that the G129E mutant blocks invasion as efficiently as wild-type PTEN transfection. In contrast to the wild-type gene, this mutant has no effect on cell clonogenicity in agar. To further establish the role of PTEN in tumor invasion, we evaluated vector- and PTEN-transfected T24T cells in an orthotopic in vivo assay that faithfully reproduces human disease. Microscopic examination of murine bladders at the completion of this experiment parallels the results obtained with the organotypic assay. Our results are the first demonstration: (1) that the inhibitory effects of PTEN on cell motility translate into suppression of in vivo invasion; (2) that PTEN can inhibit tumor invasion even in the absence of its lipid phosphatase activity; (3) how organotypic in vitro approaches can be used as surrogates of in vivo invasion allowing rapid dissection of molecular processes leading to this phenotype while reducing the number of animals used in research.

Golshani, R., S. H. Hautmann, et al. (2007). "HAS1 expression in bladder cancer and its relation to urinary HA test." *Int J Cancer* **120**(8): 1712-20.

Hyaluronic acid (HA) levels are elevated in bladder cancer tissues and regulate tumor growth and progression. Urinary HA levels measured by the HA test are an accurate marker for bladder cancer. In cells, HA is synthesized by one of the 3 HA-synthase(s) i.e., HAS1, HAS2 and HAS3. In this study, we examined HAS1 expression in bladder cancer cells and tissues. Real-time RT-PCR and northern blot analyses showed that HAS1 transcript levels are elevated 5- to 10-fold in bladder cancer tissues, when compared with normal tissues ($p < 0.001$). Among the 3 HAS1 splice variants, only HAS1-va was expressed in bladder tissues, but the expression was significantly lower than the wild type HAS1 transcript. Increased HAS1 expression in bladder tumor tissues correlated with increased tissue HA levels ($p < 0.001$). Size of the large HA species (2.0×10^6 D) present in bladder tissues was consistent with the size of the HA polymer synthesized by HAS1. The amount of HA produced by bladder cancer cell lines correlated with the

expression of HAS1 protein. Immunohistochemical analyses of bladder tumor tissues showed that HAS1 and HA expression had 79-88% sensitivity and 83.3-100% specificity. Both HAS1 and HA expression in bladder cancer tissues correlated with a positive HA urine test ($p < 0.001$). HAS1 expression correlated with tumor recurrence, prior treatment ($p < 0.05$) and possibly disease progression ($p = 0.058$). Therefore, elevated HAS1 expression in bladder tumor tissues contributes to a positive HA urine test and may have some prognostic potential.

Golshani, R., L. Lopez, et al. (2008). "Hyaluronic acid synthase-1 expression regulates bladder cancer growth, invasion, and angiogenesis through CD44." *Cancer Res* **68**(2): 483-91.

Hyaluronic acid (HA) promotes tumor metastasis and is an accurate diagnostic marker for bladder cancer. HA is synthesized by HA synthases HAS1, HAS2, or HAS3. We have previously shown that HAS1 expression in tumor tissues is a predictor of bladder cancer recurrence and treatment failure. In this study, we stably transfected HT1376 bladder cancer cells with HAS1-sense (HAS1-S), HAS1-antisense (HAS1-AS), or vector cDNA constructs. Whereas HAS1-S transfectants produced approximately 1.7-fold more HA than vector transfectants, HA production was reduced by approximately 70% in HAS1-AS transfectants. HAS1-AS transfectants grew 5-fold slower and were approximately 60% less invasive than vector and HAS1-S transfectants. HAS1-AS transfectants were blocked in G(2)-M phase of the cell cycle due to down-regulation of cyclin B1, cdc25c, and cyclin-dependent kinase 1 levels. These transfectants were also 5- to 10-fold more apoptotic due to the activation of the Fas-Fas ligand-mediated extrinsic pathway. HAS1-AS transfectants showed a approximately 4-fold decrease in ErbB2 phosphorylation and down-regulation of CD44 variant isoforms (CD44-v3, CD44-v6, and CD44-E) both at the protein and mRNA levels. However, no decrease in RHAMM levels was observed. The decrease in CD44-v mRNA levels was not due to increased mRNA degradation. Whereas CD44 small interfering RNA (siRNA) transfection decreased cell growth and induced apoptosis in HT1376 cells, HA addition modestly increased CD44 expression and cell growth in HAS1-AS transfectants, which could be blocked by CD44 siRNA. In xenograft studies, HAS1-AS tumors grew 3- to 5-fold slower and had approximately 4-fold lower microvessel density. These results show that HAS1 regulates bladder cancer growth and progression by modulating HA synthesis and HA receptor levels.

Goudopoulou, A., A. Saetta, et al. (2003). "p53 mutations detection in urinary bladder cancer in the Greek population: application of the NIRCA assay." *J Exp Clin Cancer Res* **22**(1): 99-105.

Goudopoulou et al applied the NIRCA assay, which consists of two-step PCR amplification, transcription of the amplified sequence, hybridisation of the transcripts and treatment with RNAses which recognizes mismatches due to the presence of mutations. Results of molecular analysis are correlated with immunohistochemical findings, standard clinopathological parameters and survival. p53 mutations were detected in 42.4% of the 66 examined TCCs cases. We could not demonstrate any statistical relationship between the presence of p53 mutation and p53 protein overexpression, and tumor stage or grade. A trend towards higher mutation rate in higher grade tumours was observed, although this failed to reach statistical significance. Despite the observation that the alterations of p53 gene are associated features of aggressive phenotype of transitional cell carcinomas they do not seem to offer additional prognostic information.

Gu, J., H. Zhao, et al. (2005). "Nucleotide excision repair gene polymorphisms and recurrence after treatment for superficial bladder cancer." *Clin Cancer Res* **11**(4): 1408-15.

Interindividual differences in DNA repair capacity not only modify individual susceptibility to carcinogenesis, but also affect individual response to cancer treatment. Nucleotide excision repair (NER) is one of the major DNA repair pathways in mammalian cells involved in the removal of a wide variety of DNA lesions. Polymorphisms in NER genes may influence DNA repair capacity and affect clinical outcome of bladder cancer treatment. To test the influence of NER gene polymorphisms on superficial bladder cancer outcome (recurrence and progression), we conducted a follow-up study of 288 patients with superficial bladder cancer. Median follow-up among patients who were recurrence-free at the end of observation was 21.7 months from diagnosis. The specific polymorphic loci examined include XPA [A/G at 5' untranslated region (UTR)], XPC (poly AT, Ala(499)Val, Lys(939)Gln), XPD (Asp(312)Asn, Lys(751)Gln), XPG (His(1104)Asp), ERCC 1 (G/T at 3' UTR), and ERCC6 (Met(1097)Val, Arg(1230)Pro). The ERCC6 (Met(1097)Val) polymorphism had a significant impact on recurrence: carriers of at least one variant allele (Val) had a significantly higher recurrence risk than carriers of the wild-type allele (Met/Met; hazard ratio, 1.54; 95% confidence interval, 1.02-2.33). There were no overall statistically significant differences in the distributions of the other polymorphisms between patients with and without

recurrence. However, when we combined these variant genotypes, there was a significant trend for an increased recurrence risk with an increasing number of putative high-risk alleles. Using individuals with five or fewer putative high-risk alleles as the reference group, individuals with six to seven risk alleles and individuals with eight or more risk alleles had higher recurrence risks, with hazard ratios of 0.92 (0.54-1.57) and 2.53 (1.48-4.30), respectively (P for trend < 0.001). These data suggest that interindividual differences in DNA repair capacity may have an important impact on superficial bladder cancer recurrence. A pathway-based approach is preferred to study the effects of individual polymorphism on clinical outcomes.

Habuchi, T., M. Marberger, et al. (2005). "Prognostic markers for bladder cancer: International Consensus Panel on bladder tumor markers." *Urology* **66**(6 Suppl 1): 64-74.

The International Consensus Panel on cytology and bladder tumor markers evaluated markers that have the ability to predict tumor recurrence, progression, development of metastases, or response to therapy or patient survival. This article summarizes those findings. The panel mainly reviewed articles listed in PubMed on various prognostic indicators for bladder cancer. Based on these studies, most of which were case-control retrospective studies, various prognostic indicators were classified into 6 groups: (1) microsatellite-associated markers, (2) proto-oncogenes/oncogenes, (3) tumor suppressor genes, (4) cell cycle regulators, (5) angiogenesis-related factors, and (6) extracellular matrix adhesion molecules. The panel concluded that although certain markers, such as Ki-67 and p53, appear to be promising in predicting recurrence and progression of bladder cancer, the data are still heterogeneous. The panel recommends that identifying definitive criteria for test positivity, a clearly defined patient population, standardization of techniques used to evaluate markers, and clearly specified endpoints and statistical methods will help to bring accurate independent prognostic indicators into the clinical management of patients with bladder cancer.

Hadaschik, B. A., P. C. Black, et al. (2007). "A validated mouse model for orthotopic bladder cancer using transurethral tumour inoculation and bioluminescence imaging." *BJU Int* **100**(6): 1377-84.

To describe a technique for transurethral tumour inoculation, bioluminescence imaging (BLI) and validation of this approach using ex vivo magnetic resonance imaging (MRI), as a reproducible and quantifiable model of orthotopic bladder cancer is

required to enable preclinical pharmacological studies of intravesically administered anticancer agents and the use of BLI provides a sensitive method to monitor tumour growth over time. Human KU-7 bladder tumour cells were transduced with a lentiviral construct to stably express the firefly luciferase gene. These cells were then inoculated in female nude mice by intravesical instillation. BLI was performed weekly and the mice were killed after 4 weeks. Ex vivo MRI and whole-mount step-sections were obtained to assess bladder tumour volume. KU-7 tumour cells were highly tumorigenic and were successfully inoculated in 96% of mice. After 4 weeks, all tumours were confined to the mucosa and submucosa ($\leq pT1$). There was an excellent correlation between tumour volume and BLI for both ex vivo bladder MRI ($R(2) = 0.929$) and end-point histological measurements ($R(2) = 0.836$). We have established and validated a reliable model of orthotopic bladder cancer that can be used to evaluate various methods of intravesical therapy. BLI allows excellent longitudinal surveillance and quantification of tumour burden.

Hadaschik, B. A., K. Zhang, et al. (2008). "Oncolytic vesicular stomatitis viruses are potent agents for intravesical treatment of high-risk bladder cancer." *Cancer Res* **68**(12): 4506-10.

Bladder cancer is the second most common genitourinary malignancy. At initial diagnosis, approximately 70% of cases are non-muscle-invasive; however, current treatment options for superficial disease are of limited efficacy because many patients will develop recurrent tumors. Despite using immunocompromised hosts, there was no evidence of toxicity in either group. In conclusion, VSV instillation therapy showed promising antitumor activity and safety in an orthotopic model of bladder cancer. These findings provide preclinical proof-of-principle for the intravesical use of VSV against non-muscle-invasive bladder cancer, especially in IFN-refractory patients.

Halling, K. C. and B. R. Kipp (2008). "Bladder cancer detection using FISH (UroVysion assay)." *Adv Anat Pathol* **15**(5): 279-86.

UroVysion is a fluorescence in situ hybridization assay that was developed for the detection of bladder cancer in urine specimens. It consists of fluorescently labeled DNA probes to the pericentromeric regions of chromosomes 3 (red), 7 (green), and 17 (aqua) and to the 9p21 band (gold) location of the P16 tumor suppressor gene. The UroVysion assay works by detecting urinary cells that have chromosomal abnormalities consistent with a diagnosis of bladder cancer. Studies have shown that UroVysion is more sensitive than urine cytology for

the detection of all stages and grades of bladder cancer. UroVysion is Food and Drug Administration-approved for the detection of recurrent bladder cancer in voided urine specimens from patients with a history of bladder cancer and for the detection of bladder cancer in voided urine specimens from patients with gross or microscopic hematuria, but no previous history of bladder cancer. Recent studies also suggest that UroVysion may be useful for assessing superficial bladder cancer patients' response to bacillus Calmette-Guerin therapy and in detecting upper tract urothelial carcinoma.

Havaleshko, D. M., H. Cho, et al. (2007). "Prediction of drug combination chemosensitivity in human bladder cancer." *Mol Cancer Ther* **6**(2): 578-86.

The choice of therapy for metastatic cancer is largely empirical because of a lack of chemosensitivity prediction for available combination chemotherapeutic regimens. Here, we identify molecular models of bladder carcinoma chemosensitivity based on gene expression for three widely used chemotherapeutic agents: cisplatin, paclitaxel, and gemcitabine. We measured the growth inhibition elicited by these three agents in a series of 40 human urothelial cancer cell lines and correlated the GI(50) (50% of growth inhibition) values with quantitative measures of global gene expression to derive models of chemosensitivity using a misclassification-penalized posterior approach. The misclassification-penalized posterior-derived models predicted the growth response of human bladder cancer cell lines to each of the three agents with sensitivities of between 0.93 and 0.96. We then developed an in silico approach to predict the cellular growth responses for each of these agents in the clinically relevant two-agent combinations. These predictions were prospectively evaluated on a series of 15 randomly chosen bladder carcinoma cell lines. Overall, 80% of the predicted combinations were correct ($P = 0.0002$). Together, our results suggest that chemosensitivity to drug combinations can be predicted based on molecular models and provide the framework for evaluation of such models in patients undergoing combination chemotherapy for cancer. If validated in vivo, such predictive models have the potential to guide therapeutic choice at the level of an individual's tumor.

Havaleshko, D. M., S. C. Smith, et al. (2009). "Comparison of global versus epidermal growth factor receptor pathway profiling for prediction of lapatinib sensitivity in bladder cancer." *Neoplasia* **11**(11): 1185-93.

Chemotherapy for metastatic bladder cancer is rarely curative. The recently developed small

molecule, lapatinib, a dual epidermal growth factor receptor (EGFR)/human epidermal growth factor receptor-2 receptor tyrosine kinase inhibitor, might improve this situation. Recent findings suggest that identifying which patients are likely to benefit from targeted therapies is beneficial, although controversy remains regarding what types of evaluation might yield optimal candidate biomarkers of sensitivity. Here, we address this issue by developing and comparing lapatinib sensitivity prediction models for human bladder cancer cells. After empirically determining in vitro sensitivities (drug concentration necessary to cause a 50% growth inhibition) of a panel of 39 such lines to lapatinib treatment, we developed prediction models based on profiling the baseline transcriptome, the phosphorylation status of EGFR pathway signaling targets, or a combination of both data sets. Combining microarray data and phosphoprotein profiling provided a combination model with 98% accuracy. Our findings suggest that transcriptome-wide profiling for biomarkers of lapatinib sensitivity in cancer cells provides models with excellent predictive performance and may be effectively combined with EGFR pathway phosphoprotein profiling data. These results have significant implications for the use of such tools in personalizing the approach to cancers treated with EGFR-directed targeted therapies.

Haynes, M. D., T. A. Martin, et al. (2005). "Tight junctions and bladder cancer (review)." *Int J Mol Med* **16**(1): 3-9.

Tight junctions play a critical role in the maintenance of the urine-blood barrier creating a physiological barrier to the passage of ions and solutes between the urine and blood. Alterations in this urine-blood barrier function have been demonstrated in some diseases and regulation of the tight junction function has been recognised as an important aspect of the cell biology of cancer in terms of disease progression and as a potential therapeutic target. Although tight junctions play an important role in the physiological control of bladder function, there is little published on their molecular composition or regulation in the normal or diseased bladder. The purpose of this review is to summarise current understanding on the role and regulation of tight junction function in the normal and diseased bladder.

He, L. F., K. P. Guan, et al. (2005). "Enhanced sensitivity to mitomycin C by abating heat shock protein 70 expression in human bladder cancer cell line of BIU-87." *Chin Med J (Engl)* **118**(23): 1965-72.

Bladder cancer is a relatively common tumor in the urinary system, in which mitomycin C (MMC)-based chemotherapy or combination chemotherapy

has been mainly used to treat patients with advanced bladder cancer. The prognosis of patients with advanced bladder cancer is still extremely poor in spite of recent therapeutic advances. To improve the prognosis, the sensitivity of tumor cells to mitomycin C by the induction of apoptosis with the abating heat shock protein 70 (HSP70) expression in human bladder cancer cell lines of BIU-87 was investigated. HSP70 expression was abated in BIU-87 cells by HSP mRNA antisense oligomers. MTT assay and the clone-forming test were used for evaluating the sensitivity of cells to MMC. Apoptosis was assessed using both fluorescent microscopy after staining the cells with Hoechst 33258 and DNA fragment ladder agarose electrophoresis. Thirty-two male six-week-old BALB/c nude mice, at the beginning of the experiment, were used to evaluate the effect of antisense oligomers (ASO) on the tumor formation in vivo. HSP70 expression in BIU-87 was effectively abated by HSP70 mRNA antisense oligomers. The percentage of apoptotic cells in ASO group was greater than in sense oligomers (SO) [$P < 0.05$, (18.31 \pm 2.89)% vs (1.89 \pm 0.74)%], nonsense oligomers (NO) [$P < 0.05$, (18.31 \pm 2.89)% vs (1.78 \pm 0.92)%] and blank groups [$P < 0.05$, (18.31 \pm 2.89)% vs (1.87 \pm 0.84)%], while the sensitivity of tumor cells to mitomycin C was enhanced. The in vivo tumor inhibition rate of ASO plus MMC ($> 50\%$) was more than that of ASO or MMC group alone (all $P < 0.05$). The abating level of HSP70 expression can strengthen the sensitivity of BIU-87 to MMC. One of this effect might be related to the induction of apoptosis by abating HSP70 expression.

He, X. D., Z. P. Wang, et al. (2009). "Construction of urothelium-specific recombinant adenovirus and its inhibition in bladder cancer cell." *Urol Int* **82**(2): 209-13.

To construct urothelium-specific recombinant adenovirus and investigate its inhibition in bladder cancer cell. RT-PCR analysis was used to determine expression patterns of hUPII and coxsackie adenovirus receptor on multiple cell lines. Transient transfection and luciferase detecting assay were used to detect tissue specificity of the hUPII promoter. Recombinant adenovirus Ad-UPII-E1A and Ad-UPII-Null were constructed. Restrictive enzyme digestion assay and PCR confirmed the correct construction. The adenovirus E1A protein expressed in BIU-87 was tested by Western blot after cells were infected with recombinant adenovirus. Recombinant adenovirus Ad-UPII-E1A was tested for its inhibition in bladder cancer cell line BIU-87. HUPII and CAR were expressed and the hUPII promoter is highly active in bladder cancer cell line BIU-87. Using homologous recombination in bacteria technology, the hUPII

promoter and E1A gene were inserted into the genome of type 5 recombinant adenovirus. The E1A protein was markedly positive in the samples of BIU-87 cells infected with recombinant adenovirus Ad-UPII-E1A. MTT assay demonstrated recombinant adenovirus Ad-UPII-E1A inhibited bladder cancer cell BIU-87 growth. CONCLUSION: The hUPII promoter shows high tissue specificity. Recombinant adenovirus Ad-UPII-E1A and Ad-UPII-Null were constructed and confirmed. Recombinant adenovirus Ad-UPII-E1A is effective in inhibition in bladder cancer cell line BIU-87.

Hemstreet, G. P. and W. Wang (2004). "Genotypic and phenotypic biomarker profiles for individual risk assessment and cancer detection (lessons from bladder cancer risk assessment in symptomatic patients and workers exposed to benzidine)." *Front Biosci* **9**: 2671-9.

There is a need for improved methods for detecting individuals at risk for cancer to target subsets of patients for more intensive individual screening and targeted cancer therapy and chemoprevention. One approach for accomplishing this objective is to detect premalignant molecular fingerprints in an organ at risk for cancer or to define biomarkers reflective of treatment selection and response. Bladder cancer is an excellent model for testing this approach; however, comprehending the strategy for biomarker selection and analysis is more complicated than is generally appreciated. The objective of this article is to provide a succinct overview of our experience with the selection of biomarkers for bladder cancer detection, first in symptomatic patients and then in high-risk cohorts of workers at risk for bladder cancer. Biomarker selection depends on multiple parameters, each of which must be optimized to enhance the utility of a biomarker for clinical application. Many markers that initially show promise fail in the clinical arena for a variety of reasons. Important parameters include when a biomarker is expressed in carcinogenesis (i.e., early vs. late), the sample type, and the method of analysis. The purpose of this presentation is to illustrate the fundamental concepts for selection and profile analysis of high-level phenotypic biomarkers developed for bladder cancer risk assessment, screening, and early bladder cancer detection.

Highshaw, R. A., D. J. McConkey, et al. (2004). "Integrating basic science and clinical research in bladder cancer: update from the first bladder Specialized Program of Research Excellence (SPORE)." *Curr Opin Urol* **14**(5): 295-300.

To review the progress of the genitourinary SPORE (Specialized Program of Research

Excellence) in bladder cancer. The optimal management of bladder cancer depends on the accurate assessment of the biological potential of the disease. Methotrexate, vincristine, adriamycin and cisplatin (M-VAC) chemotherapy has been the standard of therapy for over a decade. However, there has been no improvement in patient survival. Encouraging preclinical data have resulted in the rapid translation of epidermal growth factor receptor antagonists into the clinic. However, phase I and II single-agent clinical trials in head and neck, lung, and colon cancer failed to match the hope generated by laboratory investigations since only a minority of patients seemed to benefit from this approach. Nonetheless, recent data revealed that non-small-cell lung cancer tumors that responded to single-agent Iressa possessed activating epidermal growth factor receptor mutations. The integration of these new biological agents in combination with chemotherapy, in order to abrogate the progression of advanced bladder cancer, is the prime directive of our current phase II Iressa/docetaxel trial.

Hikosaka, S., I. Hara, et al. (2004). "Antitumor effect of simultaneous transfer of interleukin-12 and interleukin-18 genes and its mechanism in a mouse bladder cancer model." *Int J Urol* **11**(8): 647-52.

The objectives of this study were to evaluate the antitumor effects of the simultaneous introduction of interleukin 12 (IL-12) and IL-18 genes into a mouse bladder cancer cell line (MBT2). We intended to compare these with those of either gene alone and to investigate the mechanism of the effects induced by the transfer of IL-12 and/or IL-18 genes in this model system. We transfected the IL-12 and/or IL-18 genes into MBT2 cells by the liposome-mediated gene transfer method. We confirmed the secretion of IL-12 and/or IL-18 by enzyme-linked immunosorbent assay. Parental (MBT2/P), IL-12-transfected (MBT2/IL-12), IL-18-transfected (MBT2/IL-18) or both IL-12- and IL-18-transfected (MBT2/Both) cells were subcutaneously or intravenously injected into syngeneic C3H mice. To analyze the mechanism of tumor rejection, these clones were subcutaneously injected into naive nude mice and those depleted with natural killer (NK) cells by antibody. MBT2/IL-12, MBT2/IL-18 and MBT2/Both were completely rejected when they were injected subcutaneously or intravenously into syngeneic mice. However, MBT2/IL-12, but not MBT2/IL-18, could grow in nude mice. Moreover, the antitumor effect of MBT2/IL-18 was partially abrogated when injected into nude mice of which NK cells were depleted by antibody treatment. MBT2/Both was completely rejected in both nude mice with and without NK cells. T cells and NK cells seem to play important roles in

the antitumor effects by the secretion of IL-12 and IL-18, respectively, and MBT2/Both possesses both mechanisms.

Hinata, N., T. Shirakawa, et al. (2003). "Radiation induces p53-dependent cell apoptosis in bladder cancer cells with wild-type- p53 but not in p53-mutated bladder cancer cells." *Urol Res* **31**(6): 387-96.

It has been reported in several studies that the absence in cancer cells of the p53 tumor suppressor gene, mutations of which are frequently found in bladder cancer, increases their resistance to ionizing radiation. Other studies, however, suggest that mutations of the p53 gene could increase the radiosensitivity of cancer cells, although the evidence is still inconclusive. In the present study, we investigated the relationship between p53 status and radiation response in five different bladder cancer cell lines. 5 different human bladder cancer cell lines (KK47: with wt- p53, RT4: with wt- p53, T24: with mutated p53, 5637: with mutated p53, UM-UC-3: with mutated p53) were used in the study. Cells were irradiated with 0, 2, 4, 6 or 8 Gy, then trypsinized and re-plated for clonogenic survival assay, quantitative RT-PCR assay, flow-cytometry analysis and TUNEL assay. Results. The clonogenic assay demonstrated that KK47 and RT4 had significantly higher radiosensitivity than other cell lines. Quantitative RT-PCR analysis showed that radiation induced increased expression of p53, Bax, and p21 mRNA in KK47 and RT4. After irradiation, G1 cell-cycle arrest was observed in KK47 and RT4 under flow cytometry analysis, while T24, 5637, and UM-UC-3 showed an increase in the proportion of G2 cells. Increased cell apoptosis was also observed under TUNEL assay in KK47 and RT4, but not in other cell lines. It was demonstrated that ionizing radiation induces p53-dependent cell apoptosis in bladder cancer cells with wt- p53 but not in those with mutated p53.

Hinkel, A., S. Schmidtchen, et al. (2008). "Identification of bladder cancer patients at risk for recurrence or progression: an immunohistochemical study based on the expression of metallothionein." *J Toxicol Environ Health A* **71**(13-14): 954-9.

Despite similarities in tumor stage and grade the individual outcome of bladder cancer patients is not predictable. The ideal tool for treatment stratification has not yet been found. Metallothionein (MT) overexpression is correlated with poor tumor differentiation, resistance to chemotherapy, and impaired survival in different malignancies. The clinical relevance of MT expression for defining patients at high risk for recurrence or progression was assessed. MT was detected immunohistochemically and evaluated semiquantitatively in tumor specimens of

103 male and 19 female patients. Mean age of the patients was 68 (38-87) yr. According to histopathological features, three groups were distinguished for further analysis (pTa-1G1-2, pTis/pT1G3, and muscle invasive tumors). A cutoff value of 50% immunoreactive cells was used for further analysis. The 5-yr tumor specific survival rate was significantly lower in patients with high MT expression (32 vs. 72%). Thus, MT expression seems to be a promising marker for further risk stratification in the clinical treatment of bladder cancer patients.

Homma, I., H. Kitamura, et al. (2009). "Human leukocyte antigen class I down-regulation in muscle-invasive bladder cancer: its association with clinical characteristics and survival after cystectomy." *Cancer Sci* **100**(12): 2331-4.

Using a novel monoclonal anti-pan human leukocyte antigen (HLA) class I heavy chain antibody (EMR 8-5) reacting with paraffin-embedded sections, we examined the prognostic significance of HLA class I molecules in muscle-invasive bladder cancer patients who underwent radical cystectomy. Immunohistochemical staining for HLA class I molecules with monoclonal antibody EMR 8-5 was performed on specimens from 65 clinically muscle-invasive bladder cancer patients who underwent radical cystectomy and pelvic lymph node dissection without neoadjuvant chemotherapy. We analyzed the clinicopathological and prognostic significance of HLA class I expression. Immunohistochemical analysis revealed HLA class I down-regulation in 22 (33.8%) invasive bladder cancers. This down-regulation had no correlation with clinicopathological parameters such as pathologic stage, nodal status, and grade. The recurrence-free survival of patients with HLA class I-positive tumors was significantly better than that of those with down-regulation (log rank, $P = 0.0337$). Multivariate analysis revealed that HLA class I expression was a significant factor influencing the recurrence-free survival of bladder cancer patients after cystectomy ($P = 0.0155$). Our data demonstrate that HLA class I down-regulation in tumor cells was clearly observed in about one-third of the patients. HLA class I expression could be a prognostic marker for muscle-invasive bladder cancer patients after cystectomy.

Horiguchi, Y., K. Kuroda, et al. (2003). "Antitumor effect of a novel nuclear factor-kappa B activation inhibitor in bladder cancer cells." *Expert Rev Anticancer Ther* **3**(6): 793-8.

Nuclear factor (NF)-kappaB is a transcription factor that not only induces and controls various genes, including those of inflammatory cytokines, but also activates genes which suppress apoptosis. It has

been clearly demonstrated that certain advanced human bladder cancer cells constitutively acquire the ability to activate NF-kappaB, which not only protects cancer cells from apoptotic cell death, but also upregulates the production of various cytokines that may increase the malignant potential of the disease and cause paraneoplastic syndromes. The NF-kappaB inhibitors may therefore be useful as anticancer agents. An NF-kappaB function inhibitor, a dehydroxymethyl derivative of epoxyquinomicin C (DHMEQ), has recently been designed and synthesized. The effectiveness of DHMEQ against advanced human bladder cancer cell line KU-19-19, in which NF-kappaB is constitutively activated, has been investigated. The DNA-binding activity of NF-kappaB was completely inhibited following 2-6-h exposure to 10 microg/ml of DHMEQ. Marked levels of apoptosis were observed 48 h after DHMEQ administration. These results confirmed that NF-kappaB activation maintains the viability of KU-19-19 cells, that DHMEQ inhibited constitutively activated NF-kappaB, and, consequently, apoptosis was induced. However, it was still possible that DHMEQ caused apoptotic cell death through some other mechanism which has not yet been fully investigated. The authors conclude that DHMEQ could represent a new treatment strategy against advanced bladder cancer.

Horinaga, M., K. M. Harsch, et al. (2005). "Intravesical interleukin-12 gene therapy in an orthotopic bladder cancer model." *Urology* **66**(2): 461-6.

To evaluate the antitumor effect of intravesical cationic liposome-mediated interleukin-12 (IL-12) gene delivery in an orthotopic murine bladder cancer model, and to investigate the immunologic memory against tumors between IL-12 gene therapy and bacille Calmette-Guerin (BCG) therapy. Orthotopic murine bladder tumors were established by implanting 5×10^5 MBT-2 cells into the bladder of syngeneic female C3H mice. Intravesical IL-12 gene therapy was evaluated at varying doses: 0 microg (control) and 3, 5, and 10 microg ($n = 8$ for each group). Intravesical treatments were performed every 3 days and repeated six times beginning 5 days after tumor implantation. To compare the long-term, tumor-specific immunity between IL-12-treated mice ($n = 18$) and BCG-treated mice ($n = 20$), the animals surviving at day 60 and 10 new control mice were rechallenged with MBT-2 cells and received no additional treatment. On day 120, all surviving mice were killed and underwent necropsy. In the IL-12 groups at doses of 0, 3, 5, and 10 microg, 0, 2, 3, and 3 mice survived, respectively. Mice in the 5-microg and 10-microg IL-12 groups survived significantly longer than did the control group. All mice cured by

IL-12 treatment successfully rejected the rechallenge with MBT-2 cells; however, mice cured by BCG and the new control mice died of the rechallenged bladder tumors. Intravesical IL-12 gene therapy, which induced long-lasting tumor-specific immunologic memory compared with BCG therapy, improved survival in an orthotopic bladder cancer model.

Hou, J. Q., J. He, et al. (2006). "Effect of small interfering RNA targeting survivin gene on biological behaviour of bladder cancer." *Chin Med J (Engl)* **119**(20): 1734-9.

Bladder cancer is the most common type of urinary system tumours. It is frequently associated with genetic mutations that deregulate the cell cycle and render these tumours resistant to apoptosis. Survivin, a newly discovered member inhibitor of apoptosis protein (IAP) family in several human cancers, by inducing cell proliferation and inhibiting apoptosis is frequently activated in bladder cancer. We studied the influence of small interfering RNA (siRNA) targeting survivin on the biological behaviour of bladder cancer cells. A double strand survivin target sequence specific siRNA was designed and synthesized. After transfection of bladder cancer cell line T24 by siRNA/liposome complex with increasing concentrations (50200 nmol/L), the transfectant cells were intratumourally injected at different doses (5 microg or 50 microg). The effects were measured in vitro and in vivo. The selected siRNA efficiently down-regulated survivin mRNA expression in a dose and time dependent manner. The maximal effect was achieved at the concentration of 100 nmol/L, at which survivin expression level was down-regulated by 75.91%. The inhibition rate of cell growth was 55.29% ($P < 0.01$) and the markedly increased apoptotic rate was 45.70% ($P < 0.01$). In vivo intratumoural injection of 50 microg siRNA-survivin could notably prevent the growth of bladder cancer ($P < 0.01$) in xenografted animals. CONCLUSION: The application of siRNA-survivin could markedly inhibit survivin expression in bladder cancer cell line by inducing apoptosis and inhibiting the growth of the tumour. It may become a new gene therapy tool for bladder cancer.

Hsieh, J. L., C. L. Wu, et al. (2003). "Gene therapy for bladder cancer using E1B-55 kD-deleted adenovirus in combination with adenoviral vector encoding plasminogen kringle 1-5." *Br J Cancer* **88**(9): 1492-9.

Mutations or loss of heterozygosity of p53 are detected in approximately 50% of bladder cancers. E1B-55 kD-deleted adenovirus has been shown to kill tumour cells with defective p53 function while sparing normal cells. Here, we examined the cytolytic effect and replication of E1B-55 kD-deleted adenovirus,

designated Ad5WS1, on human bladder cancer cell lines with various p53 status. Ad5WS1 caused more severe cytolytic effect and replicated more efficiently in J82 and TCC-SUP bladder cancer cells carrying mutant p53 compared with TSGH-8301 and BFTC-905 bladder cancer cells retaining wild-type p53. Introduction of dominant negative p53 into BFTC-905 cells rendered them more susceptible to Ad5WS1-induced cytolysis. Furthermore, cells susceptible to lysis caused by Ad5WS1 were not attributable to their greater infectability by adenovirus. Finally, Ad5WS1 suppressed the growth of TCC-SUP bladder tumour xenografts, which could be augmented when combined with replication-defective adenoviral vector encoding kringles 1-5 of plasminogen (K1-5), an angiogenic inhibitor. Taken together, our results show that E1B-55 kD-deleted adenovirus replicates and hence lyses bladder cancer cells with mutant p53 much more efficient than those with wild-type p53. Thus, E1B-deleted adenovirus may have therapeutic potential, especially in combination with adenoviral vector expressing K1-5, for the treatment of bladder cancer.

Iliopoulos, D., G. Guler, et al. (2005). "Fragile genes as biomarkers: epigenetic control of WWOX and FHIT in lung, breast and bladder cancer." *Oncogene* **24**(9): 1625-33.

This study aimed to (a) determine if DNA methylation is a mechanism of WWOX (WW domain containing oxidoreductase) and FHIT (fragile histidine triad) inactivation in lung, breast and bladder cancers; (b) examine distinct methylation patterns in neoplastic and adjacent tissues and (c) seek correlation of methylation patterns with disease status. Protein expression was detected by immunohistochemistry, and methylation status by methylation-specific PCR (MSP) and sequencing, in lung squamous cell carcinomas and adjacent tissues, invasive breast carcinomas, adjacent tissues and normal mammary tissues and bladder transitional cell carcinomas. Wwox and Fhit expression was reduced in cancers in association with hypermethylation. Differential patterns of WWOX and FHIT methylation were observed in neoplastic vs adjacent non-neoplastic tissues, suggesting that targeted MSP amplification could be useful in following treatment or prevention protocols. WWOX promoter MSP differentiates DNA of lung cancer from DNA of adjacent lung tissue. WWOX and FHIT promoter methylation is detected in tissue adjacent to breast cancer and WWOX exon 1 MSP distinguishes breast cancer DNA from DNA of adjacent and normal tissue. Differential methylation in cancerous vs adjacent tissues suggests that WWOX and FHIT hypermethylation analyses could enrich a panel of DNA methylation markers.

Inoue, M., K. Tomizawa, et al. (2006). "p53 protein transduction therapy: successful targeting and inhibition of the growth of the bladder cancer cells." *Eur Urol* **49**(1): 161-8.

Virus-mediated gene therapy for bladder cancer has some problems, such as efficiency of gene delivery and safety issues. Inoue et al have reported that poly-arginine peptide (11R) has the ability to increase protein transduction in cells. Here, Inoue et al show that p53 protein transduction using 11R is useful for targeting to bladder tumors and suppressing the growth of bladder cancer cells. An 11R-fused p53 protein (11R-p53) was transduced in bladder cancer cell lines (J82 and T24) to evaluate the anti-tumor effect. Cell viability was assessed by performing the 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate (WST) assay. To investigate whether 11R-p53 enhanced the effect on anti-cancer drug-dependent apoptosis of bladder cancer cells, the cell lines were cotreated with 11R-p53 and cis-diaminedichloroplatinum (CDDP). Apoptotic cells were identified using Hoechst staining. To investigate the efficiency of protein transduction mediated by 11R in bladder tumors in vivo, SCID mice were transplanted with J82 cells in the bladder and 11R-GFP was transurethrally transduced into the bladder. The transduction of 11R-GFP in the tumor was examined by confocal microscopy. 11R-p53 inhibited the growth of both J82 and T24 cells in a dose-dependent manner. The transduction of 11R-p53 enhanced CDDP-dependent induction of apoptosis. Transurethral application of 11R-GFP resulted in transduction of GFP in bladder tumors but not in the normal bladder epithelium or subepithelial tissues. The results suggest that p53 protein transduction therapy may be a promising method for the treatment of bladder cancer.

Inoue, R., H. Matsuyama, et al. (2006). "Gefitinib-related gene signature in bladder cancer cells identified by a cDNA microarray." *Anticancer Res* **26**(6B): 4195-202.

Gefitinib significantly inhibited the proliferation of 5637 cells, while showing little inhibitory effect on T24 cells. These effects were independent of the mutation status and protein levels of EGFR. cDNA microarray analysis identified 15 feature genes classified as a cell cycle, apoptotic pathway and transcription. Notably, levels of expression of the cell invasion-related genes, YY1 and E-cadherin, were increased in 5637 cells sensitive to gefitinib. Unique genes involved in the action of gefitinib were identified. Particularly, the upregulation of YY1 and E-cadherin may account for the efficacy of gefitinib in bladder cancer.

Irie, A. (2003). "Advances in gene therapy for bladder cancer." *Curr Gene Ther* **3**(1): 1-11.

The efficacy of various currently available therapeutic strategies for bladder cancer is not always sufficient, especially for the advanced disease, recurrent superficial cancer, and treatment-resistant carcinoma in situ. Advances in genetic and molecular biology have led to novel approaches for cancer treatment. Gene therapy is currently one of the most promising strategies against various malignancies, and several clinical trials have been approved worldwide. Various strategies for modulating the genetic state have been applied in bladder cancer treatment, and encouraging results have been demonstrated both in vitro and in vivo. Although the therapeutic genes work dramatically when the transgenes are effectively expressed in the targeted cells, however, a sufficient rate of transduction cannot always be achieved. The most significant obstacle for clinical application of cancer gene therapy might be the method for sufficient delivery and expression of the therapeutic genes. Bladder is an easily accessible organ because of its anatomy; however, a glycosaminoglycan (GAG) layer on the bladder mucosa may protect integration of exo-delivered genetic vectors. Various strategies are applied for improving the transduction efficacy of the therapeutic genes into the bladder cancer cells. These strategies include the modification of adenoviral fibers, cotransduction of the materials for enhancing the viral infectivity, and disruption of the GAG layer. Recent advances in the field of gene therapy for bladder cancer are briefly summarized in this review.

Irie, A., K. Matsumoto, et al. (2006). "Growth inhibition efficacy of an adenovirus expressing dual therapeutic genes, wild-type p53, and anti-erbB2 ribozyme, against human bladder cancer cells." *Cancer Gene Ther* **13**(3): 298-305.

The altered expression of both p53 and erbB2 is strongly related to the disease status and the outcome of bladder cancers. We examined the antitumor efficacy by the modulation of these genetic alterations with a newly designed dual-gene-expressing adenovirus (Ad-p53/erbB2Rz), which expresses p53 and anti-erbB2 ribozyme simultaneously in human bladder cancer cells. Cell growth inhibition efficacy along with biological responses of this virus was compared with other viral vectors (Ad-p53, which expresses wild-type p53 cDNA, and Ad-erbB2Rz, which expresses anti-erbB2 ribozyme, solely or in combination). Sufficient transgene expression in targeted cells and the altered expression of the targeted genes and their encoded proteins were obtained by each therapeutic vector. Each of the three therapeutic viral vectors inhibited

bladder cancer cell growth, and the putative additive antitumor effect was shown by the combination of two of the therapeutic vectors. Furthermore, Ad-p53/erbB2Rz had superior therapeutic efficacy when the same titers of viruses were infected. Nonspecific vector-related toxicity was minimized by reducing the total amount of viral titers by using the dual-gene-expressing adenovirus. Modulation of multiple genetic abnormalities might enhance the therapeutic efficacy, and vector-related toxicity could be minimized when the total amount of viral titers are reduced.

Ito, M., T. Habuchi, et al. (2004). "Polymorphism within the cyclin D1 gene is associated with an increased risk of carcinoma in situ in patients with superficial bladder cancer." *Urology* **64**(1): 74-8.

To evaluate the prognostic value of CCND1 polymorphism in superficial and invasive transitional cell cancer of the bladder. CCND1 polymorphism of blood DNA from patients with transitional cell cancer of the bladder was evaluated using the polymerase chain reaction-restriction fragment length polymorphism method. No statistically significant difference was found in the recurrence-free survival of patients with superficial (pTa-T1) transitional cell cancer after transurethral resection among different genotype groups (AA versus GG, $P = 0.746$; GA versus GG, $P = 0.979$). In patients with superficial bladder cancer, the occurrence of primary carcinoma in situ was significantly greater in patients with the AA genotype compared with those with the GA or GG genotypes ($P = 0.006$, chi-square test). No statistically significant difference was found in disease-specific survival after radical cystectomy among the different genotype subgroups (AA versus GG, $P = 0.245$; GA versus GG, $P = 0.649$). Although CCND1 polymorphism is not able to serve as a prognostic marker for bladder cancer, the CCND1 variant A allele may recessively increase the risk of carcinoma in situ incidence in patients with superficial bladder cancer.

Ito, M., H. Nishiyama, et al. (2007). "P21-activated kinase 1: a new molecular marker for intravesical recurrence after transurethral resection of bladder cancer." *J Urol* **178**(3 Pt 1): 1073-9.

It is clinically important to identify bladder cancers with a high risk of intravesical recurrence after transurethral bladder tumor resection. We developed molecular markers for predicting intravesical recurrence of superficial bladder transitional cell carcinoma using oligo-microarray analysis. Gene expression profiles associated with intravesical recurrence were analyzed by oligo-microarray in 27 superficial bladder transitional cell carcinoma samples from cases treated with

transurethral resection between 2000 and 2004 at Kyoto University Hospital. Of candidate genes the expression of P21-activated kinase (Pak1) was validated by semiquantitative real-time polymerase chain reaction using another set of samples and immunohistochemistry. Furthermore, Pak1 functions in bladder cancer cells were analyzed by the transfection of constitutively active (T423E) or kinase dead (K299R) Pak1. Microarray identified 25 genes whose expression was associated with recurrence, including Pak1. Pak1 mRNA expression was statistically associated with grade and the risk of recurrence but not with stage in 86 bladder cancers. Immunohistochemistry and multivariate analysis demonstrated that high Pak1 protein expression was an independent factor associated with recurrence (relative risk 2.27, $p = 0.008$). High Pak1 expression was significantly associated with a high risk of recurrence even in low stage/grade cancers. Transfection with T423E Pak1 into 253J cells progressed cell motility on wound healing assay, whereas transfection with K299R Pak1 decreased EJ cell motility. These results suggest that Pak1 expression is associated with recurrence and it might be a useful prognostic marker for superficial bladder transitional cell carcinoma.

Junttila, T. T., M. Laato, et al. (2003). "Identification of patients with transitional cell carcinoma of the bladder overexpressing ErbB2, ErbB3, or specific ErbB4 isoforms: real-time reverse transcription-PCR analysis in estimation of ErbB receptor status from cancer patients." *Clin Cancer Res* 9(14): 5346-57.

The purpose of this research was to quantitatively analyze tumor-specific overexpression of all ErbB receptors and ErbB4 isoforms in transitional cell carcinoma (TCC) of the bladder. A real-time reverse transcription-PCR protocol was set up to simultaneously quantitate the mRNA levels of all four of the ErbB receptors and ErbB4 isoforms. Exon-intron structure of the ErbB4 gene was determined for ErbB4 isoform analysis. The assay was validated by analyzing: (a) defined ErbB cDNAs; (b) cell lines transfected with defined ErbB cDNAs; and (c) cancer cell lines with ErbB status controlled by Western blotting. ErbB mRNA expression was quantitated from 29 clinical samples representing TCC, interstitial cystitis, or histologically normal bladder. Cutoff expression levels predicting neoplasia at 95% probability were determined. ErbB expression and amplification was analyzed by immunohistochemistry and chromogenic in situ hybridization. Experiments with control cDNAs and cell lines demonstrated that the assay was both specific and sensitive, and that ErbB mRNA levels closely correlated with protein levels in cancer cell

lines. Determination of cutoff expression levels indicated tumor-specific overexpression of ErbB2, ErbB3, and specific ErbB4 isoforms in a subset of TCC patients. Significant overexpression of ErbB mRNAs was also detected in cases without amplification of the respective gene or when the protein product was not localized at the cell membrane. Bladder cancer patients with tumor-specific overexpression of ErbB receptors or their isoforms were identified. Real-time reverse transcription-PCR could be used for ErbB receptor status quantitation to produce prognostic and predictive information for cancer therapy.

Kamada, M., A. So, et al. (2007). "Hsp27 knockdown using nucleotide-based therapies inhibit tumor growth and enhance chemotherapy in human bladder cancer cells." *Mol Cancer Ther* 6(1): 299-308.

Heat shock protein 27 (Hsp27) is a cytoprotective chaperone that is phosphoactivated during cell stress that prevents aggregation and/or regulate activity and degradation of certain client proteins. Recent evidence suggests that Hsp27 may be involved in tumor progression and the development of treatment resistance in various tumors, including bladder cancer. The purpose of this study was to examine, both in vitro and in vivo, the effects of overexpression of Hsp27 and, correspondingly, the down-regulation of Hsp27 using small interfering (si) RNA and OGX-427, a second-generation antisense oligonucleotide targeting Hsp27. Hsp27 overexpression increased UMUC-3 cell growth and resistance to paclitaxel. Both OGX-427 and Hsp27 siRNA decreased Hsp27 protein and mRNA levels by >90% in a dose- and sequence-specific manner in human bladder cancer UMUC-3 cells. OGX-427 or Hsp27 siRNA treatment induced apoptosis and enhanced sensitivity to paclitaxel in UMUC-3 cells. In vivo, OGX-427 significantly inhibited tumor growth in mice, enhanced sensitivity to paclitaxel, and induced significantly higher levels of apoptosis compared with xenografts treated with control oligonucleotides. Collectively, these findings suggest that Hsp27 knockdown with OGX-427 and combined therapy with paclitaxel could be a novel strategy to inhibit the progression of bladder cancer.

Kamat, A. M. and D. L. Lamm (2004). "Antitumor activity of common antibiotics against superficial bladder cancer." *Urology* 63(3): 457-60.

The recurrence rate for superficial bladder tumors treated with complete resection averages 88%. Intravesical chemotherapy decreases the recurrence rate by only 14%; thus, new chemotherapeutic agents are needed. Antibiotics are often used to prevent infections after transurethral resection of bladder

tumors. Oral intake of antibiotics results in significantly greater concentrations in the urine than in the serum. Kamat et al evaluate four commonly used urinary antibiotics for their cytotoxic activity against bladder cancer cells at clinically relevant concentrations. Three human transitional cell carcinoma lines--HTB9 (grade 2), T24 (grade 3), and TccSup (grade 4)--were exposed to ciprofloxacin, trimethoprim-sulfamethoxazole, cefazolin, or nitrofurantoin at concentrations from 0 (control) to 1000, 1000, 5000, and 2000 microg/mL, respectively, for 96 hours. Cytotoxicity was evaluated using the MTT colorimetric assay. Six replicates were used for each data point, and the results are reported as the mean +/- standard deviation. Significant cytotoxicity ($P < 0.001$) was seen, starting at 12.5 microg/mL (HTB9, TccSup) and 50 microg/mL (T24) for ciprofloxacin, 31.25 microg/mL (HTB9, TccSup) and 62.5 microg/mL (T24) for trimethoprim-sulfamethoxazole, 19.5 microg/mL (HTB9) and 156.3 microg/mL (T24, TccSup) for cefazolin, and 7.8 microg/mL (HTB9, T24, TccSup) for nitrofurantoin. Cytotoxicity was dose dependent for all four antibiotics, and the maximal effect did not differ among antibiotics. Commonly used antibiotics exhibit significant dose-dependent cytotoxicity against bladder cancer cells at concentrations achievable in the urine after oral administration. The administration of antibiotics after transurethral resection of bladder tumors might prevent seeding of cancer cells and thereby decrease the recurrence rate. Preclinical data such as these must be considered in the design of clinical trials addressing recurrence after transurethral resection of bladder tumors.

Kamat, A. M., G. Sethi, et al. (2007). "Curcumin potentiates the apoptotic effects of chemotherapeutic agents and cytokines through down-regulation of nuclear factor-kappaB and nuclear factor-kappaB-regulated gene products in IFN-alpha-sensitive and IFN-alpha-resistant human bladder cancer cells." *Mol Cancer Ther* 6(3): 1022-30.

Bladder cancer mortality varies between the countries; whereas being highest in Western countries, it is lowest in Eastern countries, such as India. Cigarette smoking is one of the major risk factors for bladder cancer in affluent nations, such as United States. Localized early-stage bladder cancer is treated with resection and intravesical cytokine therapy, whereas metastatic cancer is typically treated with various combinations of systemic chemotherapy. Whether curcumin, a yellow curry pigment commonly consumed in countries, such as India, has any role in prevention or treatment of bladder cancer was investigated. We found that curcumin inhibited the proliferation, induced cell cycle arrest, and DNA

fragmentation in both IFN-alpha-sensitive (RT4V6) and IFN-alpha-resistant (KU-7) bladder cancer cells. Curcumin also potentiated the apoptotic effects of the chemotherapeutic agents (gemcitabine and paclitaxel) and of cytokines [tumor necrosis factor (TNF) and TNF-related apoptosis-inducing ligand]. This effect of curcumin was independent of sensitivity and resistance to IFN-alpha, commonly used for treatment of bladder cancer. Whether the effects of curcumin are mediated through modulation of the nuclear factor-kappaB (NF-kappaB) pathway known to mediate antiapoptosis was investigated. Both gemcitabine and TNF activated NF-kappaB in bladder cancer cells and curcumin suppressed this activation. Similarly, cigarette smoke, a major risk factor for bladder cancer, also activated NF-kappaB and curcumin suppressed it. Cigarette smoke-induced expression of the NF-kappaB-regulated gene products cyclooxygenase-2 and vascular endothelial growth factor, linked with proliferation and angiogenesis, respectively, was also down-regulated by curcumin.

Kanehira, M., T. Katagiri, et al. (2007). "Oncogenic role of MPHOSPH1, a cancer-testis antigen specific to human bladder cancer." *Cancer Res* 67(7): 3276-85.

To disclose the molecular mechanism of bladder cancer, the second most common genitourinary tumor, we had previously done genome-wide expression profile analysis of 26 bladder cancers by means of cDNA microarray representing 27,648 genes. Among genes that were significantly up-regulated in the majority of bladder cancers, we here report identification of M-phase phosphoprotein 1 (MPHOSPH1) as a candidate molecule for drug development for bladder cancer. Northern blot analyses using mRNAs of normal human organs and cancer cell lines indicated this molecule to be a novel cancer-testis antigen. Introduction of MPHOSPH1 into NIH3T3 cells significantly enhanced cell growth at in vitro and in vivo conditions. We subsequently found an interaction between MPHOSPH1 and protein regulator of cytokinesis 1 (PRC1), which was also up-regulated in bladder cancer cells. Immunocytochemical analysis revealed colocalization of endogenous MPHOSPH1 and PRC1 proteins in bladder cancer cells. Interestingly, knockdown of either MPHOSPH1 or PRC1 expression with specific small interfering RNAs caused a significant increase of multinuclear cells and subsequent cell death of bladder cancer cells. Our results imply that the MPHOSPH1/PRC1 complex is likely to play a crucial role in bladder carcinogenesis and that inhibition of the MPHOSPH1/PRC1 expression or their interaction should be novel therapeutic targets for bladder cancers.

Karam, J. A., J. Fan, et al. (2007). "The use of histone deacetylase inhibitor FK228 and DNA hypomethylation agent 5-azacytidine in human bladder cancer therapy." *Int J Cancer* **120**(8): 1795-802.

The long-term disease-free survival in patients with metastatic transitional cell carcinoma (TCC) is still considerably low. Novel chemotherapeutic agents are needed to decrease the morbidity and mortality of TCC. In this study, we have evaluated several epigenetic modifiers for their therapeutic application in bladder cancer. Both histone deacetylase inhibitors (FK228, TSA) and DNA hypomethylating agent (5-Azacytidine) were tested using in vitro assays such as cell viability, cell cycle analysis and western blot to determine their mechanisms of action. Drug combination experiments were also designed to study any additive or synergistic effects of these agents. In addition, two bladder cancer xenograft models (one subcutaneous and one orthotopic) were employed to assess the therapeutic efficacy of these agents in vivo. Three agents exhibited various growth inhibitory effects on 5 different TCC cell lines in a dose- and time-dependent manner. In addition to G2/M cell cycle arrest, FK228 is more potent in inducing apoptosis than the two other single agents, and combination of both FK228 and 5-Aza further enhances this effect. p21 induction is closely associated with FK228 or TSA but not 5-Aza, which is mediated via p53-independent pathway. Consistent with in vitro results, FK228 exhibited a significant in vivo growth inhibition of TCC tumor in both subcutaneous and orthotopic xenograft models. FK228 is a potent chemotherapeutic agent for TCC in vivo with minimal undesirable side effects. The elevated p21 level mediated via p53 independent pathway is a hallmark of FK228 mechanism of action.

Karashima, T., P. Sweeney, et al. (2003). "Nuclear factor-kappaB mediates angiogenesis and metastasis of human bladder cancer through the regulation of interleukin-8." *Clin Cancer Res* **9**(7): 2786-97.

Interleukin (IL)-8 is an important mediator of angiogenesis, tumorigenicity, and metastasis in transitional cell carcinoma (TCC) of the bladder. Nuclear factor kappaB (NF-kappaB)/relA regulates IL-8 expression in several neoplasms. The purpose of this study was to determine whether the organ microenvironment (hypoxia, acidosis) regulates the expression of IL-8 in TCC via NF-kappaB, and whether inhibition of NF-kappaB function by mutant IkappaB-alpha prevents induction of IL-8 expression. IL-8 mRNA expression and protein production by human TCC cell lines (UM-UC-14, HTB-9, RT-4, KU-7 and 253J B-V) were measured by Northern blot analysis and ELISA under acidic (pH 7.35-6.0) and

hypoxic (1.0% O₂) conditions. The involvement of NF-kappaB and activator protein 1 in the regulation of IL-8 production was evaluated by electrophoretic mobility shift assay. Furthermore, the tumorigenicity and metastatic potential of UM-UC-14 cells were determined after transfection with mutant IkappaB-alpha. We found that acidic and hypoxic conditions increased IL-8 mRNA expression and protein production by several, but not all, TCC cell lines evaluated. NF-kappaB, but not activator protein 1, was inducibly activated in UM-UC-14 under both acidic and hypoxic conditions, but not in UM-UC-14 mutant IkappaB-alpha transfectants. Tumor growth and lymph node metastasis were inhibited in UM-UC-14 mutant IkappaB-alpha transfectants compared with UM-UC-14 controls. This effect was associated with the inhibition of IL-8 production, cellular proliferation, and angiogenesis. The results suggest that TCCs of the bladder have heterogenic responses to physicochemical changes in the microenvironment and identify NF-kappaB as a potential molecular target for therapy.

Kasman, L. M., S. Barua, et al. (2009). "Polymer-enhanced adenoviral transduction of CAR-negative bladder cancer cells." *Mol Pharm* **6**(5): 1612-9.

The application of adenoviral gene therapy for cancer is limited by immune clearance of the virus as well as poor transduction efficiency, since the protein used for viral entry (CAR) serves physiological functions in adhesion and is frequently decreased among cancer cells. Cationic polymers have been used to enhance adenoviral gene delivery, but novel polymers with low toxicity are needed to realize this approach. We recently identified polymers that were characterized by high transfection efficiency of plasmid DNA and a low toxicity profile. In this study we evaluated the novel cationic polymer EGDE-3,3' for its potential to increase adenoviral transduction of the CAR-negative bladder cancer cell line TCCSUP. The amount of adenovirus required to transduce 50-60% of the cells was reduced 100-fold when Ad.GFP was preincubated with the EGDE-3,3' polymer. Polyethyleneimine (pEI), a positively charged polymer currently used as a standard for enhancing adenoviral transduction, also increased infectivity, but transgene expression was consistently higher with EGDE-3,3'. In addition, EGDE-3,3'-supplemented transduction of an adenovirus expressing an apoptosis inducing transgene, Ad.GFP-TRAIL, significantly enhanced the amount of cell death. Thus, our results indicate that novel biocompatible polymers may be useful in improving the delivery of adenoviral gene therapy.

Kawakami, T., H. Shiina, et al. (2004). "Inactivation of the hMSH3 mismatch repair gene in bladder cancer." *Biochem Biophys Res Commun* **325**(3): 934-42.

Deficiency in the DNA mismatch repair (MMR) is frequently involved in various cancers. The hMSH3 gene is one of the human MMR genes whose role in bladder cancer is not known. We hypothesized that down-regulation of the hMSH3 gene might be involved in bladder cancer. In this study we analyzed this gene with regard to frame-shift mutation, single nucleotide polymorphism (SNP), a 9bp repeat in exon 1, loss of heterozygosity (LOH), immunohistochemistry, and methylation status in 102 bladder cancer samples. Immunohistochemistry revealed that hMSH3 expression in bladder cancer was significant decreased compared to normal epithelium ($p < 0.0001$). An inverse correlation with pathological grade was found. The frame-shift mutation in the (A) 8 tract was lacking in bladder cancer. There was no significantly difference between bladder cancer samples and healthy controls' with regard to SNP and the 9bp repeat. In bladder cancer, presence of the codon 222 polymorphism, LOH, and the 9bp repeats in exon 1 had a correlation with either pathological stage or pathological grade. Presence of the codon 1036 polymorphism had significant correlation with pathological stage and a trend to correlation with pathological grade. After 5-aza-dC treatment, MSH3 expression was significantly enhanced in TCC and UMUC bladder cancer cells when compared to untreated cells. This is the first report suggesting that genetic and epigenetic alterations in the human MSH3 gene might play a significant role in the progression of bladder tumors.

Khin, S. S., R. Kitazawa, et al. (2009). "BAMBI gene is epigenetically silenced in subset of high-grade bladder cancer." *Int J Cancer* **125**(2): 328-38.

The bone morphogenetic protein (BMP) and activin membrane-bound inhibitor (BAMBI) is a transmembrane TGFRI/BMPRI-related pseudoreceptor, antagonizes transforming growth factor (TGF)-beta/BMP signaling by inhibiting the formation of functional authentic receptor complexes (TGFRI/BMPRI and TGFRII/BMPRII). On the assumption that BAMBI gene expression is epigenetically altered during human bladder cancer progression, we screened the expression of BAMBI protein by immunohistochemistry and the methylation status of the BAMBI promoter. In the normal or reactive urothelium, BAMBI expression was mostly overlapped with that of BMPRI, and a similar colocalization pattern was noted in low-grade papillary cancers. In high-grade and invasive cancers, however, mainly two reciprocal immunohistochemical

expression patterns were observed: BAMBI-low/BMPRI-high, and BAMBI-high/BMPRI-low, indicating that BAMBI expression is controlled such that it does not interfere with the responsiveness of high-grade cancer cells to TGF-beta/BMP signaling. Moreover, methylation of the BAMBI gene correlated significantly with negative BAMBI expression in bladder tumors. Although BAMBI overexpression significantly increased the number of apoptotic cells in T24 line, knock-down small interfering RNA showed no remarkable change. Cell motility assay revealed that on treatment with either TGF-beta1 or BMP2, T24 and HTB9 lines showed a marked increase in the number of migrated cells which, however, decreased significantly through the forced expression of BAMBI. Since certain subsets of aggressive tumors often promote cell motility, invasion and survival by inducing epithelial-to-mesenchymal transition through TGF-beta/BMP in an autocrine and paracrine manner, hypermethylation of the BAMBI gene promoter that leads to BAMBI gene suppression may be one of the epigenetic events affecting the invasiveness or aggressiveness of bladder cancers.

Kim, C. J., N. Yoshioka, et al. (2005). "Periostin is down-regulated in high grade human bladder cancers and suppresses in vitro cell invasiveness and in vivo metastasis of cancer cells." *Int J Cancer* **117**(1): 51-8.

In Kim et al examined the expression of periostin mRNA in normal bladder tissues, bladder cancer tissues and bladder cancer cell lines by Northern blot analysis and RT-PCR analysis. Although the expression of periostin mRNA was detected in 100% (5/5) of normal bladder tissues, it was not detected in 3 human bladder cancer cell lines examined. It was also detected in 81.8% (9/11) of grade 1, 40.0% (4/10) of grade 2 and 33.3% (4/12) of grade 3 bladder cancer tissues, indicating that downregulation of periostin mRNA is significantly related to higher grade bladder cancer ($p < 0.05$). To assess the tumor suppressor function of periostin, we investigated the ability of periostin gene to suppress malignant phenotypes of a bladder cancer cell line, SBT31A. Ectopic expression of periostin gene by a retrovirus vector suppressed in vitro cell invasiveness of the bladder cancer cells without affecting cell proliferation and tumor growth in nude mice. Periostin also suppressed in vivo lung metastasis of the mouse melanoma cell line, B16-F10. Mutational analysis revealed that the C-terminal region of periostin was sufficient to suppress cell invasiveness and metastasis of the cancer cells. Periostin may play a role as a suppressor of invasion and metastasis in the progression of human bladder cancers.

Kim, W. J. and Y. J. Kim (2009). "Epigenetic biomarkers in urothelial bladder cancer." *Expert Rev Mol Diagn* **9**(3): 259-69.

Bladder cancers comprise heterogeneous cell populations, and numerous factors are likely to be involved in dictating recurrence, progression and patient survival. While several molecular markers that are used to evaluate the development and prognosis of bladder cancer have been studied, the limited value of these established markers has created the need for new molecular indicators of bladder cancer prognosis. Of particular interest is the silencing of tumor-suppressor genes by epigenetic alteration. Recent progress in understanding epigenetic modification and gene silencing has led to new opportunities for the understanding, detection, treatment and prevention of cancer. Moreover, epigenetic silencing of tumor-suppressor genes is interesting from a clinical standpoint, because of the possibility of reversing epigenetic changes and restoring gene function in a cell. This review focuses on the prognostic relevance of epigenetic markers in bladder cancer.

Kim, Y. K. and W. J. Kim (2009). "Epigenetic markers as promising prognosticators for bladder cancer." *Int J Urol* **16**(1): 17-22.

Transitional cell carcinomas of the urinary bladder have diverse biological and functional characteristics. Surveillance strategies for bladder cancer recurrence have historically relied on the diagnostic combination of cystoscopy and urinary cytology. However, the accuracy of both tests depends on subjective and operator-dependent interpretations of the visible findings. In contrast, promoter hypermethylation of CpG islands is strongly associated with tumor development and prognosis of bladder cancer. Detection of DNA methylation in voided urine may be feasible and more sensitive than conventional urine cytology. Ultimately, all types of urological cancers may be screened in urine using a candidate panel of hypermethylated genes. The epigenetic silencing of tumor suppressor genes is interest from a clinical point of view because it is possible to reverse epigenetic changes and restore gene function to a cell. Methylation markers might therefore be more useful than conventional molecular markers for the treatment and prevention of bladder cancer.

Kimura, T., T. Ohashi, et al. (2003). "Antitumor immunity against bladder cancer induced by ex vivo expression of CD40 ligand gene using retrovirus vector." *Cancer Gene Ther* **10**(11): 833-9.

The interaction between CD40 ligand (CD40L) and CD40 on antigen-presenting cells is essential for the initiation of antigen-specific T-cell

responses. In order to clarify whether the expression of CD40L in tumor cells might be useful as a systemic therapy against bladder cancer, we investigated the antitumor immunity induced by CD40L in the mouse bladder cancer cell line MBT2. MBT2 was transduced by the retroviral vector expressing CD40L (MBT2-CD40L). Mouse bone marrow-derived dendritic cells cocultured with MBT2-CD40L cells produced eight times more IL-12 than those cocultured with parental MBT2 cells. In animal studies, subcutaneously inoculated MBT2-CD40L cells were rejected promptly. The vaccination of MBT2-CD40L cells induced antitumor immunity against parental tumors at a distant site. However, the antitumor effect of MBT2-CD40L inoculation was insufficient against pre-existing tumors. In the vaccination model, antibody ablation studies revealed that CD4(+) T cells were required for antitumor immunity, and tumor-specific cytotoxicity of sera was demonstrated. These data demonstrated that the antitumor immunity induced by CD40L was effective in the vaccination model and suggested that immunogene therapy using CD40L may be a new strategy of systemic therapy against bladder cancer.

Knowles, M. A. (2007). "Tumor suppressor loci in bladder cancer." *Front Biosci* **12**: 2233-51.

Large numbers of genetic and epigenetic alterations have been identified in bladder cancer in recent years. Many of these affect the function of tumour suppressor genes (TSGs), leading to partial or complete loss of protein expression or function with varied phenotypic consequences. Some of the genes implicated such as TP53 and RB1 are major players in many other tumour types. Others, particularly some on chromosome 9, show bladder-specific involvement. Other TSGs of relevance to bladder tumour development are predicted by the finding of common physical deletion or LOH in specific regions of the genome. This review summarises the approaches that have been used to identify bladder tumour suppressor genes, the current state of knowledge of the genes involved in this disease, their relationship with specific clinical features and some possible therapeutic applications.

Knowles, M. A., F. M. Platt, et al. (2009). "Phosphatidylinositol 3-kinase (PI3K) pathway activation in bladder cancer." *Cancer Metastasis Rev* **28**(3-4): 305-16.

The phosphatidylinositol 3-kinase (PI3K) pathway is a critical signal transduction pathway that regulates multiple cellular functions. Aberrant activation of this pathway has been identified in a wide range of cancers. Several pathway components including AKT, PI3K and mTOR represent potential

therapeutic targets and many small molecule inhibitors are in development or early clinical trials. The complex regulation of the pathway, together with the multiple mechanisms by which it can be activated, make this a highly challenging pathway to target. For successful inhibition, detailed molecular information on individual tumours will be required and it is already clear that different tumour types show distinct combinations of alterations. Recent results have identified alterations in pathway components PIK3CA, PTEN, AKT1 and TSC1 in bladder cancer, some of which are significantly related to tumour phenotype and clinical behaviour. Co-existence of alterations to several PI3K pathway genes in some bladder tumours indicates that these proteins may have functions that are not related solely to the known canonical pathway.

Kobayashi, T., M. Sakaguchi, et al. (2008). "Mechanistic analysis of resistance to REIC/Dkk-3-induced apoptosis in human bladder cancer cells." *Acta Med Okayama* **62**(6): 393-401.

The aim of the study by Kobayashi et al in this reference is to examine the sensitivity of bladder cancers to Ad-REIC and to clarify the molecular mechanisms that determine sensitivity/resistance. Kobayashi et al found that 2 human bladder cancer cell lines, T24 and J82, are resistant to Ad-REIC. In T24 and J82 cells, the ER stress response and activation of JNK were observed in a manner similar to that in the sensitive PC3 cells. Translocation of Bax to mitochondria occurred in PC3 cells but not in T24 and J82 cells. Bcl-2 was remarkably overexpressed in T24 and J82 compared with the expression levels in sensitive cell lines. Treatment of T24 and J82 cells with a Bcl-2 inhibitor sensitized the cells to Ad-REIC-induced apoptosis. The results indicate that some human bladder cancers are resistant to apoptosis induced by overexpression of REIC/Dkk-3, which is at least in part due to up-regulation of Bcl-2. These results provide a basis for possible use of Bcl-2 as a marker of sensitive cancers and to try to sensitize resistant cancers to Ad-REIC by down-regulation of Bcl-2.

Kolla, S. B., A. Seth, et al. (2008). "Prognostic significance of Her2/neu overexpression in patients with muscle invasive urinary bladder cancer treated with radical cystectomy." *Int Urol Nephrol* **40**(2): 321-7.

The aim of the study was to evaluate the status of Her2/neu protein expression in patients with muscle-invasive urothelial carcinomas of the bladder treated with radical cystectomy and to determine its prognostic significance. Kolla et al retrospectively analyzed the data of 90 patients who had undergone

cystectomy for invasive transitional cell carcinoma of the urinary bladder. Immunohistochemical analysis for Her2/neu was done on paraffin-fixed tissues with CB11 antibodies (BioGenex, San Ramon, CA, USA). Sections with grade 2 and grade 3 staining were considered positive for Her2/neu. Over a median follow-up period of 46 months (24-96 months) 46 patients are living without disease recurrence and six with recurrent disease either at the local site or with distant metastases. The remaining 38 patients have died. The median overall survival time was 50 months, and median disease-free survival time was 40 months. The Her2/neu status was significantly related to the tumor stage ($P = 0.001$), lymph node involvement (77% in N+ vs 23% in N0; $P = 0.001$) and the grade of the disease (32% of grade 2 vs 71% of grade 3; $P = 0.037$). Kaplan-Meier curves showed a significantly worse disease-related survival period (log rank $P = 0.011$) for patients with Her2 overexpressing tumors than for those without overexpression. In addition to tumor stage [$P = 0.001$; relative risk (RR) = 2.62] and lymph node status ($P = 0.0001$; RR = 2.95), Her2 status ($P = 0.020$; RR = 2.22) was identified as an independent predictor for disease-related survival in a multivariate analysis. These results suggest that Her2 expression might provide additional prognostic information for patients with muscle-invasive bladder cancer. Future studies on Her2 expression with chemosensitivity and the efficacy of Her2-targeted therapies in urothelial carcinomas are warranted.

Konstantakou, E. G., G. E. Voutsinas, et al. (2009). "Human bladder cancer cells undergo cisplatin-induced apoptosis that is associated with p53-dependent and p53-independent responses." *Int J Oncol* **35**(2): 401-16.

Cisplatin is a first-line chemotherapeutic agent and a powerful component of standard treatment regimens for several human malignancies including bladder cancer. DNA-Pt adducts produced by cisplatin are mainly responsible for cellular toxicity and induction of apoptosis. Identification of the mechanisms that control sensitivity to cisplatin is central to improving its therapeutic index and to successfully encountering the acquired resistance frequently emerging during therapy. In the present study, using MTT-based assays, Western blotting and semi-quantitative RT-PCR, we examined the apoptosis-related cellular responses to cisplatin exposure in two human urinary bladder cancer cell lines characterized by different malignancy grade and p53 genetic status. Both RT4 (grade I; wild-type p53) and T24 (grade III; mutant p53) cell types proved to be vulnerable to cisplatin apoptotic activity, albeit in a grade-dependent and drug dose-specific manner, as

demonstrated by the proteolytic processing profiles of Caspase-8, Caspase-9, Caspase-3, and the Caspase repertoire characteristic substrates PARP and Lamin A/C, as well. The differential resistance of RT4 and T24 cells to cisplatin-induced apoptosis was associated with an RT4-specific phosphorylation (Ser15; Ser392) pattern of p53, together with structural amputations of the Akt and XIAP anti-apoptotic regulators. Furthermore, cisplatin administration resulted in a Granzyme B-mediated proteolytic cleavage of Hsp90 molecular chaperone, exclusively occurring in RT4 cells. To generate functional networks, expression analysis of a number of genes, including Bik, Bim, Bcl-2, FAP-1, Fas, FasL, TRAIL, Puma, Caspase-10, ATP7A, ATP7B and MRP1, was performed, strongly supporting the role of p53-dependent and p53-independent transcriptional responses in cisplatin-induced apoptosis of bladder cancer cells.

Kramer, M. W., M. A. Kuczyk, et al. (2008). "Decreased expression of galectin-3 predicts tumour recurrence in pTa bladder cancer." *Oncol Rep* **20**(6): 1403-8.

Galectin-3 (gal-3) is a glycoprotein involved in various physiological cellular processes. Altered expression/loss of function of gal-3 is suggested to be involved in the pathogenesis and further progression of various human cancer entities. The aim of the present investigation was to elucidate the role of galectin-3 in the development and/or progression of non-muscle invasive (pTa, pT1) transitional cell carcinoma (TCC) of the urinary bladder. Gal-3 was analyzed by immunohistochemistry in 162 randomly selected non-muscle invasive bladder cancer specimens (pTa, 91; pT1, 71) using tissue microarray technique. It was compared with various patient and tumour characteristics (t-test). In addition, the role of gal-3 in association with tumour recurrence and progression was investigated (Log-rank test, Cox regression analysis). Gal-3 was found to be negatively correlated with tumour grade ($p < 0.02$). Within the group of non-muscle invasive TCC, gal-3 could not differentiate between pTa and pT1 tumours ($p = 0.50$), and within the subgroup of pTa tumours, loss of gal-3 determined the likelihood for the development of recurrent disease ($p < 0.03$; Student's t-test). Furthermore, as demonstrated by Kaplan-Meier analysis, the expression level of gal-3 was identified to predict the duration of recurrence-free survival ($p = 0.01$). In the multivariate analysis, gal-3 was found as an independent prognostic marker for predicting recurrence among the cohort of bladder tumours classified as pTa. In conclusion, loss of galectin-3 appears to be involved in the carcinogenesis of TCC and to serve as a valuable biological variable to

identify a subgroup of Ta bladder cancer patients at high risk for the development of recurrent disease.

Kunze, D., D. Wuttig, et al. (2008). "Multitarget siRNA inhibition of antiapoptotic genes (XIAP, BCL2, BCL-X(L)) in bladder cancer cells." *Anticancer Res* **28**(4B): 2259-63.

The knockdown of XIAP, BCL2 and BCL-X(L) by siRNAs represents a promising treatment option for bladder cancer (BCa) since the overexpression of antiapoptotic genes is often associated with tumor progression and treatment resistance. EJ28 BCa cells were transfected with siRNAs--separately and combined--followed by analysis of target expression, viability, clonogenic survival, apoptosis and cell cycle. Furthermore, a possible chemosensitization by siRNA pretreatment was investigated. The siRNA-mediated inhibition of these targets--either separately or combined--reduced the targets' expression, reduced cell growth and sensitized cells to a subsequent chemotherapy. Since tumor cells may bypass the inhibition of a single gene by changing their expression profile, e.g. switch from BCL2 to BCL-X(L), the combined knockdown of multiple genes of the same pathway might be more effective in killing cancer cells. The siRNAs used represent appropriate tools for this aim since they reduced their targets' expression significantly and long-lastingly.

Kurahashi, T., I. Hara, et al. (2005). "Detection of micrometastases in pelvic lymph nodes in patients undergoing radical cystectomy for locally invasive bladder cancer by real-time reverse transcriptase-PCR for cytokeratin 19 and uroplakin II." *Clin Cancer Res* **11**(10): 3773-7.

The objective of this study was to clarify the significance of micrometastases in pelvic lymph nodes in patients who underwent radical cystectomy for bladder cancer. Kurahashi et al studied 40 patients with locally invasive bladder cancer who underwent radical cystectomy and pelvic lymphadenectomy. Expression of cytokeratin 19 (CK19), uroplakin II (UP II), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in 760 lymph nodes were assessed by a fully quantitative real-time reverse transcription-PCR (RT-PCR) assay. The quantification value of CK19 or UP II mRNA was described as each value relative to GAPDH mRNA. In this study, we regarded specimen in which either CK19 or UP II mRNA was positive as "presence of micrometastasis." Routine pathologic examinations detected tumor cells in 29 lymph nodes from six patients. Real-time RT-PCR identified positive expression of CK19 and UP II mRNAs in 49 lymph nodes from 10 patients and 98 lymph nodes from 16 patients, respectively. Of 633 lymph nodes

from 34 patients with no pathologic evidence of nodal involvement, 13 nodes from five patients and 58 nodes from 10 patients were diagnosed as positive for CK19 and UP II mRNAs expression, respectively, by real-time RT-PCR. Presence of micrometastases was significantly associated with other conventional prognostic variables, including pathologic stage and microvascular invasion. Disease recurrence was occurred in eight patients, among whom four patients were negative for lymph node metastasis by routine pathologic examination and diagnosed as having micrometastasis by real-time RT-PCR assay. Furthermore, cause-specific survival rate in patients without micrometastasis was significantly higher than that in those with micrometastasis, irrespective of the presence of pathologic-positive nodes. Approximately 30% of locally invasive bladder cancer shed cancer cells to pelvic lymph nodes, and disease recurrence after radical cystectomy could be explained, at least in part, by micrometastases in pelvic lymph nodes.

Lee, C. F., S. Y. Chang, et al. (2004). "Immunotherapy for bladder cancer using recombinant bacillus Calmette-Guerin DNA vaccines and interleukin-12 DNA vaccine." *J Urol* **171**(3): 1343-7.

Lee et al investigated the efficacy of recombinant bacillus Calmette-Guerin (BCG) DNA (poly-rBCG) and murine interleukin (IL)-12 (mIL-12) vaccines in inducing T helper 1 polarized cytokines and suppressing bladder tumor growth in mice. 4 mycobacteria candidate genes (Ag85A, Ag85B, Mpt64 and PstS3) were cloned, fused with ESAT6 and ligated into eukaryotic expression vectors. Combined poly-rBCG and mIL-12 vaccines were transferred into a murine bladder tumor model. The efficiency of gene expression was detected using Western blotting, flow cytometry and semiquantitative reverse transcriptase-polymerase chain reaction. Systemic cytokine responses, tumor growth and cumulative survival rates were monitored. Transfected bladder cancer cells showed high in vitro and in vivo expression of the recombinant subcomponents. Mice with tumors injected with poly-rBCG plus mIL-12 produced serum interferon-gamma significantly within 21 days but no significant elevations in tumor necrosis factor-alpha, IL-2, IL-4 or IL-5 were found. On day 28 after electroporation the growth of MBT-2 implants treated with poly-rBCG, mIL-12 or poly-rBCG plus mIL-12 was significantly inhibited. The cumulative survival of mice treated with poly-rBCG plus mIL-12 was significantly higher than that of the other 3 groups. Highly immunopotent recombinant vaccines of bacillus Calmette-Guerin DNA were produced that elicited T helper 1 immune responses with a high serum interferon-gamma level, inhibited tumor growth

and prolonged the survival of tumor bearing mice. Thus, electroporation immunogene therapy using poly-rBCG plus mIL-12 may be an attractive regimen for the treatment of bladder cancer.

Lee, S. J., Y. H. Cho, et al. (2008). "Magnolol elicits activation of the extracellular signal-regulated kinase pathway by inducing p27KIP1-mediated G2/M-phase cell cycle arrest in human urinary bladder cancer 5637 cells." *Biochem Pharmacol* **75**(12): 2289-300.

Magnolol has been reported to play a role in antitumor activity. However, the relevant pathway integrating cell cycle regulation and signaling pathways involved in growth inhibition in cancer cells remains to be identified. In the present study, magnolol treatment of these cells resulted in significant dose-dependent growth inhibition together with apoptosis, G1- and G2/M-phase cell cycle arrest at a 60 microM (IC50) dose in 5637 bladder cancer cells. In addition, magnolol treatment strongly induced p27KIP1 expression, and down-regulated expression of cyclin-dependent kinases (CDKs) and cyclins. Moreover, treatment with magnolol-induced phosphorylation of ERK, p38 MAP kinase, and JNK. Among the pathway inhibitors examined, only PD98059, an ERK-specific inhibitor, blocked magnolol-dependent p27KIP1 expression. Blockade of ERK function consistently reversed magnolol-mediated inhibition of cell proliferation and decreased G2/M cell cycle proteins, but not G1 cell cycle proteins. Furthermore, magnolol treatment increased both Ras and Raf activation. Transfection of cells with dominant negative Ras (RasN17) and Raf (RafS621A) mutant genes suppressed magnolol-induced ERK activity and p27KIP1 expression. Finally, the magnolol-induced reduction in cell proliferation and G2/M cell cycle proteins was also abolished in the presence of RasN17 and RafS621A mutant genes. These data demonstrate that the Ras/Raf/ERK pathway participates in p27KIP1 induction, leading to a decrease in the levels of cyclin B1/Cdc2 complexes and magnolol-dependent inhibition of cell growth. Overall, these novel findings concerning the molecular mechanisms of magnolol in 5637 bladder cancer cells provide a theoretical basis for therapeutic treatment of malignancies.

Lee, S. J., S. S. Park, et al. (2008). "Activation of matrix metalloproteinase-9 by TNF-alpha in human urinary bladder cancer HT1376 cells: the role of MAP kinase signaling pathways." *Oncol Rep* **19**(4): 1007-13.

The expression of matrix metalloproteinase-9 (MMP-9) has been implicated in tumor invasion and metastasis. In this study, the factors and signaling pathways that are involved in the regulation of the

MMP-9 expression were examined in urinary bladder cancer HT1376 cells. Tumor necrosis factor-alpha (TNF-alpha) stimulated the secretion of MMP-9 in HT1376 cells, as shown by zymography and immunoblot analysis. At the level of transcription, TNF-alpha also stimulated 5'-flanking promoter activity of MMP-9. Transcription factor NF-kappaB, AP-1 and Sp-1 binding sites were identified by a gel shift assay to be cis-elements for TNF-alpha activation of the MMP-9 promoter. TNF-alpha activates multiple signaling pathways in HT1376 cells, including the extracellular signal-regulated kinase (ERK1/2), p38 MAP kinase and JNK pathways. Chemical inhibitors, which specifically inhibit each of these TNF-alpha-activated pathways, were used to examine the signaling pathways involved in TNF-alpha-mediated MMP-9 expression. The ERK1/2 inhibitor, U0126 and the p38 MAP kinase inhibitor, SB203580, significantly down-regulated TNF-alpha-induced MMP-9 expression and promoter activity. The transactivation of TNF-alpha-stimulated NF-kappaB, AP-1 and Sp-1 were inhibited by U0126 and SB203580 treatment. In conclusion, the findings of the present study indicate that TNF-alpha induces MMP-9 expression in HT1376 cells by activating transcription factors, which are involved in the ERK1/2- and p38 MAP kinase-mediated control of MMP-9 regulation, namely, NF-kappaB, AP-1 and Sp-1.

Li, G. C., X. Zhang, et al. (2006). "Histone deacetylase inhibitor trichostatin A inhibits the growth of bladder cancer cells through induction of p21WAF1 and G1 cell cycle arrest." *Int J Urol* **13**(5): 581-6.

To investigate whether Trichostatin A (TSA) possesses antitumor activity against human bladder cancer cells, and if any, its mechanism. A human bladder cancer cell line, BIU-87, was treated with different concentrations of TSA. After treatment, cell growth was measured by MTT assay. Cell apoptosis and cell cycle changes were examined by means of flow cytometry (FCM). Apoptosis was confirmed by apoptotic ladder formation assay. mRNA expression of p21WAF1 and p53 was assessed by differential reverse transcription-polymerase chain reaction. Trichostatin A significantly inhibited the proliferation of bladder cancer cell at nanomolar concentrations in a time- and dose-dependent fashion. TSA treatment caused cell cycle arrest at the G1 phase and increased apoptotic cell death as shown by FCM and DNA fragmentation analysis, accompanied by increased p21WAF1 mRNA expression. In addition, TSA treatment did not alter p53 mRNA expression. **CONCLUSION:** Our results indicate that TSA is able to inhibit bladder cancer cell growth in vitro, possibly

through p21WAF1 mediated cell cycle arrest and apoptotic cell death. This study suggests that TSA may be a potential therapeutic agent for the treatment of bladder cancer.

Li, L. and D. L. He (2005). "Transfection of promyelocytic leukemia in retrovirus vector inhibits growth of human bladder cancer cells." *Acta Pharmacol Sin* **26**(5): 610-5.

To construct a recombinant retrovirus vector carrying human promyelocytic leukemia (PML) cDNA and identify its expression and biology role in bladder cancer UM-UC-2 cells for future gene therapy. PML full-length cDNA was inserted into the EcoR I and BamH I site of pLXSN vector containing the long terminal repeat (LTR) promoter. The vector was identified by restriction enzyme digestion and then transfected into PA317 packaging cell line by calcium phosphate coprecipitation. PML cDNA was detected by polymerase chain reaction (PCR) and the protein was identified by laser confocal microscopy and Western blot in bladder cancer cells, respectively. The morphology was observed by inverted phase contrast microscope, and MTT assay determined growth curve of the bladder cancer cells. Restriction enzyme digestion proved that a 2.1 kb PML cDNA was inserted into the pLXSN vector. PCR assay demonstrated that 304 bp fragments were found in UM-UC-2/pLPMLSN transfects. Laser confocal microscopy showed speck dots fluorescence in the UM-UC-2/pLPMLSN nucleus. A 90 kD specific band was found by Western blot. MTT assay demonstrated the UM-UC-2/pLPMLSN bladder cancer growth inhibition. **CONCLUSION:** The retrovirus pLPMLSN vector was successfully constructed and could generate high effective expression of human PML in bladder cancer cell UM-UC-2, suggesting that PML recombinant retrovirus have potential utility in the gene therapy for bladder cancer.

Li, T. M., G. W. Chen, et al. (2005). "Ellagic acid induced p53/p21 expression, G1 arrest and apoptosis in human bladder cancer T24 cells." *Anticancer Res* **25**(2A): 971-9.

It is well known that dietary phenolic compounds can elicit vital cellular responses such as cytotoxicity, cell cycle arrest and apoptosis by activating a cascade of molecular events. Ellagic acid is one of these phenolic compounds, but the exact mechanism of its action is still unclear. The objective of this study was to investigate ellagic acid-induced cell cycle arrest and apoptosis in T24 human bladder cancer cells in vitro. Assays were performed to determine cell viability, cell cycle arrest, apoptosis, caspases-3 activity and gene expression, measured by

flow cytometric assay, polymerase chain reaction (PCR) and determination of caspase-3 activity. Ellagic acid significantly reduced the viable cells, induced G0/G1-phase arrest of the cell cycle and apoptosis. Ellagic acid also increased p53 and p21 and decreased CDK2 gene expression, that may lead to the G0/G1 arrest of T24 cells. Ellagic acid also promoted caspase-3 activity after exposure for 1, 3, 6, 12 and 24 h, which led to induction of apoptosis. Furthermore, the ellagic acid-induced apoptosis on T24 cells was blocked by the broad-spectrum caspase inhibitor (z-VAD-fmk).

Liu, H. B., C. Z. Kong, et al. (2009). "Livin may serve as a marker for prognosis of bladder cancer relapse and a target of bladder cancer treatment." *Urol Oncol* **27**(3): 277-83.

To evaluate the expression of Livin in bladder cancer, investigate its clinical and prognostic implications, and explore the effect of gene Livin transfection on the proliferation and apoptosis in bladder cancer cells. The expression of Livinalpha and beta was detected in 48 bladder cancer samples (G(1) in 23 cases, G(2) in 17 cases, and G(3) in 8 cases. Of the 48 cases, 17 developed relapse) and 15 non-tumor bladder tissues by Western blot and reverse transcription PCR (RT-PCR). Livinalpha-pcDNA3.1(+) was constructed and transfected into T24, BIU-87 and EJ bladder cancer cells. The clone activity of the transfected cells was detected by colony formation analysis. MTT was used to determine the cell proliferation assay. Flow cytometry and acridine orange staining were used to examine apoptosis. Caspase 3 activity assay was also measured. Expression of Livinalpha, but not beta, was detected in 19 of the 48 bladder cancer samples; G(1) was 39.13%, G(2) and G(3) were 41.18% and 37.50%, respectively, which showed no significant ($P > 0.05$), but not in 15 non-tumor bladder tissues. The positive rate of Livinalpha was significant higher in relapse tumors (58.82%) than in primary tumors (29.03%) ($P < 0.05$). By the end of 2 years follow-up, the relapse rate in Livin positive patients was 68.42%, and 37.93% in Livin negative group. The difference between the two groups was significant ($P < 0.05$). Additionally, overexpression of Livinalpha clearly stimulated cell proliferation and inhibited chemical induced apoptosis in bladder cancer cells. Livin may serve as a promising marker to identify the relapse risk in bladder cancer, and targeting Livin could offer a therapeutic benefit in apoptosis-inducing treatment.

Liu, J., Q. Wang, et al. (2003). "Apoptosis of bladder cancer cells induced by short-term and low-dose Mitomycin-C: potential molecular mechanism and clinical implication." *Int J Mol Med* **11**(3): 389-94.

Adjuvant chemotherapies have been used to prevent recurrence of bladder transitional cell carcinoma (TCC), but their efficacies are variable due to the side effects of anti-cancer agents and the drug resistant property of the target cells. To provide experimental evidence for improving clinical management of TCCs, two human TCC cell lines, EJ and BIU, were treated for 1 or 2 h by 50, 100, 150 and 200 micro g/ml, respectively and their growth and death patterns were elucidated in 12-h intervals. The status of Fas, FasL and caspase-3 in the two cell lines were analyzed with immunocytochemical staining and Western blot hybridization, and their potential link to MMC-induced cell death was investigated by treating the cells with anti-Fas antibody (150 ng/ml) and by incubating the cells with an inhibitor of caspase-3 related proteases Ac-DEVO-CHO (250 micro M) 1 h before 100 micro g/ml MMC treatment. The results demonstrated that the lower dose (100 micro g/ml) and short-term (1 h) MMC treatment could induce sufficient apoptosis in EJ and BIU populations within 48 or 60 h. Constitutive soluble and membrane Fas and FasL were found in both cell lines, and caspase-3 could be upregulated after MMC treatment. Anti-Fas antibody could commit the target cells to die of apoptosis, while Ac-DEVD-CHO inhibited MMC-induced apoptosis. Our data thus suggest that MMC-induced apoptosis in EJ and BIU cells is mediated by Fas and upregulation and activation of caspase-3 is an essential element for the apoptotic process. Reduced dose and short-term MMC strategy would be of practical value either in determining the apoptotic susceptibility of individual TCC cases or in the clinical instillation of urothelial cancers.

Lokeshwar, V. B., V. Estrella, et al. (2006). "HYAL1-v1, an alternatively spliced variant of HYAL1 hyaluronidase: a negative regulator of bladder cancer." *Cancer Res* **66**(23): 11219-27.

Tumor cells express HYAL1 hyaluronidase, which degrades hyaluronic acid. HYAL1 expression in bladder cancer cells promotes tumor growth, invasion, and angiogenesis. We previously described five alternatively spliced variants of HYAL1 that encode enzymatically inactive proteins. The HYAL1-v1 variant lacks a 30-amino acid sequence that is present in HYAL1. In this study, we examined whether HYAL1-v1 expression affects bladder cancer growth and invasion by stably transfecting HT1376 bladder cancer cells with a HYAL1-v1 cDNA construct. Although HYAL1-v1 transfectants expressed equivalent levels of enzymatically active HYAL1 protein when compared with vector transfectants, their conditioned medium had 4-fold less hyaluronidase activity due to a noncovalent complex formed between HYAL1 and HYAL1-v1

proteins. HYAL1-v1 transfectants grew 3- to 4-fold slower due to cell cycle arrest in the G(2)-M phase and increased apoptosis. In HYAL1-v1 transfectants, cyclin B1, cdc2/p34, and cdc25c levels were > or =2-fold lower than those in vector transfectants. The increased apoptosis in HYAL1-v1 transfectants was due to the extrinsic pathway involving Fas and Fas-associated death domain up-regulation, caspase-8 activation, and BID cleavage, leading to caspase-9 and caspase-3 activation and poly(ADP-ribose) polymerase cleavage. When implanted in athymic mice, HYAL1-v1-expressing tumors grew 3- to 4-fold slower and tumor weights at day 35 were 3- to 6-fold less than the vector tumors (P < 0.001). Whereas vector tumors were infiltrating and had high mitoses and microvessel density, HYAL1-v1 tumors were necrotic, infiltrated with neutrophils, and showed low mitoses and microvessel density. Therefore, HYAL-v1 expression may negatively regulate bladder tumor growth, infiltration, and angiogenesis.

Loskog, A., C. Ninalga, et al. (2005). "Optimization of the MB49 mouse bladder cancer model for adenoviral gene therapy." *Lab Anim* **39**(4): 384-93.

Bladder cancer is regarded as a promising candidate for innovative therapies in the field of immune and gene therapy. In this paper, we present the subcutaneous, metastatic and a novel orthotopic model of murine MB49 bladder cancer in C57BL/6 mice. We further show the potential of using adenoviral vectors together with different transduction enhancers to augment in vivo gene delivery. Finally, we present candidate genes for tumour detection, therapy or targeting. The MB49 tumour grew rapidly in mice. The subcutaneous model allowed for tumour detection within a week and the possibility to monitor growth rate on a day-by-day basis. Injection of MB49 cells intravenously into the tail vein gave rise to lung metastases within 16 days, while instillation of tumour cells into pretreated bladders led to a survival time of 20-40 days. Adenoviral vectors can be used as a vehicle for gene transfer to the bladder. By far, the most potent transduction enhancer was Clorpactin, also known as oxychlorosene. Last, we show that MB49 cells express tumour-associated antigens like bladder cancer-4, prostate stem cell antigen and six-transmembrane epithelial antigen of the prostate. Given the possibility for efficient genetic modification of the bladder and the presence of known tumour antigens, the MB49 models can be used in innovative ways to explore immunogene therapy.

Loskog, A. S., M. E. Fransson, et al. (2005). "AdCD40L gene therapy counteracts T regulatory cells and cures aggressive tumors in an orthotopic

bladder cancer model." *Clin Cancer Res* **11**(24 Pt 1): 8816-21.

The aim of this study was to develop an immunostimulating gene therapy for the treatment of orthotopic bladder carcinoma by transferring the gene for CD40L into the tumor site. CD40L stimulation of dendritic cells induces interleukin-12 expression that drives Th1 type of immune responses with activation of cytotoxic T cells. The gene for murine CD40L was transferred into bladders of tumor-bearing mice using an adenoviral vector construct. To facilitate viral uptake, the bladders were pretreated with Clorpactin. Survival of mice as well as transgene expression and immunologic effect, such as resistance to tumor challenge and presence of T regulatory cells, were monitored. On viral vector instillation, CD40L expression could be detected by reverse transcription-PCR. As a sign of transgene function, interleukin-12 (IL-12) expression was significantly increased. AdCD40L gene therapy cured 60% of mice with preestablished tumors. The cured mice were completely resistant to subcutaneous challenge with MB49 tumor cells, whereas the growth of a syngeneic irrelevant tumor was unaltered. Furthermore, the mRNA expression level of the T regulatory cell transcription factor Foxp3 was evaluated both in tumor biopsies and lymph nodes. There were no differences within the tumors of the different treatment groups. However, Foxp3 mRNA levels were down-regulated in the lymph nodes of AdCD40L-treated mice. Correspondingly, T cells from AdCD40L-treated mice were not able to inhibit proliferation of naive T cells as opposed to T cells from control-treated, tumor-bearing mice. AdCD40L gene therapy evokes Th1 cytokine responses and counteracts T regulatory cell development and/or function.

Macoska, J. A., S. Adsule, et al. (2008). "-(-)Gossypol promotes the apoptosis of bladder cancer cells in vitro." *Pharmacol Res* **58**(5-6): 323-31.

Dysregulation of Bcl2 family member proteins has been associated with poor chemotherapeutic response in bladder cancer, suggesting that agents targeting these crucial proteins may provide an interventional strategy to slow or halt bladder cancer progression and metastasis. In this study, we investigated whether the cottonseed polyphenol, -(-)gossypol, a BH3 mimetic, can reduce the expression of pro-survival, or increase the expression of pro-apoptotic, Bcl2 family proteins and thereby effectively sensitize otherwise resistant bladder cancer cells to the standard chemotherapeutic drugs gemcitabine, paclitaxel and carboplatin. These studies show that gossypol induced apoptosis in both chemosensitive UM-UC2 and chemoresistant resistant

UM-UC9 bladder cancer cells in vitro in a dose and time dependent manner via a caspase mediated death signaling pathway. Moreover, in combined treatments, gossypol synergized with gemcitabine and carboplatin to induce apoptosis in chemoresistant bladder cancer cells. This effect was associated with the down-regulation the Bcl-xl and Mcl-1 pro-survival Bcl2 family proteins and up-regulation of the Bim and Puma BH3-only Bcl2 family proteins. Overall, these studies show that gossypol sensitizes bladder cancer cells to standard chemotherapeutic drugs and may provide a promising new strategy for bladder cancer treatment.

Malats, N. (2008). "Genetic epidemiology of bladder cancer: scaling up in the identification of low-penetrance genetic markers of bladder cancer risk and progression." *Scand J Urol Nephrol Suppl*(218): 131-40.

Bladder cancer is an increasingly important international public health problem. As a multifactorial disease, both environmental and genetic factors are involved in its development and progression. This neoplasm is a paradigm for the participation of low-penetrance genetic variants (GSTM1-null and NAT2-slow) and provides the best established gene-environment interaction in cancer (NAT2-slow * tobacco). Genetic variants in nucleotide excision and double strand break DNA repair pathways have provided promising results, ERCC2-XPD rs238406 being the most consistent variant associated with an increased of bladder cancer risk, by itself and by interacting with tobacco. Variants in other pathways such as cell-cycle control, 1-C metabolism and inflammation have been studied, although the results are inconsistent. Three very large whole genome association studies are being undertaken using the same genotyping platform. Their results will be available soon. Genetic variants have seldom been considered as markers of prognosis or response to therapy in this tumour. The results of these studies are inconclusive. Other issues that need to be addressed are the role of genetic variants in different population subgroups--defined by ethnicity, gender and age, among others--and the association with bladder cancer subphenotypes according to clinical, pathological and molecular characteristics of the tumour. This endeavour can only be achieved by integrating multidisciplinary tools and information. Can this information be applied better to identify high-risk populations? Can the information be used to better assess prognosis or predict response to therapy? These questions require large, well-designed, multicentre studies to be conducted. Funding agencies should be aware of these needs.

Maluf, F. C., C. Cordon-Cardo, et al. (2006). "Assessing interactions between mdm-2, p53, and bcl-2 as prognostic variables in muscle-invasive bladder cancer treated with neo-adjuvant chemotherapy followed by locoregional surgical treatment." *Ann Oncol* 17(11): 1677-86.

Tumor proliferation and apoptosis may be influenced by the mdm-2 gene product, which can block the antiproliferative effects of p53. bcl-2, one of a family of related genes that regulates the apoptotic pathway, exhibits a negative influence. Both individual and cooperative effects of these gene products may affect the biological behavior of primary bladder cancers and long-term outcome to standard therapy. This study retrospectively evaluated the association with survival of mdm-2, p53, and bcl-2 expression in 59 patients with muscle-invasive, node-negative transitional cell carcinoma (TCC) treated with neo-adjuvant chemotherapy followed by locoregional surgery. Each marker was defined as an altered phenotype if $\geq 20\%$ malignant cells in the primary tumor exhibited staining; normal or minimal expression was defined as $< 20\%$ cells exhibiting staining. Altered mdm-2, p53, and bcl-2 expression was observed in 37%, 54%, and 46% of patients, respectively. In single marker analysis, altered p53 expression correlated with long-term survival ($P = 0.05$) but mdm-2 ($P = 0.42$) or bcl-2 ($P = 0.17$) did not. In the multiple-marker analysis, a prognostic index simultaneously assessing mdm-2, p53, and bcl-2 correlated with survival ($P = 0.01$). The 5-year survival for patients in which all markers were normally expressed was 54% compared with 25% in those with all three markers aberrantly expressed. Patients with aberrant expression of either one or two markers had an intermediate 5-year survival (49%). There was no association of molecular markers either alone or in combination with pathologic downstaging after neo-adjuvant chemotherapy. CONCLUSION: The cooperative effects of phenotypes determined by mdm-2, p53, and bcl-2 expression may predict survival in patients with muscle-invasive TCC of the bladder.

Mangsbo, S. M., C. Ninalga, et al. (2008). "CpG therapy is superior to BCG in an orthotopic bladder cancer model and generates CD4+ T-cell immunity." *J Immunother* 31(1): 34-42.

Bacillus Calmette Guerin (BCG) immunotherapy has been successful in extending tumor remission in bladder cancer, the fifth most common cancer in men. However, relapses are frequent and some patients develop resistance to BCG. CpGs were previously demonstrated to be effective in the murine MB49 model. In this paper, we modeled a more aggressive orthotopic bladder cancer than

previously studied. Moreover, we compared standard BCG immunotherapy side-by-side with the Toll-like receptor-9 agonist CpG. MB49 tumor-bearing mice were treated with BCG or CpG and survival as well as tumor progression were observed over time. Urine, blood, and tumors were collected and analyzed. Mice were rechallenged and evaluated for tumor-specific immunity. In this study, CpGs induced a complete response of large aggressive orthotopic MB49 bladder tumors, resulting in tumor-specific systemic immunity. Further, data indicated that this potent antitumor effect required T cells. A comparison of CpGs and BCG in both a highly and less aggressive orthotopic tumor model, and in a subcutaneous tumor model, demonstrated that CpGs were superior to BCG. In the orthotopic model, BCG induced a local cytokine storm during treatment initiation whereas CpG affected a more refined cytokine pattern over time. Increased levels of cytokines in serum correlated with enhanced survival in the subcutaneous model. Further, immune cell depletion studies demonstrated that CpG-induced protective immunity was CD4+ T-cell dependent. Taken together, our data suggest that CpGs are superior to BCG for bladder cancer immunotherapy. Thus, this potent new drug may be an attractive therapeutic alternative and should be evaluated in bladder cancer patients.

Mansure, J. J., R. Nassim, et al. (2009). "Peroxisome proliferator-activated receptor gamma in bladder cancer: a promising therapeutic target." *Cancer Biol Ther* 8(7): 6-15.

Peroxisome Proliferator-Activated Receptors (PPARs) are ligand-activated intracellular transcription factors, members of the nuclear hormone receptor superfamily. The PPAR subfamily consist of three subtypes encoded by distinct genes denoted PPARalpha, PPARbeta/delta, and PPARgamma. The peroxisome proliferator-activated receptor gamma (PPARgamma) is the most extensively studied subtype of the PPARs. Over the last decade, research on PPARgamma unveiled its role in important biological processes, including lipid biosynthesis, glucose metabolism, anti-inflammatory response, and atherosclerosis. Recently, PPARgamma has been shown to be expressed in many cancers including, lung, prostate, bladder, colon, breast, duodenal, thyroid, and has been demonstrated to potentially play an important role in carcinogenesis. In bladder cancer, PPARgamma ligands such as troglitazone and 15d-PGJ2 have shown to inhibit tumor growth. We have recently published the first report to show that a new class of PPARgamma agonists, PPARgamma-active C-DIMs, which are more potent than the previous generation of drugs, exhibit anti-tumorigenic activity against bladder cancer cells in vitro and bladder

tumors in vivo. The following review will discuss the molecular structure of PPARgamma, its function and its role in cancer biology and how it is emerging as a promising therapeutic target in bladder cancer.

Mao, Q., Y. Li, et al. (2008). "Up-regulation of E-cadherin by small activating RNA inhibits cell invasion and migration in 5637 human bladder cancer cells." *Biochem Biophys Res Commun* 375(4): 566-70.

Recent studies have reported that chemically synthesized small duplex RNAs complementary to promoters of target genes can specifically induce gene expression in several cancer cell lines. Such dsRNA, referred to as small activating RNA (saRNA), are involved in the recently described phenomenon called RNA activation (RNAa). Recent findings show that saRNA can inhibit cell proliferation and viability via up-regulation of p21(WAF1/CIP1) (p21) in human bladder cancer cells. In the present study, we demonstrate that induction of E-cadherin expression by saRNA leads to suppression of migration and invasion of 5637 human bladder cancer cells in vitro. The elevated E-cadherin expression was confirmed at transcriptional and protein levels after transfection of a 21-nucleotide dsRNA targeting the E-cadherin promoter (dsEcad). Furthermore, this inhibitory effect was associated with relocalization of beta-catenin from the nucleus to the plasma membrane and decreased beta-catenin-mediated transactivation. These data suggest that activation of E-cadherin by saRNA may have a therapeutic benefit for bladder and other types of cancer.

Margulis, V., S. F. Shariat, et al. (2006). "Ki-67 is an independent predictor of bladder cancer outcome in patients treated with radical cystectomy for organ-confined disease." *Clin Cancer Res* 12(24): 7369-73.

To determine the association of the cell proliferative marker Ki-67 with pathologic features and disease prognosis in patients with transitional cell carcinoma (TCC) of the urinary bladder. Immunohistochemical staining for Ki-67 was done on serial cuts from tissue microarrays containing cystectomy specimens from 9 patients without bladder cancer and 226 consecutive patients with bladder TCC. We also assessed malignant lymph nodes from 50 of the 226 cystectomy patients. Ki-67 expression was increased in 42.5% cystectomy specimens and in 54% metastatic lymph nodes. In contrast, it was absent in all nine benign cystectomy specimens. Ki-67 overexpression was associated with advanced pathologic stage, higher grade, lymphovascular invasion, and metastases to lymph nodes ($P = 0.001, 0.040, 0.031, \text{ and } 0.036$, respectively). In multivariate analyses, pathologic stage and lymph node metastases

were independent predictors of disease recurrence and bladder cancer-specific mortality. In the subgroup of patients with organ-confined disease (<pT(3) N(0); n = 91), excluding patients who received neoadjuvant or adjuvant chemotherapy, Ki-67 status was an independent predictor of both disease recurrence (risk ratio, 7.591; P = 0.001) and bladder cancer-specific mortality (risk ratio, 4.045; P = 0.041). Ki-67 overexpression is associated with features of aggressive bladder TCC and adds independent prognostic information to standard pathologic features for prediction of clinical outcome after radical cystectomy.

Marsit, C. J., M. R. Karagas, et al. (2005). "Epigenetic inactivation of SFRP genes and TP53 alteration act jointly as markers of invasive bladder cancer." Cancer Res **65**(16): 7081-5.

In the United States each year, almost 13,000 deaths are attributable to bladder cancer, with the majority of these deaths related to higher stage, muscle-invasive solid tumors. Epigenetic silencing of the secreted frizzled receptor proteins (SFRP), antagonists of the WNT pathway, leads to constitutive WNT signaling, altering cell morphology and motility. Identifying alterations in this pathway in bladder cancer may prove useful for defining the invasive phenotype and provide targets for guiding therapy. Using a population-based study of bladder cancer (n = 355), we examined epigenetic alterations, specifically gene promoter hypermethylation, of four SFRP genes in addition to immunohistochemical staining of TP53, which has been previously shown to be a predictor of invasive disease. We observed a significant linear trend (P < 0.0004) in the magnitude of the risk of invasive disease with the number of SFRP genes methylated. Both TP53 alteration and SFRP gene methylation showed significant independent associations with invasive bladder cancer. Strikingly, in examining the joint effect of these alterations, we observed a >30-fold risk of invasive disease for patients with both altered SFRP gene methylation and intense TP53 staining (odds ratio, 32.1; P < 10(-13)). Overall patient survival was significantly poorer in patients with any SFRP genes methylated (P < 0.0003) and in proportional hazards modeling, patients with methylation of any SFRP gene had significantly poorer overall survival (hazard ratio, 1.78; P < 0.02) controlled for TP53 staining intensity and other survival-associated factors. Classifying tumors based on SFRP methylation status and TP53 protein staining intensity may be a clinically powerful predictor of invasive, deadly disease.

Marzoni, D., T. Lorenzi, et al. (2009). "Expression of basic fibroblast growth factor, its receptors and

syndecans in bladder cancer." Int J Immunopathol Pharmacol **22**(3): 627-38.

Basic fibroblast growth factor (bFGF) is a heparin-binding cationic protein involved in a variety of pathological conditions including angiogenesis and solid tumour growth. The basic fibroblast growth factor receptor (FGFR) family comprises at least 4 high affinity tyrosine kinase receptors that require syndecans for their function. Mounting evidence indicates that syndecans, that bind both bFGF and their FGFRs, will act as stimulators, whereas syndecans that only bind bFGF will act as inhibitors of signaling by sequestering the growth factor. Recent findings have highlighted the importance of syndecans in urological cancers. The aim of this study is to investigate the expression of bFGF, its receptors (R1 and R2) and syndecans (1-4) in invasive urothelial carcinoma and normal-looking urothelium by Western blotting, RT-PCR, and immunohistochemistry analyses. Interestingly, bFGF, FGFR1 and FGFR2 protein levels statistically increased in bladder cancer tissues. mRNA of FGFR1 and syndecans (1-4), showed a statistically significant increase while an mRNA increase in the other molecules analysed was not significant. bFGF, its receptors and syndecan immunostaining were mainly present in the urothelium both in normal-looking tissues and urothelial neoplastic cells. In conclusion, our data report that the bFGF, FGFR and syndecan expressions are altered in bladder tumours.

Matsumoto, H., H. Matsuyama, et al. (2004). "Allelic imbalance at 1p36 may predict prognosis of chemoradiation therapy for bladder preservation in patients with invasive bladder cancer." Br J Cancer **91**(6): 1025-31.

Invasive bladder cancers have been treated by irradiation combined with cis-platinum (CDDP) as a bladder preservative option. The aim of this study was to find a marker for predicting patient outcome as well as clinical response after chemoradiation therapy (CRT) by investigating allelic loss of apoptosis-related genes. A total of 67 transitional cell carcinomas of the bladder treated by CRT (median dose: 32.4 Gy of radiation and 232 mg of CDDP) were studied. We investigated allelic imbalances at 14 loci on chromosomes 17p13 and 1p36 including the p53 and p73 gene regions by fluorescent multiplex PCR based on DNA from paraffin-embedded tumour specimens and peripheral blood. The response to CRT was clinical response (CR) in 21 patients (31%), partial response (PR) in 31 (46%), and no change (NC) in 15 (22%). There was no statistical correlation between treatment response and clinical parameters, such as tumour grade, stage, radiation dose, or CDDP dose. The frequencies of allelic imbalance for TP53 and

TP73 were 21 and 56%, respectively; neither was correlated with clinical treatment response and tumour stage or grade. There was no statistical correlation between treatment response and allelic imbalance at the other 12 loci. We found a significant correlation between cancer-specific survival and an imbalance of D1S243 ($P=0.0482$) or TP73 ($P=0.0013$) using a Log-rank test, although other loci including TP53 did not correlate with survival ($P=0.4529$). Multivariate analysis showed performance status ($P=0.0047$), recurrence ($P=0.0017$), and radiation doses ($P=0.0468$) were independent predictive factors for cancer-specific survival. However, an allelic imbalance of TP73 was the most remarkable independent predictive factor of poor patient survival ($P=0.0002$, risk ratio: 3382). Our results suggest that the allelic loss of the p73 gene predicts a clinical outcome of locally advanced bladder cancer when treated by CRT.

Matsumoto, K., A. Irie, et al. (2007). "Expression of S100A2 and S100A4 predicts for disease progression and patient survival in bladder cancer." *Urology* **70**(3): 602-7.

To determine the expression patterns and prognostic value of S100A2 and S100A4 in surgical specimens from radical cystectomy for transitional cell carcinoma of the urinary bladder. Immunohistochemical staining for S100A2 and S100A4 was performed in 92 archived radical cystectomy and 38 normal specimens. The immunoreactivity of these proteins was stratified on a 0 to 6 scale and then correlated with the pathologic features and clinical outcome. S100A2 expression was significantly decreased in the bladder cancer specimens compared with the controls ($P < 0.0001$), and S100A4 expression was significantly greater in the bladder cancer specimens ($P = 0.03$). The loss of expression of S100A2 and increased expression of S100A4 were associated with muscle invasion ($P < 0.05$). These alterations in expression were also associated with a greater risk of disease progression and a decreased chance of cancer-specific survival at a median follow-up of 25.3 months ($P < 0.0001$ for both). After adjusting for the effects of the pathologic findings, S100A4 expression remained a significant predictor of disease progression ($P < 0.0001$) and cancer-specific survival ($P < 0.0001$). S100A4 appeared to be an independent predictor for the treatment outcome in bladder cancer. The expression patterns of S100A2 and S100A4 correlated well with the pathologic stage, disease progression, and cancer-specific mortality. This finding could aid in identifying more biologically aggressive cancers and thus patients who might benefit from more intensive adjuvant therapy.

Matsumoto, K., T. Satoh, et al. (2008). "Loss expression of uroplakin III is associated with clinicopathologic features of aggressive bladder cancer." *Urology* **72**(2): 444-9.

Tissue-specific expression is of key importance in gene therapy and can be achieved by using tissue-specific promoters to drive therapeutic gene expression. The uroplakin (UP) promoter is a powerful tool for bladder cancer gene therapy, but the role of UP protein in the bladder remains unknown. This study aimed to detect UP III expression in transitional cell carcinoma (TCC) of the bladder and to determine whether the role of UP III heterogeneity is associated with predicting disease recurrence and patient survival. Immunohistochemical staining for UP III was carried out in 92 archival radical cystectomy and 38 normal specimens and correlated with pathologic features and clinical outcomes. UP III expression was significantly decreased in bladder cancer tissues compared with normal controls ($P < 0.0001$). Loss of UP III expression was associated with high-grade, muscle-invasive cancer, lymphovascular invasion ($P < 0.01$), and decreased cancer-specific survival at a median follow-up of 25.3 months ($P = 0.04$). When adjusted for the effects of standard pathologic features, only lymph node metastases were associated with bladder cancer progression ($P = 0.01$) and mortality ($P = 0.04$). Loss of UP III expression is associated with established markers of biologically aggressive bladder cancer such as lymphovascular invasion, pathologic stage, and grade. UP III expression has limited prognostic value in patients with bladder TCC, but gene therapy viral vectors driven by the UP promoter would drive therapeutic gene expression in high-UP-expressing TCC cells, but not in aggressive low-UP-expressing TCC cells.

McConkey, D. J., W. Choi, et al. (2009). "Role of epithelial-to-mesenchymal transition (EMT) in drug sensitivity and metastasis in bladder cancer." *Cancer Metastasis Rev* **28**(3-4): 335-44.

Epithelial-to-mesenchymal transition (EMT) is a process that plays essential roles in development and wound healing that is characterized by loss of homotypic adhesion and cell polarity and increased invasion and migration. At the molecular level, EMT is characterized by loss of E-cadherin and increased expression of several transcriptional repressors of E-cadherin expression (Zeb-1, Zeb-2, Twist, Snail, and Slug). Early work established that loss of E-cadherin and increased expression of MMP-9 was associated with a poor clinical outcome in patients with urothelial tumors, suggesting that EMT might also be associated with bladder cancer progression and metastasis. More

recently, we have used global gene expression profiling to characterize the molecular heterogeneity in human urothelial cancer cell lines (n = 20) and primary patient tumors, and unsupervised clustering analyses revealed that the cells naturally segregate into two discrete "epithelial" and "mesenchymal" subsets, the latter consisting entirely of muscle-invasive tumors. Importantly, sensitivity to inhibitors of the epidermal growth factor receptor (EGFR) or type-3 fibroblast growth factor receptor (FGFR3) was confined to the "epithelial" subset, and sensitivity to EGFR inhibitors could be reestablished by micro-RNA-mediated molecular reversal of EMT. The results suggest that EMT coordinately regulates drug resistance and muscle invasion/metastasis in urothelial cancer and is a dominant feature of overall cancer biology.

McKnight, J. J., S. B. Gray, et al. (2005). "Apoptosis and chemotherapy for bladder cancer." *J Urol* **173**(3): 683-90.

We discuss the role of apoptosis, that is gene directed self-destruction of a cell, in the response of bladder transitional cell carcinoma cells to chemotherapy. A directed MEDLINE literature search of apoptosis, bladder cancer and chemotherapy was performed to extract relevant information for review. The characteristics of apoptotic cells were defined and the methods in common use to detect these traits is described. The role of the key mediators of the apoptotic process in bladder cancer is discussed in the context of chemosensitivity and disease stage. The importance of the apoptosis induction after chemotherapy is highlighted. On stimulus by appropriate external or internal signals a cell may alter the expression of genes encoding for proteins associated with the apoptotic process. The development of apoptosis depends on the balance between pro-apoptotic and anti-apoptotic proteins. Key alterations in genes and proteins related to apoptosis within bladder cancer result in a shift away from the default state of apoptosis toward a cell with increased survival properties that is chemoresistant. Much current research in bladder cancer is aimed at restoring chemosensitivity by shifting the cell toward a pro-apoptotic phenotype. Successful translation of this work into clinical practice may improve survival in patients in whom prognosis is currently poor.

Melquist, J. J., M. Kacka, et al. (2006). "Conditionally replicating adenovirus-mediated gene therapy in bladder cancer: an orthotopic in vivo model." *Urol Oncol* **24**(4): 362-71.

INTRODUCTION: The effects of a conditionally replicating adenovirus on various bladder cancer lines were explored, a truncated bone

sialoprotein (BSP) promoter controlling the E1a/lytic-regulating sequence was used, since BSP protein is found in many osteotropic neoplasms, including bladder cancer. Reverse transcriptase polymerase chain reaction analysis was used to determine expression patterns of BSP and Coxsackie adenovirus receptor, a receptor known to interact with adenovirus, on multiple lines of bladder cancer (253J, 253J B-V, RT4, transitional cell carcinoma, T24, UMUC3, and WH). Ad-BSP-E1a was tested in vitro for lytic activity on 4 of these cell lines. The 253J B-V cell line was used and inoculated into female nude mice either subcutaneously in the flank or orthotopically into the bladder, and treated with control or Ad-BSP-E1a virus. BSP is expressed in RT4, transitional cell carcinoma, and WH. Meanwhile, Coxsackie adenovirus receptor was expressed in all lines except T24. Ad-BSP-E1a had the most impact on 253J and 253J B-V cells; cell density declined significantly when compared to phosphate-buffered saline and Ad-BSP-TK "dummy" virus-treatment groups. The 253J B-V tumors treated with Ad-BSP-E1a revealed a decreased percent change of size in the subcutaneous model when compared to controls at week 3. The orthotopic murine model showed decreased end tumor mass in the Ad-BSP-E1a treated group over controls. Histologic examination of in vivo tumors showed evidence of fibrosis and apoptosis in the Ad-BSP-E1a treated groups using hematoxylin-eosin, trichrome, and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling (TUNEL) staining. Control groups only had viable tumor in in vivo models. **CONCLUSION:** Adenovirus therapy of orthotopic murine bladder tumors is feasible. Ad-BSP-E1a is effective in treating very aggressive yet sensitive bladder tumor cells. Further study of this conditionally replicating adenovirus treatment (Ad-BSP-E1a) with chemotherapeutic combination is warranted, and future translation of such combination therapy into human beings is a possibility.

Mey, V., E. Giovannetti, et al. (2006). "In vitro synergistic cytotoxicity of gemcitabine and pemetrexed and pharmacogenetic evaluation of response to gemcitabine in bladder cancer patients." *Br J Cancer* **95**(3): 289-97.

The present study was performed to investigate the capability of gemcitabine and pemetrexed to synergistically interact with respect to cytotoxicity and apoptosis in T24 and J82 bladder cancer cells, and to establish a correlation between drug activity and gene expression of selected genes in tumour samples. The interaction between gemcitabine and pemetrexed was synergistic; indeed, pemetrexed favoured gemcitabine cytotoxicity by increasing cellular population in S-phase, reducing Akt

phosphorylation as well as by inducing the expression of a major gemcitabine uptake system, the human equilibrative nucleoside transporter-1 (hENT1), and the key activating enzyme deoxycytidine kinase (dCK) in both cell lines. Bladder tumour specimens showed a heterogeneous gene expression pattern and patients with higher levels of dCK and hENT1 had better response. Moreover, human nucleoside concentrative transporter-1 was detectable only in 3/12 patients, two of whom presented a complete response to gemcitabine. These data provide evidence that the chemotherapeutic activity of the combination of gemcitabine and pemetrexed is synergistic against bladder cancer cells in vitro and that the assessment of the expression of genes involved in gemcitabine uptake and activation might be a possible determinant of bladder cancer response and may represent a new tool for treatment optimization.

Meyer-Siegler, K. L., E. C. Leifheit, et al. (2004). "Inhibition of macrophage migration inhibitory factor decreases proliferation and cytokine expression in bladder cancer cells." *BMC Cancer* 4: 34.

The importance of various inflammatory cytokines in maintaining tumor cell growth and viability is well established. Increased expression of the proinflammatory cytokine macrophage migration inhibitory factor (MIF) has previously been associated with various types of adenocarcinoma. MIF IHC was used to localize MIF in human bladder tissue. ELISA and Western blot analysis determined the synthesis and secretion of MIF by human bladder transitional cell carcinoma cells. The effects of MIF inhibitors (high molecular weight hyaluronate (HA), anti-MIF antibody or MIF anti-sense) on cell growth and cytokine expression were analyzed. Human bladder cancer cells (HT-1376) secrete detectable amounts of MIF protein. Treatment with HA, anti-MIF antibody and MIF anti-sense reduced HT-1376 cell proliferation, MIF protein secretion, MIF gene expression and secreted inflammatory cytokines. Our evidence suggests MIF interacts with the invariant chain, CD74 and the major cell surface receptor for HA, CD44. This study is the first to report MIF expression in the human bladder and these findings support a role for MIF in tumor cell proliferation. Since MIF participates in the inflammatory response and bladder cancer is associated with chronic inflammatory conditions, these new findings suggest that neutralizing bladder tumor MIF may serve as a novel therapeutic treatment for bladder carcinoma.

Mhawech-Fauceglia, P., R. T. Cheney, et al. (2006). "FGFR3 and p53 protein expressions in patients with pTa and pT1 urothelial bladder cancer." *Eur J Surg Oncol* 32(2): 231-7.

AIMS: This study is designed to evaluate the expression and prognostic value of FGFR3 protein expression in patients with pTa/pT1 tumours and to determine the significance of the combinations of FGFR3 and p53 protein expressions in bladder pathogenesis. A tissue microarray (TMA) of 107 pTa, and 147 pT1 tumours was constructed. The TMA sections were immunostained with FGFR3 and p53 monoclonal antibodies. There were significant associations between loss of FGFR3 and tumour stage ($p < 0.001$) and grade ($p < 0.001$) and between p53 overexpression and tumour stage and grade ($p < 0.001$ and $p < 0.001$, respectively). There was no association between FGFR3 and p53 proteins ($p = 0.107$). In addition, tumours with FGFR3+/p53- phenotype have slower recurrence rate than other (FGFR3+/p53+, FGFR3-/p53- and FGFR3-/p53+). CONCLUSION: 1-FGFR3 expression is significantly associated with two important prognostic factors; stage and grade. 2-FGFR3 protein expression is not an independent predictive factor for pTa/pT1 tumour recurrence and progression. 3-Tumours with FGFR3+/p53- phenotype seem to have a distinctive pathway in bladder tumorigenesis.

Mian, B. M., C. P. Dinney, et al. (2003). "Fully human anti-interleukin 8 antibody inhibits tumor growth in orthotopic bladder cancer xenografts via down-regulation of matrix metalloproteinases and nuclear factor-kappaB." *Clin Cancer Res* 9(8): 3167-75.

We previously demonstrated that overexpression of interleukin 8 (IL-8) in human transitional cell carcinoma (TCC) resulted in increased tumorigenicity and metastasis. This increase in tumor growth and metastasis can be attributed to the up-regulation in the expression and activity of the metalloproteinases MMP-2 and MMP-9. To investigate whether targeting IL-8 with a fully human anti-IL-8 antibody (ABX-IL8) could be a potential therapeutic strategy for controlling TCC growth, we studied its effects on TCC growth in vitro and in an in vivo mouse model. Human TCC cell lines 253J B-V and UM UC3 (high IL-8 producers), 253J (low IL-8), and 253J transfected with the IL-8 gene (high producer) were used. ABX-IL8 had no effect on TCC cell proliferation in vitro. However, in the orthotopic nude mouse model, after 4 weeks of treatment (100 micro g/week, i.p.), a significant decrease in tumor growth of both cell lines was observed. IL-8 blockade by ABX-IL8 significantly inhibited the expression, activity, and transcription of MMP-2 and MMP-9, resulting in decreased invasion through reconstituted basement membrane in vitro. The down-regulation of MMP-2 and MMP-9 in these cells could be explained by the modulation of nuclear factor-kappaB expression and transcriptional activity by ABX-IL8.

Our data point to the potential use of ABX-IL8 as a modality to treat bladder cancer and other solid tumors, either alone or in combination with conventional chemotherapy or other antitumor agents.

Mian, C., M. Lodde, et al. (2006). "Multiprobe fluorescence in situ hybridisation: prognostic perspectives in superficial bladder cancer." *J Clin Pathol* **59**(9): 984-7.

To establish independent prognostic factors on a chromosomal basis in superficial bladder cancer, using a multicolour fluorescence in situ hybridisation (FISH) probe mix. In 2002, voided urine from 75 consecutive patients (mean age 71.7, range 52-93) years under follow-up for superficial urothelial cancer was studied prospectively. The patients were observed for a mean (standard deviation (SD)) period of 39.3 (6.8) months (range 27-58) until July 2005. A multicolour FISH on liquid-based voided urinary cytology was carried out on all patients. Univariate analysis, using a log rank test, was used to determine the prognostic relevance of a low-risk pattern and a high-risk pattern. Progression-free survival time was calculated from the date of first diagnosis to first recurrence or progression according to the Kaplan-Meier product-limit method. One patient was lost to follow-up. 27 of the 74 remaining (36.8%) patients showed recurrent disease. In 9 (33.3%) patients with a low-risk pattern disease recurred after a mean (SD) observation time of 29.7 (1.9) months (range 8.3-52.3, median 30.8 (12.4)). 18 (66.7%) patients with a high-risk pattern developed recurrence within a mean (SD) of 17.6 (2.0) months (range 4-38.8, median 16.7 (11.6)). The Kaplan-Meier curve for progression-free survival showed marked differences between the low-risk and the high-risk groups. CONCLUSION: Patients with a high-risk chromosomal pattern have a markedly shorter disease-free survival time and higher progression rate than patients with a low-risk pattern. High-risk patients can therefore be treated more aggressively to prevent tumour spreading.

Michel, M. S., P. Erben, et al. (2004). "Acoustic energy: a new transfection method for cancer of the prostate, cancer of the bladder and benign kidney cells." *Anticancer Res* **24**(4): 2303-8.

Clinical use of gene therapy is limited by the poor efficacy and accuracy of intracellular DNA delivery. Known concepts of DNA transfection have not yet become clinical routine in the treatment of human disorders. We therefore focused on new transfection methods using different forms of acoustic energy as potentially safe and topographically applicable methods for gene delivery in the field of urology. Three different cell lines (prostatic and urothelial cancer, benign kidney) were transfected by

different forms of acoustic energy. The effect of several parameters of electromagnetic shock wave treatment (number and frequency of impulses, energy flow density and plasmid concentration) as well as focused ultrasound on the transfection rate was assessed in a standardized experimental setup. The transfection rate was measured through reporter genes (pEGFP) by FACScan. Transfection by lipofectamine and electroporation served as positive controls. All cell lines were transfectable by acoustic energy. Maximum transfection rate was achieved using focused ultrasound (49.50%; 200 W, 500 ms, 200 microg/ml DNA). 31.3% of kidney cells were transfected by electromagnetic shock waves (1500 impulses, 200 microg/ml DNA, 0.5 mJ/mm² energy density, 2 Hz). Plasmid strand breaks were identified as a limiting factor of the transfection rate. CONCLUSION: Transfection by acoustic energy, especially focused ultrasound, can be achieved at a high level in different cell lines. The possible topical application to urological organs and the low level of side-effects make acoustic energy a promising new gene therapy treatment option in urology.

Mitra, A. P., M. Birkhahn, et al. (2007). "p53 and retinoblastoma pathways in bladder cancer." *World J Urol* **25**(6): 563-71.

A majority of the aggressive, invasive bladder carcinomas have alterations in the p53 and retinoblastoma genes and pathways. Examination of the alterations in the molecules in these pathways that regulate the cell cycle and their effects on the prognosis of bladder cancer are areas of active research. While defects in the p53-Mdm2-p14 axis have been implicated in urothelial cancer, perturbations in the cyclin-dependent kinases and their inhibitors have also been extensively studied in this context. Genetic alterations of the retinoblastoma gene and aberrant post-translational modifications of its protein have also been incriminated in invasive bladder cancer. This article reviews the individual prognostic roles of alterations in these molecules in the context of bladder cancer. Additionally, we review findings from recent studies that are attempting to analyze these markers in combination in an effort to construct molecular panels that can serve as more robust outcome predictors. More importantly, alterations in these molecules are now becoming enticing targets for novel therapeutics. We also review some of these agents that can restore the tumor cells' altered homeostatic mechanisms, thereby having potential in transitional cell carcinoma therapy. Future management of bladder cancer will employ validated marker panels for outcome prediction, and novel genetic and pharmacologic agents that will be able to

target molecular alterations in individual tumors based on their respective profiles.

Mitra, A. P. and R. J. Cote (2009). "Molecular pathogenesis and diagnostics of bladder cancer." *Annu Rev Pathol* **4**: 251-85.

Despite elaborate characterization of the risk factors, bladder cancer is still a major epidemiological problem whose incidence continues to rise each year. Urothelial carcinoma is now recognized as a disease of alterations in several cellular processes. The more prevalent, less aggressive, recurrent, noninvasive tumors are characterized by constitutive activation of the Ras-MAPK pathway. The less common but more aggressive invasive tumors, which have a higher mortality rate, are characterized by alterations in the p53 and retinoblastoma pathways. Several diagnostic tests have attempted to identify these molecular alterations in tumor cells exfoliated in the urine, whereas prognostic tests have tried to identify aberrations so as to predict tumor behavior and identify therapeutic targets. The future of bladder cancer patient management will rely on the use of molecular tests to reliably diagnose the presence of disease, predict individual tumor behavior, and suggest potential targeted therapeutics.

Mitra, A. P., H. Lin, et al. (2006). "Molecular biology of bladder cancer: prognostic and clinical implications." *Clin Genitourin Cancer* **5**(1): 67-77.

The role of various molecular determinants involved in the genesis, progression, and outcome of bladder cancer has been the focus of investigations for the past 2 decades. Increasingly, the analysis of the interplay between these molecular factors is taking center stage. We review herein the studies examining the effects of deregulation of the various molecules implicated in the cell cycle, apoptosis, and angiogenesis pathways and analyze the central role of p53 in regulating these pathways. Technological advancements enable detection and quantification of gene transcripts and protein products, helping us move toward achieving the goal of establishing diagnostic, prognostic, and therapeutic marker panels. Recent studies have therefore focused on multiple-marker analyses to generate informative panels that can have greater clinical value for bladder cancer management. The use of molecular marker panels can provide a more objective alternative to clinical parameters for diagnosis and treatment decisions. Clinical trials aimed at treating urothelial carcinoma based on a patient's molecular profile can be predicted to empower clinicians to personalize patient management through increased therapeutic efficacy.

Miyake, H., H. Eto, et al. (2004). "Synergistic antitumor activity by combined treatment with gemcitabine and antisense oligodeoxynucleotide targeting clusterin gene in an intravesical administration model against human bladder cancer kotcc-1 cells." *J Urol* **171**(6 Pt 1): 2477-81.

We investigated whether antisense (AS) oligodeoxynucleotide (ODN) targeting the clusterin gene enhances the cytotoxic effect of gemcitabine in human bladder cancer KoTCC-1 cells in vitro and in vivo, and evaluated the usefulness of the combined administration of AS clusterin ODN and gemcitabine using an intraperitoneal tumor cell injection model. The cytotoxic effect of combined treatment with AS clusterin ODN and gemcitabine on in vitro KoTCC-1 growth was examined using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The in vivo growth inhibitory effects of combined AS clusterin ODN and gemcitabine therapy on subcutaneous KoTCC-1 tumor was also examined. The intraperitoneal tumor cell injection model, which mimics intravesical administration therapy against bladder cancer, was used to evaluate the efficacy of combined AS clusterin ODN and gemcitabine therapy. AS clusterin ODN treatment of KoTCC-1 cells significantly enhanced gemcitabine chemosensitivity in a dose dependent manner, decreasing gemcitabine IC50 by approximately 90%. In vivo systemic administration of AS clusterin ODN and gemcitabine significantly decreased subcutaneous KoTCC-1 tumor volume compared with scramble control ODN plus gemcitabine. Furthermore, combined administration of AS clusterin ODN plus gemcitabine resulted in significantly delayed formation of hemorrhagic ascites compared with scramble control ODN plus gemcitabine in an intraperitoneal tumor cell injection model. These findings suggest that AS clusterin ODN may be useful for enhancing the cytotoxicity of gemcitabine in patients with bladder cancer, particularly as a novel therapeutic strategy for intravesical instillation therapy.

Miyake, H., K. Yamanaka, et al. (2005). "Therapeutic efficacy of adenoviral-mediated p53 gene transfer is synergistically enhanced by combined use of antisense oligodeoxynucleotide targeting clusterin gene in a human bladder cancer model." *Neoplasia* **7**(2): 171-9.

To establish a more effective therapeutic strategy against advanced bladder cancer, we investigated the effects of combined treatment with antisense (AS) oligodeoxynucleotide (ODN) targeting the anti-apoptotic gene clusterin and adenoviral-mediated p53 gene transfer (Ad5CMV-p53) using the human bladder cancer KoTCC-1 model. Clusterin expression in KoTCC-1 cells was highly upregulated

by Ad5CMV-p53 treatment; however, AS clusterin ODN treatment further suppressed clusterin expression in KoTCC-1 cells after Ad5CMV-p53 treatment. AS clusterin ODN treatment synergistically enhanced the cytotoxic effect of Ad5CMV-p53, and DNA fragmentation characteristic of apoptosis was observed only after combined treatment with AS clusterin ODN and Ad5CMV-p53, but not after treatment with either agent alone. Administration of AS clusterin ODN and Ad5CMV-p53 into nude mice resulted in a significant inhibition of KoTCC-1 tumor growth as well as lymph node metastases compared to administration of either agent alone. Furthermore, combined treatment with AS clusterin ODN, Ad5CMV-p53, and cisplatin completely eradicated KoTCC-1 tumors and lymph node metastases in 60% and 100% of mice, respectively. These findings suggest that combined treatment with AS clusterin ODN and Ad5CMV-p53 could be a novel strategy to inhibit bladder cancer progression, and that further additional use of a chemotherapeutic agent may substantially enhance the efficacy of this combined regimen.

Mori, K., H. Enokida, et al. (2009). "CpG hypermethylation of collagen type I alpha 2 contributes to proliferation and migration activity of human bladder cancer." *Int J Oncol* **34**(6): 1593-602.

In our microarray screening of methylated genes in bladder cancer (BC), the collagen type I alpha 2 (COL1A2) gene was the most up-regulated among the 30,144 genes screened. We hypothesize that inactivation of the COL1A2 gene through CpG methylation contributes to proliferation and migration activity of human BC. We subjected a bladder cancer cell line (BOY) and 67 BC specimens and 10 normal bladder epitheliums (NBEs) to conventional or real-time methylation quantitative polymerase chain reaction (PCR) and to real-time reverse transcriptase (RT)-PCR. We also established a stable COL1A2 transfectant for evaluating cell proliferation and migration activity. After 5-aza-dC treatment, the expression levels of COL1A2 mRNA transcript markedly increased in BOY. Our cell proliferation assays consistently demonstrated growth inhibition in the COL1A2 transfectant compared with control and wild-type BOY cells ($p < 0.0001$). Wound healing assays also showed significant wound healing inhibition in the COL1A2 transfectant compared to the counterparts ($p = 0.0016$). We demonstrated by bisulfite DNA sequencing that the promoter hypermethylation of COL1A2 was a frequent event in clinical BCs. The methylation index of COL1A2 was significantly higher in the 67 BCs than in the 10 NBEs ($p = 0.0011$). Conversely, COL1A2 mRNA transcript was significantly lower in the BCs than in the NBEs

($p = 0.0052$). The mechanism of COL1A2 down-regulation in BC is through CpG hypermethylation of the promoter region. COL1A2 gene inactivation through CpG hypermethylation may contribute to proliferation and migration activity of BC.

Moussa, O., J. M. Riker, et al. (2008). "Inhibition of thromboxane synthase activity modulates bladder cancer cell responses to chemotherapeutic agents." *Oncogene* **27**(1): 55-62.

Recently, we reported prognostic significance of thromboxane synthase (TXAS) gene expression in invasive bladder cancer. The positive correlation between elevated TXAS expression and shorter patient survival supports a potential role for TXAS-regulated pathways in tumor metastases. In this study, using immunohistochemical analysis, we found an increased expression of TXAS protein in bladder cancer. Treatment of T24 and transitional cell carcinoma TCC-SUP bladder cancer cells with the TXAS inhibitors furegrelate or ozagrel induced an apoptotic effect measured as an increase in caspase-3 activation and cell death, and decreased survivin expression. Pharmacological inhibition of TXAS using the TXAS inhibitor furegrelate increased sensitivity to the chemotherapeutic agents cisplatin and paclitaxel. Molecular inhibition of TXAS expression by siRNA significantly decreased cell growth and migration. In concordance with the pharmacological data, siRNA-mediated reduction of TXAS expression increased sensitivity to cisplatin and paclitaxel in T24 and TCC-SUP cells. In summary, the data support a role for the thromboxane A(2) pathway in the pathogenesis of bladder cancer and the potential utility of modulation of this signaling pathway for cancer chemotherapy.

Moussa, O., J. S. Yordy, et al. (2005). "Prognostic and functional significance of thromboxane synthase gene overexpression in invasive bladder cancer." *Cancer Res* **65**(24): 11581-7.

Thromboxane synthase (TXAS) is one of the enzymes downstream from cyclooxygenase-2 and catalyzes the synthesis of thromboxane A(2) (TXA(2)). TXAS was among the genes we identified based on its overexpression in invasive bladder tumors. TXAS is overexpressed in common forms of bladder tumors: 69 of 97 (71.1%) transitional cell carcinoma (TCC), 38 of 53 (71.6%) squamous cell carcinoma, and 5 of 11 (45.5%) adenocarcinoma relative to nontumor tissue. Overall, 112 of 161 (69.5%) invasive tumors exhibited elevated expression. Significantly, patients with tumors having >4-fold levels of TXAS expression showed significant statistical evidence of lower overall survival expressed by the estimated hazard ratio of 2.74 with $P = 0.009$ in

Cox's regression analysis. TXAS mRNA expression was found to be an independent prognostic marker for patients with bladder cancer. Treatment of bladder cancer cell lines (T24 and TCC-SUP) with TXAS inhibitors and TXA(2) (TP) receptor antagonists reduced cell growth, migration, and invasion, whereas TP agonists stimulated cell migration and invasion. The positive correlation between elevated TXAS expression and shorter patient survival supports a potential role for TXAS-regulated pathways in tumor invasion and metastases and suggests that modulation of the TXAS pathway may offer a novel therapeutic approach.

Muramaki, M., H. Miyake, et al. (2003). "Introduction of midkine gene into human bladder cancer cells enhances their malignant phenotype but increases their sensitivity to antiangiogenic therapy." *Clin Cancer Res* **9**(14): 5152-60.

Midkine (MK) is a member of a family of heparin-binding growth factors, which was reported to have an important role in angiogenesis. Although MK was reported to be associated with bladder cancer progression, the functional significance of MK expression in bladder cancer progression has not been elucidated. The objectives of this study were to determine whether overexpression of MK in bladder cancer cells enhances their malignant potential and to evaluate the inhibitory effect of the antiangiogenic agent TNP-470 on the growth of MK-overexpressing bladder cancer cells in vivo. We introduced the MK gene into human bladder cancer UM-UC-3 cells that do not secrete a detectable level of MK protein and generated the MK-overexpressing cell line UM-UC-3/MK. The biological activity of secreted MK was evaluated using a human umbilical vein endothelial cell proliferation assay. To investigate the in vivo effects of MK overexpression on tumor growth, each cell line was injected s.c. and orthotopically into nude mice. To evaluate the therapeutic effects of the antiangiogenic agent, mice were given TNP-470 after s.c. injection of each cell line. The microvessel density of tumors was quantitated by immunohistochemistry of CD31. The heparin affinity-purified conditioned media of UM-UC-3/MK cells significantly enhanced human umbilical vein endothelial cell proliferation. MK expression had no effect on in vitro growth but conferred a growth advantage on both s.c. and orthotopic tumors in vivo. Furthermore, enhanced tumor growth was closely associated with increased microvessel density. Significant inhibition of tumor growth by TNP-470 treatment was observed only in UM-UC-3/MK tumors and not in control tumors. We demonstrated that overexpression of the MK gene causes an increase in the angiogenic activity of cells through vascular endothelial cell growth, resulting in

enhanced malignant potential of human bladder cancer cells. Moreover, the present findings suggest that TNP-470 could be used as a novel therapeutic adjunct to conventional agents for patients with advanced bladder cancer overexpressing MK.

Muramaki, M., H. Miyake, et al. (2004). "Over expression of CD44v8-10 in human bladder cancer cells decreases their interaction with hyaluronic acid and potentiates their malignant progression." *J Urol* **171**(1): 426-30.

Muramaki et al evaluated the effects of the over expression of CD44v8-10, one of the high molecular form of CD44, on the malignant potential of UM-UC-3 human bladder cancer cells in vitro and in vivo. Muramaki et al introduced CD44v8-10 complementary DNA into UM-UC-3 cells, which do not express detectable level of CD44v8-10 protein, and generated the CD44v8-10 over expressing cell line UM-UC-3/v8-10 and the vector only transfected cell line UM-UC-3/C. The effects of the introduction of CD44v8-10 into UM-UC-3 cells on the ability to bind hyaluronic acid (HA) were analyzed by the cell adhesion assay and the cell migration assay. We then evaluated tumor progression of UM-UC-3 sublines after subcutaneous and orthotopic injection into athymic nude mice. There were no significant differences in in vitro growth rates. UM-UC-3/v8-10 cells showed significantly decreased binding and migration ability to HA but not to other extracellular matrix proteins. Furthermore, UM-UC-3/v8-10 cells demonstrated significantly increased tumor growth after subcutaneous as well as orthotopic injection into athymic nude mice and enhanced lymph node metastasis after orthotopic injection compared with UM-UC-3/C cells. These findings suggest that CD44v8-10 over expression in human bladder cancer cells decreases their interaction with HA and potentiates their malignant progression.

Muramaki, M., A. So, et al. (2009). "Chemosensitization of gemcitabine-resistant human bladder cancer cell line both in vitro and in vivo using antisense oligonucleotide targeting the anti-apoptotic gene, clusterin." *BJU Int* **103**(3): 384-90.

To characterize changes in clusterin (sCLU-2) expression in bladder cancer cells after continuous treatment with gemcitabine and to determine whether knockdown of sCLU-2 can re-introduce sensitivity of gemcitabine-resistant cells to treatment with gemcitabine. A human bladder cancer cell line, UM-UC-3, was continuously exposed to increasing doses of gemcitabine in vitro, and a gemcitabine-resistant cell line UM-UC-3R was developed. The role of sCLU-2 in chemoresistant phenotype acquired in both in vitro and in vivo was then analysed using antisense

oligonucleotide targeting the sCLU-2 gene (OGX-011). Treatment of parental UM-UC-3 cells (UM-UC-3P) with gemcitabine induced transient up-regulation of sCLU-2 protein. There was a sustained increase in sCLU-2 expression levels in UM-UC-3R compared with UM-UC-3P cells (6.4-fold). Treatment of UM-UC-3R cells with OGX-011 resulted in a dose-dependent and sequence-specific inhibition in sCLU-2 expression. Furthermore, OGX-011 chemosensitized UM-UC-3R cells to gemcitabine in vitro with a reduction in the concentration that reduces the effect by 50% (IC₅₀) from 100 nm to 10 nm. Tumour volume and the incidence of metastasis in nude mice injected with UM-UC-3R cells was significantly greater than those of nude mice injected with UM-UC-3P cells; however, systemic administration of OGX-011 plus a low dose of gemcitabine significantly suppressed tumour volume and the incidence of metastasis in both groups. CONCLUSION: These findings suggest that sCLU-2 plays a significant role in the acquisition of chemoresistant phenotype in bladder cancer cells and the knockdown of sCLU-2 using OGX-011 combined with a chemotherapeutic agent could be an attractive approach for advanced bladder cancer through the enhancement of chemosensitivity.

Nagabhushan, T. L., D. C. Maneval, et al. (2007). "Enhancement of intravesical delivery with Syn3 potentiates interferon-alpha2b gene therapy for superficial bladder cancer." *Cytokine Growth Factor Rev* **18**(5-6): 389-94.

Intravesical administration of interferon alpha-2b protein (IFN) has been successfully used in the treatment of patients with superficial bladder tumors. Local dosing of IFN minimizes well-known systemic side effects of the drug, but exposure to bladder tumors is limited by the duration of instillation and transient concentrations achieved in the urothelium. Intravesical delivery of the gene encoding interferon results in an alternative strategy for IFN-based therapy of the disease, enabling sustained exposure of IFN protein that results from production by tumor and non-tumor cells in the urothelium. Efficient gene delivery and expression of IFN has been achieved using a recombinant adenovirus gene delivery system (rAd-IFN) in conjunction with the novel small molecule excipient Syn3. Studies with rAd-IFN/Syn3 in animal models result in urine concentrations of IFN that persisted for weeks and correlated with potent anti-tumor effects. The objective of this review is to communicate the rationale and preclinical findings that support ongoing clinical investigation of intravesical rAd-IFN/Syn3 in superficial bladder cancer.

Nawrocki, S., T. Skacel, et al. (2003). "From microarrays to new therapeutic approaches in bladder cancer." *Pharmacogenomics* **4**(2): 179-89.

Bladder cancer is a model tumor progressing from superficial to locally invasive and finally metastatic disease. The likelihood of progression is determined to a large extent by the molecular profile of the tumor. From the pre-genomic era only p53 emerged as the molecular prognostic factor able to add value to existing clinical and pathological features of bladder cancer, however it was not introduced to the clinic. Microarray technologies enable us to study expression of thousands of genes in the tumor tissue. This method has already proven to add information to clinical classifiers, to find new tumor suppressor genes and to define p53 related pathways of cell-cycle regulation. In the last decade, progress in the treatment of locally invasive and metastatic bladder cancer was minimal; large Phase III trials with neo/adjuvant chemotherapy were inconclusive. The new paradigm of treatment tailored to an individual patient could be realized in bladder cancer for his chronic clinical course with opportunities to obtain tumor samples for microarray studies. Molecular profiling of two samples taken at the superficial stage and at cystectomy should enable us to study the microevolution of the tumor, to tailor existing treatment options, and to introduce new biologicals to the clinic. Introduction

Nicholson, B. E., H. F. Frierson, et al. (2004). "Profiling the evolution of human metastatic bladder cancer." *Cancer Res* **64**(21): 7813-21.

Pulmonary metastases frequently develop in patients with aggressive bladder cancer, yet investigation of this process at the molecular level suffers from the poor availability of human metastatic tumor tissue and the absence of suitable animal models. To address this, we developed progressively more metastatic human bladder cancer cell lines and an in vivo bladder-cancer lung-metastasis model, and we successfully used these to identify genes of which the expression levels change according to the degree of pulmonary metastatic potential. By initially intravenously injecting the poorly metastatic T24T human urothelial cancer cells into nude mice, and then serially reintroducing and reisolating the human tumor cells from the resultant mouse lung tumors, three derivative human lines with increasingly metastatic phenotypes, designated FL1, FL2, and FL3, were sequentially isolated. To identify the genes associated with the most lung-metastatic phenotype, the RNA complement from the parental and derivative cells was evaluated with oligonucleotide microarrays. In doing so, we found 121 genes to be progressively up-regulated during the transition from T24T to FL3,

whereas 43 genes were progressively down-regulated. As expected, many of the genes identified in these groups could, according to the ascribed functions of their protein product, theoretically participate in tissue invasion and metastasis. In addition, the magnitude of gene expression changes observed during the metastatic transition correlated with the in vivo propensity for earlier lung colonization and decreased host survival. To additionally define which genes found in the experimental system were of relevance to human bladder cancer lung metastasis, we evaluated gene expression profiles of 23 primary human bladder tumors of various stages and grades, and then we compared these gene expression profiles to the altered profiles in our model cell lines. Here we found that the expression of epiregulin, urokinase-type plasminogen activator (uPA), matrix metalloproteinase (MMP)14, and tissue inhibitor of metalloproteinase (TIMP-2) were consistently and progressively up-regulated when viewed as a function of tumor stage in tissues of patients versus the metastatic potential seen in the mouse lung model. The strong correlation of these four markers between the experimental and clinical situations helps validate this system as a useful tool for the study of lung metastasis and defines targets of therapy that may reduce the incidence of this process in patients.

Ning, Z. F., Y. J. Huang, et al. (2009). "Subpopulations of stem-like cells in side population cells from the human bladder transitional cell cancer cell line T24." *J Int Med Res* **37**(3): 621-30.

Cancer stem cells can be isolated from human tumours using specific cell surface markers. Bladder cancer cells, however, lack specific cell surface markers, making this approach impracticable. In this study an alternative method was used, involving isolation of side population cells to explore the stem cell characteristics of bladder cancer. Side population cells were isolated from the bladder transitional cell cancer cell line T24 and examined for potential stem cell characteristics related to proliferation, cell cycle distribution, self-renewal and differentiation. It was observed that T24 side population cells have stronger proliferative and colony formation abilities than non-side population cells. Side population cells were also more resistant to chemotherapy and radiotherapy, which may be due to expression of the ATP-binding cassette half-transporter, sub-family G, member 2 protein. Overall, the results suggest that side population cells from the human bladder transitional cell cancer cell line T24 harbour stem-like cells.

Nishiyama, H., J. Watanabe, et al. (2008). "p53 and chemosensitivity in bladder cancer." *Int J Clin Oncol* **13**(4): 282-6.

Urothelial carcinoma is the second most common genitourinary malignancy. Although the majority of patients present with superficial bladder tumors, there are several clinical problems, such as progression to invasive tumors, poor prognosis of invasive tumors, and chemosensitivity. Alterations in p53 represent one of the most common genetic events in patients with invasive urothelial carcinoma and are suggested to be linked to tumor progression, prognosis, and chemosensitivity. p53 possesses various functions, including induction of cell-cycle arrest, apoptosis, DNA repair, and antioxidants; it acts as a killer and a healer. In this article, we review the roles of p53 pathways in bladder carcinogenesis and findings from recent studies of ours and other groups, and we discuss the clinical significance of the abrogation of p53 pathways in the treatment of urothelial carcinoma.

Nixdorf, S., M. O. Grimm, et al. (2004). "Expression and regulation of MIM (Missing In Metastasis), a novel putative metastasis suppressor gene, and MIM-B, in bladder cancer cell lines." *Cancer Lett* **215**(2): 209-20.

It has been proposed that a 356 amino acid protein encoded by the MIM (Missing In Metastasis) gene on Chromosome 8q24.1, is a bladder cancer metastasis suppressor. Recently, Machesky and colleagues [*Biochem. J.* 371 (2003) 463] identified MIM-B, a 759 amino acid protein, of which the C-terminal 356 amino acids are almost identical to MIM. Importantly, PCR primers and Northern Blotting probes used in the studies of MIM in bladder cancer did not distinguish between sequences specific for MIM or MIM-B, thus the importance of either protein to bladder cancer remains unclear. We have used primer sequences specific for either MIM or MIM-B to explore the possible functional significance of MIM and MIM-B to bladder cancer cell behaviour. We have compared MIM and MIM-B mRNA levels in a non-tumourigenic, non-invasive, transformed uroepithelial cell line versus 15 bladder cancer cell lines of differing in vitro invasive abilities, as well as in five cell lines clonally isolated from the BL17/2 bladder tumour cell line, whose in vitro and in vivo invasive abilities have been determined. MIM and MIM-B mRNA levels varied widely between cell lines. Down-regulation of MIM and MIM-B occurred in 6/15 (40%) lines but lines showing down-regulation differed between MIM and MIM-B. Reduced levels of MIM and MIM-B in BL17/2 were further reduced in 2/5 (40%) sublines (MIM and MIM-B). Importantly, there was no association between MIM or MIM-B

expression and invasive behaviour in vivo or in vitro. Treatment of representative cell lines with 5-aza-2-deoxycytidine failed to induce MIM or MIM-B expression. Furthermore, there was no association between MIM or MIM-B mRNA levels and p53 functional status. Our data indicate that down-regulation of MIM and/or MIM-B expression can occur in bladder cancer cell lines but is not associated with increased invasive behaviour. Our data also suggest that in those cell lines with reduced levels of MIM and MIM-B mRNA, down-regulation is unlikely to be due to promoter hypermethylation or loss of p53 function.

Nogawa, M., T. Yuasa, et al. (2005). "Intravesical administration of small interfering RNA targeting PLK-1 successfully prevents the growth of bladder cancer." *J Clin Invest* **115**(4): 978-85.

The mainstay in the management of invasive bladder cancer continues to be radical cystectomy. With regard to improvement of quality of life, however, therapies that preserve the bladder are desirable. We investigated the use of intravesical PLK-1 small interfering RNA (siRNA) against bladder cancer. Patients with bladder cancers expressing high levels of PLK-1 have a poor prognosis compared with patients with low expression. Using siRNA/cationic liposomes, the expression of endogenous PLK-1 could be suppressed in bladder cancer cells in a time- and dose-dependent manner. As a consequence, PLK-1 functions were disrupted. Inhibition of bipolar spindle formation, accumulation of cyclin B1, reduced cell proliferation, and induction of apoptosis were observed. In order to determine the efficacy of the siRNA/liposomes in vivo, we established an orthotopic mouse model using a LUC-labeled bladder cancer cell line, UM-UC-3(LUC). PLK-1 siRNA was successfully transfected into the cells, reduced PLK-1 expression, and prevented the growth of bladder cancer in this mouse model. This is the first demonstration, to our knowledge, of inhibition of cancer growth in the murine bladder by intravesical siRNA/cationic liposomes. We believe intravesical siRNA instillation against bladder cancer will be useful as a therapeutic tool.

Nomura, T., M. Nakagawa, et al. (2002). "Clinical significance of thymidylate synthase expression in bladder cancer." *Int J Urol* **9**(7): 368-76.

The purpose of the present paper was to investigate the clinical significance of thymidylate synthase (TS) expression in bladder cancer and its association with proliferation markers, such as p53, Ki-67, and proliferating cell nuclear antigen (PCNA). Thymidylate synthase gene expression in 54 patients

with bladder cancer was measured by the reverse transcription polymerase chain reaction (RT-PCR) method using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal standard. The TS expression was also examined immunohistochemically. Immunohistochemical studies of p53, Ki-67, and PCNA expression were carried out to examine the correlation between TS expression and the expression of proliferation markers in the tumors. Prognostic and clinical outcome factors such as vascular invasion and distant metastasis were also examined along with TS expression. Twenty-four patients with invasive bladder cancer had TS levels of 5.07 +/- 0.77 (mean +/- SE), while 30 patients with superficial bladder cancer had TS levels of 2.28 +/- 0.38. There was a significant difference in TS levels between invasive and superficial bladder cancer ($P = 0.001$). There was a positive correlation between TS expression and each proliferation marker of p53 ($r = 0.686$, $P < 0.01$), Ki-67 ($r = 0.715$, $P < 0.0001$) or PCNA expressions ($r = 0.670$, $P < 0.0001$) in these patients. Patients with high TS levels (TS ≥ 2.63 , the median value) had significantly higher rates of vascular invasion and distant metastasis. Kaplan-Meier analysis demonstrated that patients with high TS levels (TS ≥ 2.63) had unfavorable prognosis compared to patients with low TS levels (TS < 2.63 ; $P = 0.034$). Furthermore, patients with high TS staining had a significantly poorer prognosis than those with low staining ($P = 0.012$). **CONCLUSION:** Determination of level of TS expression may help in the selection of an appropriate treatment for bladder cancer because TS expression influences the biological characteristic of bladder tumor.

Ogishima, T., H. Shiina, et al. (2005). "Promoter CpG hypomethylation and transcription factor EGR1 hyperactivate heparanase expression in bladder cancer." *Oncogene* **24**(45): 6765-72.

Heparanase plays a critical role in the degradation of extracellular matrix and cell membrane and is frequently upregulated in malignant tumors. Transcription factor, early growth response 1 (EGR1), is closely associated with inducible transcription of the heparanase gene. We hypothesized that promoter CpG hypomethylation with increased EGR1 expression could determine heparanase expression during the pathogenesis of bladder cancer. Bladder cancer cell lines (J82, T24 and transitional cell carcinoma) significantly restored heparanase expression after 5-Aza-dC treatment. Transfection of EGR1 siRNA with T24 bladder cancer cell line significantly downregulated heparanase expression compared to the control siRNA transfection. In 54 bladder cancer and paired normal bladder samples, heparanase expression was significantly higher in

bladder cancer than in normal bladder ($P < 0.01$). We performed methylation-specific PCR targeting the CpG sites within the core-binding consensus motifs of EGR1 (GGCG) and Sp1 (GGGCGG). Methylation prevalence was significantly higher in normal bladder than in bladder cancer ($P < 0.05$) and inversely correlated with heparanase expression ($P = 0.055$). In the total series of bladder cancer and normal bladder samples, the combination of promoter CpG methylation and EGR1 expression regulated heparanase expression in a stepwise manner, where heparanase expression was the lowest in methylation-positive and EGR1-negative samples and the highest in methylation-negative and EGR1-positive samples. To our knowledge, this is the first study demonstrating that increased heparanase expression during the pathogenesis of bladder cancer is due to promoter hypomethylation and transcription factor EGR1.

O'Kane, H. F., C. J. Watson, et al. (2006). "Targeting death receptors in bladder, prostate and renal cancer." *J Urol* **175**(2): 432-8.

O'Kane et al describe key components of normal and aberrant death receptor pathways, the association of these abnormalities with tumorigenesis in bladder, prostate and renal cancer, and their potential application in novel therapeutic strategies targeted toward patients with cancer. A MEDLINE literature search of the key words death receptors, TRAIL (tumor necrosis factor related apoptosis inducing ligand), FAS, bladder, prostate, renal and cancer was done to obtain information for review. A brief overview of the TRAIL and FAS death receptor pathways, and their relationship to apoptosis is described. Mechanisms that lead to nonfunction of these pathways and how they may contribute to tumorigenesis are linked. Current efforts to target death receptor pathways as a therapeutic strategy are highlighted. Activation of tumor cell expressing death receptors by cytotoxic immune cells is the main mechanism by which the immune system eliminates malignant cells. Death receptor triggering induces a caspase cascade, leading to tumor cell apoptosis. Receptor gene mutation or hypermethylation, decoy receptor or splice variant over expression, and downstream inhibitor interference are examples of the ways that normal pathway functioning is lost in cancers of the bladder and prostate. Targeting death receptors directly through synthetic ligand administration and blocking downstream inhibitor molecules with siRNA or antisense oligonucleotides represent novel therapeutic strategies under development. Research into the death receptor pathways has demonstrated the key role that pathway aberrations have in the initiation and progression of malignancies of the bladder, prostate and kidney. This

new understanding has resulted in exciting approaches to restore the functionality of these pathways as a novel therapeutic strategy.

Oliveira-Ferrer, L., D. Tilki, et al. (2004). "Dual role of carcinoembryonic antigen-related cell adhesion molecule 1 in angiogenesis and invasion of human urinary bladder cancer." *Cancer Res* **64**(24): 8932-8.

Here, we show that carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) is expressed in umbrella cells of bladder urothelium but is down-regulated in superficial bladder cancer, such as histologic tumor stage a (pTa) and transitional cell carcinoma in situ (pTis). Concurrently, CEACAM1 is up-regulated in the endothelia of adjacent angiogenic blood vessels. Mimicking the CEACAM1 down-regulation in the urothelium, CEACAM1 was silenced in bladder cancer cell lines 486p and RT4 using the small interfering RNA technique. CEACAM1 down-regulation was confirmed at the protein level by Western blot analyses. CEACAM1 silencing leads to a significant up-regulation of vascular endothelial growth factor (VEGF)-C and VEGF-D in quantitative reverse transcription-PCR. Correspondingly, supernatants from the CEACAM1-overexpressing bladder cancer cell lines reduce, but those from CEACAM1 silencing induce endothelial tube formation and potentiate the morphogenetic effects of VEGF. These data suggest that the epithelial down-regulation of CEACAM1 induces angiogenesis via increased expression of VEGF-C and VEGF-D. Inversely, CEACAM1 is up-regulated in endothelial cells of angiogenic blood vessels. This in turn is involved in the switch from noninvasive and nonvascularized to invasive and vascularized bladder cancer. CEACAM1 appears to be a promising endothelial target for bladder cancer therapy.

Orenes-Pinero, E., M. Corton, et al. (2007). "Searching urinary tumor markers for bladder cancer using a two-dimensional differential gel electrophoresis (2D-DIGE) approach." *J Proteome Res* **6**(11): 4440-8.

Aiming at identifying biomarkers for bladder cancer, the urinary proteome was explored through a two-dimensional gel-based proteomic approach (2D-DIGE) coupled with mass spectrometry and database interrogation. The increased expression of proteins differentially expressed between patients with bladder tumors and controls such as Reg-1 and keratin 10 was confirmed to be associated with bladder cancer progression on bladder cancer cell lines by immunoblotting, and bladder tumors by immunohistochemistry. Moreover, the association of these proteins, especially Reg-1, with tumor staging

and clinical outcome was confirmed by immunohistochemistry using an independent series of bladder tumors contained in tissue microarrays (n=292). Furthermore, Reg-1 was quantified using an independent series of urinary specimens (n=80) and provided diagnostic utility to discriminate patients with bladder cancer and controls (area under the curve (AUC=0.88)). Thus, the 2D-DIGE approach has identified Reg-1 as a biomarker for bladder cancer diagnostics, staging, and outcome prognosis.

Osman, I., D. F. Bajorin, et al. (2006). "Novel blood biomarkers of human urinary bladder cancer." *Clin Cancer Res* **12**(11 Pt 1): 3374-80.

Recent data indicate that cDNA microarray gene expression profile of blood cells can reflect disease states and thus have diagnostic value. We tested the hypothesis that blood cell gene expression can differentiate between bladder cancer and other genitourinary cancers as well as between bladder cancer and healthy controls. We used Affymetrix U133 Plus 2.0 GeneChip (Affymetrix, Santa Clara, CA) to profile circulating blood total RNA from 35 patients diagnosed with one of three types of genitourinary cancer [bladder cancer (n = 16), testicular cancer (n = 10), and renal cell carcinoma (n = 9)] and compared their cDNA profiles with those of 10 healthy subjects. We then verified the expression levels of selected genes from the Affymetrix results in a larger number of bladder cancer patients (n = 40) and healthy controls (n = 27). Blood gene expression profiles distinguished bladder cancer patients from healthy controls and from testicular and renal cancer patients. Differential expression of a combined set of seven gene transcripts (insulin-like growth factor-binding protein 7, sorting nexin 16, chondroitin sulfate proteoglycan 6, and cathepsin D, chromodomain helicase DNA-binding protein 2, nell-like 2, and tumor necrosis factor receptor superfamily member 7) was able to discriminate bladder cancer from control samples with a sensitivity of 83% (95% confidence interval, 67-93%) and a specificity of 93% (95% confidence interval, 76-99%). CONCLUSION: We have shown that the gene expression profile of circulating blood cells can distinguish bladder cancer from other types of genitourinary cancer and healthy controls and can be used to identify novel blood markers for bladder cancer.

Pagliaro, L. C., A. Keyhani, et al. (2003). "Adenoviral p53 gene transfer in human bladder cancer cell lines: cytotoxicity and synergy with cisplatin." *Urol Oncol* **21**(6): 456-62.

Mutations of the tumor suppressor gene p53 are common in bladder cancer. To determine whether p53 gene transfer would lead to decreased viability of

bladder cancer cells, we studied the effect of p53 gene transfer in human bladder cancer cell lines with either mutant or wild-type p53. Bladder cancer cell lines 5637 and J82 (which express only mutant p53) and 253J-BV (which expresses wild-type p53) were transduced with vectors containing the beta-galactosidase gene (Ad5-lacZ), wild-type human p53 gene (Ad5CMV-p53), or no foreign gene (DL312 or Ad5-polyA). X-gal staining of cells exposed to Ad5-lacZ showed that the adenoviral vector was capable of transducing each of the cell lines. Increases in p53, p21(waf1/cip1) and bax protein were demonstrated following exposure to Ad5CMV-p53, and there was a dose-dependent increase in the number of apoptotic cells. Cell viability was decreased in all three cell lines, although J82 was less sensitive than either 5637 or 253J-BV. To determine whether cisplatin increases sensitivity of J82 cells to Ad5CMV-p53, we performed median effect analysis for cisplatin combined with Ad5CMV-p53 or DL312. The combination index for cisplatin plus Ad5CMV-p53 revealed synergy, whereas cisplatin and DL312 were only additive. These results suggest that forced p53 gene expression is cytotoxic to human bladder cancer cells with either p53 mutant or wild-type background, and that combination with cisplatin is a potential method for overcoming resistance.

Pagliaro, L. C., A. Keyhani, et al. (2003). "Repeated intravesical instillations of an adenoviral vector in patients with locally advanced bladder cancer: a phase I study of p53 gene therapy." *J Clin Oncol* **21**(12): 2247-53.

We investigated the feasibility, safety, and biologic activity of adenovirus-mediated p53 gene transfer in patients with locally advanced bladder cancer. Patients with measurable, locally advanced transitional-cell carcinoma of the bladder who were not candidates for cystectomy were eligible. On a 28-day cycle, intravesical instillations of INGN 201 (Ad5CMV-p53) were administered on days 1 and 4 at three dose levels (10(10) particles to 10(12) particles) or on either 4 or 8 consecutive days at a single dose level (10(12) particles). Thirteen patients received a total of 22 courses without dose-limiting toxicity. Specific transgene expression was detected by reverse transcriptase polymerase chain reaction in bladder biopsy tissue from two of seven assessable patients. There were no changes in p53, p21waf1/cip1, or bax protein levels in bladder epithelium evident from immunohistochemical analysis of 11 assessable patients. Outpatient administration of multiple courses was feasible and well tolerated. A patient with advanced superficial bladder cancer showed evidence of tumor response. CONCLUSION: Intravesical instillation of Ad5CMV-p53 is safe, feasible, and

biologically active when administered in multiple doses to patients with bladder cancer. Observations from this study indicate that this treatment has an antitumor effect in superficial transitional-cell carcinoma. Improvements in the efficiency of gene transfer and the levels of gene expression are required to develop more effective gene therapy for bladder cancer.

Palit, V., R. M. Phillips, et al. (2005). "Expression of HIF-1alpha and Glut-1 in human bladder cancer." *Oncol Rep* **14**(4): 909-13.

HIF-1 is a heterodimer consisting of the HIF-1alpha and HIF-1beta subunits, and HIF-1alpha is the unique oxygen regulated subunit that determines HIF-1 activity. HIF-1alpha upgrades many gene products which include the glucose transporter protein 1 (Glut-1). Immunohistochemical studies using a monoclonal antibody specific for HIF-1alpha indicate that the overexpression of HIF-1alpha occurs in the most common forms of human cancer, including bladder cancer. The expression of Glut-1 in human bladder cancer is associated with poor prognosis and a low survival rate. To our knowledge, this is the first study to compare the expression of both HIF-1alpha and Glut-1 with clinicopathological characteristics in superficial and invasive human bladder cancer (all invasive bladder cancer patients received radical radiotherapy). The Kaplan-Meier survival analysis curve shows a significant association of HIF-1alpha expression with recurrence and survival in superficial bladder cancer and shows a significant association of Glut-1 with survival in invasive bladder cancer [$\chi^2(4)=10.52$; $P > \chi^2=0.0012$].

Park, H. S., W. S. Park, et al. (2008). "Quantitation of Aurora kinase A gene copy number in urine sediments and bladder cancer detection." *J Natl Cancer Inst* **100**(19): 1401-11.

Chromosome missegregation and the resulting aneuploidy is a common change in neoplasia. The Aurora kinase A (AURKA) gene, which encodes a key regulator of mitosis, is frequently amplified and/or overexpressed in cancer cells, and the level of AURKA amplification is associated with the level of aneuploidy. We examined whether AURKA gene amplification is a biomarker for the detection of bladder cancer. The effect of ectopic expression of Aurora kinase A (AURKA) using an adenoviral vector in simian virus 40-immortalized urothelial cells (SV-HUC) on centrosome multiplication and chromosome copy number was measured in vitro by immunofluorescence and fluorescence in situ hybridization (FISH), respectively. The FISH test was also used to examine AURKA gene copy number in exfoliated cells in voided urine samples from 23

patients with bladder cancer and 7 healthy control subjects (training set), generating a model for bladder cancer detection that was subsequently validated in an independent set of voided urine samples from 100 bladder cancer patients and 148 control subjects (92 healthy individuals and 56 patients with benign urologic disorders). An AURKA gene score (the proportion of cells with three or more AURKA signals) was used to produce receiver operating characteristic (ROC) curves and to calculate the specificity and sensitivity of the AURKA FISH test. Differences between mean AURKA scores in different pathogenetic groups of bladder cancer stratified according to histological grade and stage were tested by unpaired Mann-Whitney t tests or one-way Wilcoxon tests. All statistical tests were two-sided. Forced overexpression of AURKA in urothelial cells induced amplification of centrosomes, chromosome missegregation, and aneuploidy, and natural overexpression was detectable in in situ lesions from patients with bladder cancer. The FISH test for the AURKA gene copy number performed on the validation set yielded a specificity of 96.6% (95% confidence interval [CI] = 92.3% to 98.5%) and sensitivity of 87% (95% CI = 79.0% to 92.2%) and an area under the ROC curve of 0.939 (95% CI = 0.906 to 0.971; $P < .001$). CONCLUSION: Overexpression of AURKA can cause aneuploidy in urothelial cells, and the AURKA gene copy number is a promising biomarker for detection of bladder cancer.

Patard, J. J., A. Rodriguez, et al. (2003). "The current status of intravesical therapy for superficial bladder cancer." *Curr Opin Urol* **13**(5): 357-62.

PURPOSE OF REVIEW: To analyse recent advances in intravesical instillation therapy for superficial bladder cancer. **RECENT FINDINGS:** Although intravesical bacillus Calmette-Guerin has been used for many years in the treatment of superficial bladder cancer, its mechanism of action remains unclear, its poor tolerance remains a problem, the prediction of its efficacy has still to be validated, and its long-term effects on progression and survival are controversial. The exact timing and place of intravesical chemotherapy needs to be better defined, as well as the place of some new molecules. Finally, new approaches need to be explored for overcoming the limitations of the usual intravesical agents. **SUMMARY:** No dramatic advances have been made in understanding the mechanisms of action of bacillus Calmette-Guerin during the past year. However, a careful dissection of this complex immunological pathway continues and immunological criteria are promising for predicting the response to bacillus Calmette-Guerin. Evidence has been accumulating to suggest that a dose reduction during the initial

treatment remains effective and reduces side-effects. In addition, bacillus Calmette-Guerin maintenance therapy is useful for high-risk patients. However, long-term tolerance remains an important issue, and the optimal protocol has not yet been defined. On the other hand, it has been proved that intravesical chemotherapy, when administered early after transurethral resection, is effective in preventing frequent recurrences, whereas maintenance chemotherapy is ineffective. Finally, new approaches, including instillations of activated immune cells or targeted gene therapy, are being explored.

Philips, B. J., C. H. Coyle, et al. (2009). "Induction of apoptosis in human bladder cancer cells by green tea catechins." *Biomed Res* **30**(4): 207-15.

Cell culture and animal studies have demonstrated strong chemopreventative effects of green tea and its associated polyphenols in multiple cancers, though the exact mechanisms of action are not well understood. This *in vitro* study examined the antiproliferative/pro-apoptotic potential of green tea extract (GTE), polyphenon-60 (PP-60), (-)-epicatechin gallate (ECG) and (-)-epigallocatechin-3-gallate (EGCG) in both normal and malignant human bladder cells. Cell growth (proliferation/apoptosis) was measured in UROtsa (normal), SW780 (tumorigenic; low-grade), and TCCSUP (tumorigenic; high-grade) human bladder urothelial cells by cell proliferation (XTT) assay after treatment with 0-80 microg/mL of GTE, PP-60, ECG and EGCG for 72 h. Molecular signaling pathways of catechin-induced apoptosis were analyzed using Human signal transduction RT(2) Profiler PCR array (SuperArray). Compared to control-treated cells, treatment with catechin agents significantly suppressed cell growth in a dose-dependent fashion ($P < 0.01$), with strongest effects evoked by ECG and EGCG in UROtsa cells, ECG in low-grade RT4 and SW780 cells, and PP-60 and EGCG in high-grade TCCSUP and T24 cells. Microarray analysis indicated distinct differences in mRNA gene expression regarding growth signaling pathway activation induced by EGCG in normal/tumorigenic human bladder cell lines, providing a rationale for the putative therapeutic usage of green tea polyphenols against bladder disease.

Picard, V., A. Bergeron, et al. (2007). "MAGE-A9 mRNA and protein expression in bladder cancer." *Int J Cancer* **120**(10): 2170-7.

In a previous analysis, we showed that MAGE-As were the most frequently expressed cancer-testis antigens in human bladder tumours. Here, we further characterized by RT-PCR the expression of this family of genes by analyzing specifically MAGE-A3, -A4, -A8 and -A9 mRNAs in

46 bladder tumours and 10 normal urothelia. We found that they were expressed in 30, 33, 56 and 54% of tumours, respectively. Although MAGE-A8 was the most frequent, its expression was low and was also found in most normal urothelia. The other MAGE-A mRNAs were all tumour-specific but MAGE-A9 mRNA was expressed at a higher level and was two times more frequent in superficial than in invasive tumours. To study the expression of the protein, we produced 2 MAGE-A9-specific monoclonal antibodies (mAbs) presenting no cross-reactivity with other MAGE-A proteins. MAb 14A11, was used to analyse the expression of the antigen in testis and tumour samples by immunohistochemistry. In testis, MAGE-A9 expression was restricted to primary spermatocytes. Most bladder tumours that expressed the MAGE-A9 transcript were positive with mAb 14A11. Staining was heterogeneous but half of the tumours showed over 75% positive cells. Finally, we showed that treatment of bladder cancer cells with the methylation inhibitor, 5-aza-2'-deoxycytidine, alone or in combination with the histone deacetylase inhibitors MS-275 and 4-phenylbutyrate could strongly induce the expression of MAGE-A9. These results show that MAGE-A9 is frequently expressed in superficial bladder cancer and could be a relevant target for immunotherapy or chemoimmunotherapy because its expression can be induced by chemotherapeutic drugs.

Platt, F. M., C. D. Hurst, et al. (2009). "Spectrum of phosphatidylinositol 3-kinase pathway gene alterations in bladder cancer." *Clin Cancer Res* **15**(19): 6008-17.

The phosphatidylinositol 3-kinase (PI3K) pathway can be activated by alterations affecting several pathway components. For rational application of targeted therapies, detailed understanding of tumor biology and approaches to predict efficacy in individual tumors are required. Our aim was to assess the frequency and distribution of pathway alterations in bladder cancer. We examined the pathway components (PIK3CA, PTEN, TSC1, RHEB, and LKB1) and putative upstream regulators (FGFR3 and RAS genes) for mutation, allelic loss, copy number alteration, and expression in bladder tumors and cell lines. No mutations were found in RHEB and only a single mutation in LKB1. PIK3CA mutations were detected in 25% of tumors and 26% of cell lines with a significant excess of helical domain mutations (E542K and E545K). There was over-representation but not amplification of the gene. Loss of heterozygosity of the PTEN region and homozygous deletion were found in 12% and 1.4% of tumors, and reduced expression in 49%. Forty-six percent of cell lines showed alterations that implicated PTEN. Sixteen percent of tumors and 11% of cell lines

showed TSC1 mutation, and 9q loss of heterozygosity was common (57%). Pathway alterations were independently distributed, suggesting that the mutation of two pathway members may have additive or synergistic effects through noncanonical functions. PI3K pathway alterations are common in bladder cancer. The lack of redundancy of alterations suggests that single-agent PI3K-targeted therapy may not be successful in these cancers. This study provides a well-characterized series of cell lines for use in preclinical studies of targeted agents.

Pong, R. C., R. Roark, et al. (2006). "Mechanism of increased coxsackie and adenovirus receptor gene expression and adenovirus uptake by phytoestrogen and histone deacetylase inhibitor in human bladder cancer cells and the potential clinical application." *Cancer Res* **66**(17): 8822-8.

Coxsackie and adenovirus receptor (CAR) is known as a principal receptor for adenovirus commonly used as a gene delivery vector. Down-regulation of CAR is often detected in several cancer types. Epigenetic modifiers such as histone deacetylase inhibitor FK228 (depsipeptide) have been shown to increase CAR expression as well as the uptake of adenovirus in bladder cancer in vivo and in vitro, indicating that altered transcriptional regulation of CAR is the key mechanism responsible for the decreased CAR levels in this cancer. In this study, we screened agents that could induce CAR expression in bladder cancer cells. Fifty-eight drugs with various chemical properties were tested. Ipriflavone and plant isoflavones were found to exhibit the ability to induce CAR gene expression in combination with FK228. Genistein, the natural isoflavone found in soybean, when combined with FK228, exerts a synergistic effect on CAR gene and protein expression in bladder cancer cells. Chromatin immunoprecipitation results showed an increased histone acetylation in the CAR promoter gene, which is due to the suppression of histone deacetylase activity by both agents. Also, our data indicated that combination treatment is a potent chemotherapeutic regimen for bladder cancer cells and the subsequent administration of recombinant adenovirus could further eliminate the remaining cells. Taken together, our results provide a strong rationale for combining chemotherapeutic and gene therapeutic agents to enhance the therapeutic efficacy in bladder cancer.

Pook, S. H., J. N. Rahmat, et al. (2007). "Internalization of Mycobacterium bovis, Bacillus Calmette Guerin, by bladder cancer cells is cytotoxic." *Oncol Rep* **18**(5): 1315-20.

Instillation of Bacillus Calmette Guerin (BCG) into the bladder is the standard treatment for

superficial bladder cancer. It leads to a local inflammatory response due to the release of cytokines and influx of immune cells to the tumor site. Although the presence of an intact immune system is an essential criterion for successful therapy, attachment of the bacteria to the bladder urothelial is just as important. The purpose of our study is to determine the role of bacterial internalization by epithelial cells. Transfection of the alpha5 integrin gene into the BCG unresponsive bladder cancer cell line, RT4, caused an increase in bacterial uptake and also increased cell death. Treatment of cells with cycloheximide did not prevent bacterial internalization but blocked its cytotoxic effect suggesting that unlike cell death, the process of bacterial internalization does not require new protein synthesis. Our data also show that the bacteria secretory products can prevent its own internalization. The extract prepared from lyophilized BCG altered the phosphorylation status of the focal adhesion kinase which is responsible for cellular endocytosis. Therefore, bacterial phosphatases may be present in the bacterial extract. Their activity may inhibit BCG internalization. Thus washing the reconstituted bacteria to remove the enzymes before instillation into the bladder might improve the therapeutic outcome of intravesical BCG therapy.

Puzio-Kuter, A. M., M. Castillo-Martin, et al. (2009). "Inactivation of p53 and Pten promotes invasive bladder cancer." *Genes Dev* **23**(6): 675-80.

Although bladder cancer represents a serious health problem worldwide, relevant mouse models for investigating disease progression or therapeutic targets have been lacking. We show that combined deletion of p53 and Pten in bladder epithelium leads to invasive cancer in a novel mouse model. Inactivation of p53 and PTEN promotes tumorigenesis in human bladder cells and is correlated with poor survival in human tumors. Furthermore, the synergistic effects of p53 and Pten deletion are mediated by deregulation of mammalian target of rapamycin (mTOR) signaling, consistent with the ability of rapamycin to block bladder tumorigenesis in preclinical studies. Our integrated analyses of mouse and human bladder cancer provide a rationale for investigating mTOR inhibition for treatment of patients with invasive disease.

Qin, J., J. Yuan, et al. (2009). "In vitro and in vivo inhibitory effect evaluation of cyclooxygenase-2 inhibitors, antisense cyclooxygenase-2 cDNA, and their combination on the growth of human bladder cancer cells." *Biomed Pharmacother* **63**(3): 241-8.

Overexpression of cyclooxygenase (COX)-2 is associated with the progression of various malignancies, but the contribution of COX-2

expression, bioactivity or their cooperation to bladder cancer growth calls for further clarification. In this study, we investigated the inhibitory effect of COX-2 inhibitors, antisense COX-2 nucleotide, and their combination on the growth of bladder cancer cells (5637, 5637-P and 5637-AS). Suppression of either COX-2 expression or activity caused reduced cell proliferation, enhanced cell numbers in G(1) phase, and increased apoptosis; the joint suppression of COX-2 expression and bioactivity enhanced the degree of cell growth inhibition. COX-2 antisense-expressing 5637-AS tumors showed a 41.42 \pm 3.08% growth inhibition as compared with 5637 controls. Oral administration of indomethacin (3mg/kg) or celecoxib (15 mg/kg) caused tumor growth inhibition by 31.5 \pm 14.87% or 83.17 \pm 1.17%, respectively. When COX-2 antisense cDNA and COX-2 inhibitor celecoxib were combined, the tumor growth inhibition rate was further increased up to 88.78 \pm 3.10%. These results provide evidence that celecoxib has potential therapeutic effect on bladder cancer, and the joint use of COX-2 antisense cDNA with celecoxib may improve their individual therapeutic effect, especially significantly increase the growth inhibitory effect of COX-2 antisense cDNA.

Quek, M. L., D. I. Quinn, et al. (2003). "Molecular prognostication in bladder cancer--a current perspective." *Eur J Cancer* **39**(11): 1501-10.

The optimal management of bladder cancer depends on the accurate assessment of the tumour's biological potential. Advances in molecular biology and cytogenetics have spurred intense research in identifying and characterising prognostic markers for patients with transitional cell carcinoma (TCC) of the bladder. The molecular changes that occur can be categorised into (1) chromosomal alterations leading to carcinogenesis, (2) cellular proliferation as a result of dysregulation of cell cycle control, and (3) growth control processes such as angiogenesis leading to metastasis. The accumulation of these changes ultimately determines a tumour's clinical behaviour and response to therapy. As the understanding of bladder cancer evolves, novel molecular markers for prognostication will make their way from the research laboratory to the clinical setting with the promise to improve patient care and outcomes.

Raghavan, D. (2003). "Molecular targeting and pharmacogenomics in the management of advanced bladder cancer." *Cancer* **97**(8 Suppl): 2083-9.

Bladder cancer is one of the malignancies for which considerable information is available regarding molecular pathogenesis and genetic predictors of natural history, as well as response to treatment. Loss of heterozygosity of chromosome 9 appears to be

essential to the genesis of superficial bladder cancer, and mutation of the p53 suppressor gene frequently is associated with progression to invasive and metastatic disease. Many oncogenes, gene products, and suppressor gene mutations, including those of Ras, Myc, p53, Rb, p16, p21, thrombospondin-1, glutathione, and factors controlling expression and function of the epidermal growth factor receptor, have been shown to be involved in the biology of this disease. Retrospective studies have demonstrated that some of these factors have important roles as independent prognostic determinants or predictors of response to chemotherapy, and clinical trials have now been established to validate the utility of molecular prognostication in bladder cancer. Paradigms developed from the treatment of colorectal malignancy, in which the metabolism of cytotoxic agents is affected by genetic and racial factors, now are being applied to the management of bladder cancer. This review summarizes current knowledge in these evolving domains.

Rao, J. (2002). "Targeting actin remodeling profiles for the detection and management of urothelial cancers--a perspective for bladder cancer research." *Front Biosci* **7**: e1-8.

The actin cytoskeleton and numerous proteins associated with its regulation and function constitute over 25% of total proteins in the cell. Growing evidence from this laboratory and others shows that alterations of actin polymerization, or actin remodeling, plays a pivotal role in regulating the morphologic and phenotypic events of a malignant cell. The actin remodeling is the result of activation of oncogenic actin signaling pathways (e.g., Ras and Src), or inactivation of several important actin-binding proteins that have tumor suppressive functions (e.g., Gelsolin, E-Cadherin, etc.). Recently distinctive protein expression patterns of some of these genes in bladder cancer carcinogenic and progressive processes have been observed. Specific actin-pathway antagonists that have growth inhibitory effect on transformed cells, but not normal cells, have been developed. Our overall hypothesis is that actin alterations are progressive and that distinctive actin remodeling profiles are associated with different stages of cancer development and progression. These patterns can be used as markers for cancer early detection and prognostic indication. On the other hand, detection of specific types of actin-signaling pathway alterations also enables a targeted preventive or therapeutic intervention with specific actin signaling pathway blockers, thereby providing an actin-based paradigm for individualized monitoring and intervention of human bladder cancer.

Ribeiro-Filho, L. A., J. Franks, et al. (2002). "CpG hypermethylation of promoter region and inactivation of E-cadherin gene in human bladder cancer." *Mol Carcinog* **34**(4): 187-98.

Several studies have shown that E-cadherin expression is lost during malignant transformation. We hypothesized that CpG methylation in the promoter region may inactivate the expression of the E-cadherin gene in human bladder cancer. Normal and bladder cancer samples from 51 patients were compared in terms of E-cadherin gene expression and methylation status by immunohistochemistry, methylation-specific polymerase chain reaction (MSP), and bisulfite genome-sequencing techniques. Ten different CpG sites (nt 863, 865, 873, 879, 887, 892, 901, 918, 920, and 940) in the promoter region were studied. Thirty-five of 51 (69%) bladder cancer samples lacked E-cadherin expression, whereas only six of 51 (12%) normal bladder samples lacked E-cadherin immunoreactivity. MSP analysis of bladder cancer samples suggested that 43 of 51 (84%) showed methylation of the promoter region, whereas only 12 of 51 (24%) normal bladder samples showed hypermethylation. Sodium bisulfite genome-sequencing analysis revealed that of 10 CpG sites, two sites (nt 892 and nt 940) showed 100% methylation in all the cancer samples analyzed. Other CpG sites were partially methylated (47-91%). Normal tissue showed only 12% methylation (range, 1-33%) on various CpG sites. Also supporting these data, E-cadherin-negative bladder cancer cell lines restored expression of the E-cadherin gene after treatment with the demethylating agent 5-aza-2'-deoxycytidine. The present study showed that CpG hypermethylation was an important mechanism of E-cadherin gene inactivation in bladder cancer and also that specific CpG sites consistently presented higher methylation levels than others. These findings may provide a better strategy for the diagnosis and management of bladder cancer.

Romero, J. M., P. Jimenez, et al. (2005). "Coordinated downregulation of the antigen presentation machinery and HLA class I/beta2-microglobulin complex is responsible for HLA-ABC loss in bladder cancer." *Int J Cancer* **113**(4): 605-10.

Downregulation of MHC class I expression is a widespread phenomenon used by tumor cells to escape antitumor T-cell-mediated immune responses. These alterations may play a role in the clinical course of the disease. The aim of our study was to investigate the molecular mechanism underlying the absence of HLA-class I molecule expression in bladder cancer cells. Microdissected tumor tissues were characterized by real-time quantitative PCR for the expression of HLA-ABC, beta2-microglobulin and the members of the antigen processing machinery (APM) of HLA

class I molecules (LMP2, LMP7, TAP1, TAP2 and tapasin). Our results showed that irreversible HLA loss by mutations in the beta2-microglobulin gene was not the cause of low HLA class I expression in bladder cancers. In contrast, we observed a coordinated transcription downregulation of HLA-ABC and beta2-microglobulin and APM genes in microdissected tumor tissue derived from bladder carcinomas. This mechanism may represent a major factor for the downregulation of HLA class I expression and in the subsequent direct recognition of cancer cells by cytolytic T lymphocytes. Because this regulatory mechanism is frequently reversible by IFN-gamma treatment, we conclude that HLA class I expression should be a major consideration for immunotherapeutic purposes in patients with bladder cancer.

Rotterud, R., S. D. Fossa, et al. (2007). "Protein networking in bladder cancer: immunoreactivity for FGFR3, EGFR, ERBB2, KAI1, PTEN, and RAS in normal and malignant urothelium." *Histol Histopathol* **22**(4): 349-63.

A panel of markers, selected for the suspected bladder cancer relevance of their corresponding genes, were explored for their expression and subcellular location in urinary bladder tissue. The expression in normal urothelium, in non-metastasised transitional cell carcinomas (TCC), and in primary metastasised TCC with corresponding metastases was mapped. Potential associations between the proteins were identified. The observations were then combined in a set of hypotheses aimed at further hypothesis testing. Membranous ERBB4 and cytoplasmic p21RAS were downregulated in carcinoma cells compared with normal urothelium cells. FGFR3 was translocated from the cytoplasm to the nucleus. ERBB2 was translocated to the membrane and seemingly upregulated in one subgroup and conversely downregulated in another. EGFR, KAI1 and possibly PTEN revealed increased membranous immunoreactivity in non-metastasised tumours. The metastases showed decreased nuclear FGFR3 and membranous PTEN staining compared with corresponding primary tumours. EGFR expression was positively correlated with the expression of PTEN and FGFR3. The expression of ERBB2 was negatively correlated with p21RAS expression. According to our results, bladder carcinogenesis comprises FGFR3 translocation to the nucleus, upregulation of EGFR, ERBB2, KAI1 and PTEN; downregulation of p21RAS; and translocation of EGFR, ERBB2, and possibly PTEN to the membrane. Our results support the hypotheses regarding PTEN and KAI1 functioning as tumour suppressors in bladder cancer. EGFR and KAI1 may

discriminate between non-metastasised and metastasised cancers. A complex network of associations between the factors is suggested.

Ruifa, H., L. Liwei, et al. (2006). "Additional gene therapy with rAAV-wt-p53 enhanced the efficacy of cisplatin in human bladder cancer cells." *Urol Int* 77(4): 355-61.

Gene therapy is defined as the treatment of an acquired or inherited disease by transfer of genetic material. The most common strategies in gene therapy of bladder cancer are corrective, inductive and cytotoxic gene therapy. Mutations in the p53 tumor suppressor gene are the most common molecular genetic abnormalities in bladder cancer and p53 gene transfer in the human bladder cancer cell line by adenoviral or other vectors was demonstrated to be cytotoxic. However, so far there has been no report of adeno-associated virus-2 vector-mediated p53 gene deliveries in bladder cancer. In this study, wild-type p53 cDNA was transfected into the bladder cancer cells, using the adeno-associated virus-2 vector, and the capability of rAAV-wt-p53 gene therapy in bladder cancer was evaluated in vitro. **METHOD:** Bladder cancer cell lines 5637 were transduced with adeno-associated virus-2 vectors containing wild-type human p53 gene (rAAV-wt-p53). Gene expression and transcriptional activation of p53 was determined by Western blot analysis. The cellular growth inhibition and apoptosis of rAAV-mediated p53 transfection were assessed by flow cytometry. The combination effect of rAAV-wt-p53 and cisplatin was measured by MTT assay. The virus rAAV efficiently enters the cells and expresses its gene products. The gene product of rAAV-wt-p53 is cytotoxic to bladder cancer cells. The bladder cell line 5637 was found to experience a synergistic killing effect when rAAV-wt-p53 was used in combination with cisplatin. **CONCLUSION:** rAAV-mediated p53 gene transfer could offer a powerful novel therapeutic approach in bladder cancer.

Sachs, M. D., M. Ramamurthy, et al. (2004). "Histone deacetylase inhibitors upregulate expression of the coxsackie adenovirus receptor (CAR) preferentially in bladder cancer cells." *Cancer Gene Ther* 11(7): 477-86.

Studies on bladder cancer cell lines have shown that low adenoviral (Ad) infectivity is associated with low-level coxsackie adenovirus receptor (CAR) expression. Recently, we and others demonstrated a tumor stage- and grade-dependent downregulation of CAR expression in a large series of clinical bladder cancer specimens. Here, we demonstrate adenoviral gene transfer can be markedly enhanced in bladder cancer cells by upregulation of

CAR through the use of certain differentiating agents, including the histone deacetylase inhibitors (HDACI) trichostatin A and sodium phenylbutyrate. CAR upregulation to supraphysiologic levels was demonstrated by quantitative rt-PCR, Western blotting, flow cytometry and adenoviral gene transfer. Normal urothelial cells and CAR-positive papilloma cells (RT4) failed to demonstrate upregulation under the same conditions. Upregulation was cell cycle dependent, associated with increased adenoviral gene transfer and persisted for at least 7 days after a single treatment. Such upregulation, however, appears to be tumor cell specific, as other CAR-negative cell lines failed to demonstrate enhanced adenoviral gene transfer with the same treatments. These results provide a rational basis for combining HDACI therapy with gene therapy as a method of augmenting activity in bladder cancer, but this strategy may not be universally applicable to other cell types.

Sachs, M. D., K. A. Rauen, et al. (2002). "Integrin alpha(v) and coxsackie adenovirus receptor expression in clinical bladder cancer." *Urology* 60(3): 531-6.

To evaluate the expression of the coxsackie and adenovirus receptor (CAR) and alpha(v) integrins in clinical specimens of bladder cancer to determine the susceptibility to adenoviral gene therapy. Efficient adenovirus-based gene therapy requires binding of the virus to CAR and involves the alpha(v) integrins. Studies on bladder cancer cell lines have shown that low adenoviral transduction rates were associated with low-level expression of CAR. Integrin alpha(v) expression increases in various tumors suggest its importance in differentiation, proliferation, and migration. CAR is structurally a member of the Ig-type superfamily of cell-cell adhesion molecules, suggesting that its expression may also be related to the state of tumor differentiation. We performed immunohistochemistry for CAR and integrin alpha(v) expression in bladder cancer specimens in 50 paraffin-embedded tumor-normal pairs and confirmed the results by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of 11 separate bladder tumors and 4 separate normal bladder controls. Immunohistochemistry demonstrated a stage and grade-dependent decrease in CAR expression (90.0%, 83.3%, and 31.3% of normal urothelium and superficial and invasive transitional cell carcinoma [TCC] and 83.3% and 39.5% of low and high-grade TCC, respectively). Furthermore, we found a stage and grade-dependent increase in alpha(v) integrin expression (13.3%, 46.0%, and 56.3% of normal urothelium, superficial TCC, and invasive TCC and 25% and 52.6% of low and high-grade TCC, respectively). Quantitative RT-PCR analysis confirmed a downregulation at the CAR gene

expression level. This down-regulation may have a major impact on developing adenoviral-based gene therapy modalities. In addition, we propose that loss of CAR expression decreases rigid cell adhesion, possibly increasing the migratory potential. Loss of CAR expression correlates with the invasive phenotype in our analysis of bladder cancer. Simultaneously, the finding of increased alpha(v) expression in invasive cancer suggests a pathogenesis that involves heterophilic adhesion and migration of these cells on various extracellular ligands.

Saint, F., M. A. Le Frere Belda, et al. (2004). "Pretreatment p53 nuclear overexpression as a prognostic marker in superficial bladder cancer treated with Bacillus Calmette-Guerin (BCG)." *Eur Urol* **45**(4): 475-82.

INTRODUCTION: Altered p53 gene product correlates with the stage and grade of bladder tumor, but its value as a predictor of BCG response has been disappointing. In order to revisit the prognostic value of pretreatment p53 nuclear overexpression for the BCG response, we studied a large cohort of consecutive patients with superficial bladder cancer treated with BCG. From 1988 to 2001, 102 patients with a history of multifocal, recurrent, and/or high-risk papillary transitional cell carcinoma or carcinoma in situ, were treated for the first time with BCG. p53 immunostaining was performed on paraffin-embedded tissues using monoclonal antibody DO7 and an automated immunostainer. Special attention was paid to the conditions of tumor fixation. p53 overexpression was defined as more than 20% tumor cells with p53-stained nuclei. Immunostaining was significantly higher for Ta/T1 G3 +/- Cis ($p < 0.001$), tumoral substage T1b ($p = 0.001$), grade 3 ($p = 0.0001$), and Cis ($p = 0.002$). Times to recurrence, progression and cancer death were shorter among patients with p53 overexpression ($p = 0.03$; $p < 0.0001$; $p = 0.0003$). In multivariate analysis, p53 overexpression was an independent predictor of recurrence ($p = 0.0003$) [RR = 0.15; 95%CI, 0.06 to 0.42]. **CONCLUSION:** Pretreatment p53 nuclear overexpression in superficial bladder tumors is associated with a high risk of disease recurrence, progression and cancer death after BCG therapy. Applying antibody DO7 with an automated immunostainer and stringent fixative conditions, p53 nuclear immunostaining yields clinically relevant information and may be a useful tool for selecting patients with superficial bladder cancer who might be resistant to BCG.

Sak, S. C., P. Harnden, et al. (2005). "APE1 and XRCC1 protein expression levels predict cancer-

specific survival following radical radiotherapy in bladder cancer." *Clin Cancer Res* **11**(17): 6205-11.

INTRODUCTION: Radiotherapy offers the potential of bladder preservation in muscle-invasive bladder cancer, but only a proportion of tumors respond, and there are no accurate predictive methods. The ability of tumor cells to repair DNA damage induced by ionizing radiation influences radiosensitivity. We therefore investigated the prognostic value of the DNA repair proteins APE1 and XRCC1 in patients with muscle-invasive bladder cancer treated by radical radiotherapy. The tumors of 90 patients with muscle-invasive transitional cell carcinoma and known clinical outcomes were immunostained with APE1 and XRCC1 antibodies. Levels of protein expression were assessed as a percentage of tumor cells with positive nuclear staining (1,000 cells per tumor). The median percentage of nuclear staining for APE1 was 98.7% (range, 42.2-100%) and for XRCC1 was 96.5% (range, 0.6-99.6%). High expression levels of APE1 or XRCC1 ($>$ or $=$ 95% positivity) were associated with improved patient cancer-specific survival (log-rank, $P = 0.02$ and 0.006 , respectively). In a multivariate Cox regression model, APE1 and XRCC1 expression and hydronephrosis were the only independent predictors of patient survival. Expression levels of both APE1 and XRCC1 proteins were strongly associated with patient outcome following radiotherapy, separating patients with good outcome from the 50% with poor outcome (82% and 44%, 3-year cause-specific survival, respectively). If prospectively validated, this simple test could be incorporated into clinical practice to select patients likely to respond to radiotherapy and consider alternative forms of therapy for those unlikely to respond.

Sakano, S., T. Wada, et al. (2006). "Single nucleotide polymorphisms in DNA repair genes might be prognostic factors in muscle-invasive bladder cancer patients treated with chemoradiotherapy." *Br J Cancer* **95**(5): 561-70.

DNA repair enzymes repair DNA damaged by platinum agents and ionising radiation. Single nucleotide polymorphisms (SNPs) in DNA repair genes modulate the repair capacity and might affect response and prognosis following platinum-based chemoradiotherapy (CRT). We investigated associations between the functional SNPs in DNA repair genes and response and survival in muscle-invasive bladder cancer patients treated with CRT to determine the predictive value of the SNPs in patient selection for bladder conservation therapy. The study group comprised 78 patients who underwent CRT for transitional cell carcinoma of the bladder. Single

nucleotide polymorphisms in xeroderma pigmentosum complementation groups C (Lys939Gln, A/C), D (XPD; Lys751Gln, A/C), and G (Asp1104His, G/C), and X-ray repair cross-complementing groups 1 (XRCC1; Arg399Gln, G/A) and 3 (Thr241Met, T/C) genes were genotyped. Combined genotypes with at least one variant allele in XPD or XRCC1 were significantly associated with improved cancer-specific survival compared with remaining groups ($P=0.009$). In multivariate analysis, only the combined XPD and XRCC1 genotypes were independently associated with cancer-specific survival ($P=0.04$). The association was stronger in stage T3/T4 patients ($P=0.0008$). These results suggest that combined XPD and XRCC1 genotypes might be prognostic factors in muscle-invasive bladder cancer patients treated with CRT.

Sanchez-Carbayo, M., P. Capodiecici, et al. (2003). "Tumor suppressor role of KiSS-1 in bladder cancer: loss of KiSS-1 expression is associated with bladder cancer progression and clinical outcome." *Am J Pathol* **162**(2): 609-17.

The expression profiles of nine bladder cancer cell lines were compared against a pool containing equal total RNA quantities of each of them. Lower expression of KiSS-1 was revealed in cells derived from the most advanced bladder tumors. When comparing 15 primary bladder tumors versus a pool of four bladder cancer cell lines, lower transcript levels of KiSS-1 were observed in the invasive bladder carcinomas as compared to superficial tumors. KiSS-1 expression ratios provided prognostic information. The expression pattern of KiSS-1 transcripts was analyzed using in situ hybridization in nine bladder cancer cells, paired normal urothelium and bladder tumor samples ($n = 25$), and tissue microarrays of bladder tumors ($n = 173$). We observed complete loss of KiSS-1 in all invasive tumors under study as compared to their respective normal urothelium. The expression of KiSS-1 was found to be significantly associated with histopathological stage. Patients with lower KiSS-1 expression showed a direct correlation with overall survival in a subset of bladder tumors whose follow-up was available ($n = 69$). We did not observe any significant differential KiSS-1 expression along cell cycle by sorting analysis. A potential tumor suppressor role in bladder cancer was revealed for KiSS-1. Moreover, it showed predictive value by identifying patients with poor outcome.

Sanchez-Carbayo, M., N. D. Socci, et al. (2007). "A polymorphism in HDM2 (SNP309) associates with early onset in superficial tumors, TP53 mutations, and

poor outcome in invasive bladder cancer." *Clin Cancer Res* **13**(11): 3215-20.

The HDM2 gene represents one of the central nodes in the p53 pathway. A recent study reported the association of a single nucleotide polymorphism (SNP309) in the HDM2 promoter region with accelerated tumor formation in both hereditary and sporadic cancers. In this study, we aim to evaluate the SNP309 in bladder cancer and to link it to TP53 status. SNP309 genotyping and TP53 mutation status were done on 141 bladder tumors and 8 bladder cancer cell lines using a RFLP strategy and TP53 genotyping arrays, respectively. Transcript profiling of a subset of cases ($n = 41$) was done using oligonucleotide arrays to identify genes differentially expressed regarding their SNP309 status. Of 141 bladder tumors analyzed, 36.9% displayed the SNP309 wild-type (WT; T/T) genotype, whereas 11.3% were homozygous (G/G) and 51.8% were heterozygous (T/G) cases. Patients with superficial disease and the G/G genotype had an earlier age on onset than those with the T/G or T/T genotypes ($P = 0.029$). Tumors with SNP309 WT genotype significantly displayed TP53 mutations when compared with tumors harboring G/G or T/G genotypes ($P < 0.05$). SNP309 WT cases had a poorer overall survival than cases with G/G and T/G genotypes ($P < 0.05$). TP53 mutation status provided enhanced prognostic value ($P < 0.001$). Transcript profiling identified TP53 targets among those differentially expressed between tumors displaying G/G or T/G SNP309 versus WT cases. SNP309 is a frequent event in bladder cancer, related to earlier onset of superficial disease and TP53 mutation status. SNP309 genotypes were found to be associated with clinical outcome.

Sayan, A. E., T. R. Griffiths, et al. (2009). "SIP1 protein protects cells from DNA damage-induced apoptosis and has independent prognostic value in bladder cancer." *Proc Natl Acad Sci U S A* **106**(35): 14884-9.

The epithelial-mesenchymal transition (EMT) contributes to cancer metastasis. Two ZEB family members, ZEB1 and ZEB2(SIP1), inhibit transcription of the E-cadherin gene and induce EMT in vitro. However, their relevance to human cancer is insufficiently studied. Here, we performed a comparative study of SIP1 and ZEB1 proteins in cancer cell lines and in one form of human malignancy, carcinoma of the bladder. Whereas ZEB1 protein was expressed in all E-cadherin-negative carcinoma cell lines, being in part responsible for the high motility of bladder cancer cells, SIP1 was hardly ever detectable in carcinoma cells in culture. However, SIP1 represented an independent factor of

poor prognosis ($P = 0.005$) in a series of bladder cancer specimens obtained from patients treated with radiotherapy. In contrast, ZEB1 was rarely expressed in tumor tissues; and E-cadherin status did not correlate with the patients' survival. SIP1 protected cells from UV- and cisplatin-induced apoptosis in vitro but had no effect on the level of DNA damage. The anti-apoptotic effect of SIP1 was independent of either cell cycle arrest or loss of cell-cell adhesion and was associated with reduced phosphorylation of ATM/ATR targets in UV-treated cells. The prognostic value of SIP1 and its role in DNA damage response establish a link between genetic instability and metastasis and suggest a potential importance for this protein as a therapeutic target. In addition, we conclude that the nature of an EMT pathway rather than the deregulation of E-cadherin per se is critical for the progression of the disease and patients' survival.

Sazawa, A., T. Watanabe, et al. (2002). "Adenovirus mediated gelsolin gene therapy for orthotopic human bladder cancer in nude mice." *J Urol* **168**(3): 1182-7.

Gelsolin is an actin regulatory protein that is undetectable or reduced in human bladder tumors compared with normal epithelial cells. Whether the over expression of gelsolin could inhibit tumor growth was investigated in an orthotopic bladder cancer nude mouse model using recombinant adenovirus encoding wild-type gelsolin (Ad-GSN). The 2 human bladder cancer cell lines KU-7 and UMUC-2 were transduced with Ad-GSN in vitro. Flow cytometric analysis was done to examine the cell cycle after transducing the adenovirus. Cell growth was compared with control groups of these cells transduced with adenovirus containing the Escherichia coli beta-galactosidase gene Ad-beta-gal. In vivo KU-7 cells were introduced into the bladder of nude mice (day 0), followed by 3 injections into the urethra (days 2 to 4) with Ad-GSN or Ad-beta-gal (1×10^8 pfu). At 8 days after initial adenovirus exposure (day 10) each bladder was sectioned and stained, and the mass of the tumor was digitally determined. Bladder cancer cell growth (KU-7 and UMUC-2) was inhibited after these cells were transduced with Ad-GSN in vitro. Based on flow cytometric analysis over expression of gelsolin may cause these cells to arrest or delay at the G2/M phase of the cell cycle. In the orthotopic bladder cancer model the mass of the tumor was approximately 90% less in Ad-GSN treated animals than in controls. Ad-GSN provides a significant tumor suppressive effect on human bladder cancer cells in this orthotopic nude mouse model. Adenovirus mediated over expression of gelsolin may be useful therapy for human bladder cancer.

Schaaf, A., S. Langbein, et al. (2003). "In vitro transfection of human bladder cancer cells by acoustic energy." *Anticancer Res* **23**(6C): 4871-5.

The objective of this study was to examine and quantify the shock-wave-induced transfection of human bladder carcinoma cells. Cell suspensions were transfected with different concentrations of the pEGFP-N1 plasmid. Shock-waves were applied in a degassed water bath with different numbers of impulses at different energy levels. Additionally, the effects of different DNA concentrations, frequencies and the absence/presence of a liquid air border were examined. After shock-wave application, the transfection rate increased up to a maximum of 27.10% after 1000 impulses at an energy level of 0.5 mJ/mm². In comparison negative control groups were transfected significantly below 1%. An increase in acoustic power and frequency and of DNA concentration and the presence of a liquid-air border resulted in an increasing transfection rate. CONCLUSION: The results demonstrate that naked plasmid DNA can easily and effectively be delivered to malignant urothelial cells in vitro upon exposure to lithotripter-generated shock-waves.

Schaaf, A., S. Sagi, et al. (2004). "Cytotoxicity of cisplatin in bladder cancer is significantly enhanced by application of bcl-2 antisense oligonucleotides." *Urol Oncol* **22**(3): 188-92.

The aim of this study was to examine the effects of the combined application of cisplatin and bcl-2 antisense oligonucleotide on human bladder cancer cell lines to determine the possible synergistic effects in cytotoxicity and to estimate its potential value for subsequent in vivo trials. Human bladder cancer cell lines (UM-UC 3, RT 112, T24/83 and HT 1197) were treated with bcl-2 antisense oligonucleotide, cisplatin, or a combination of both and incubated for 48 h under standard conditions. Cell survival was determined using a Neubauer haemocytometer or standard MTT assay. BCL-2 expression was verified using western blotting. The combined treatment resulted in significant lower cell survival rates compared to individual treatment. Additionally, there was a decrease in cell survival rate with an increase in cisplatin concentration in combined treatment that was not observed in cisplatin mono treatment. For the combined treatment with oligonucleotides and cisplatin a synergistic effect can be strongly suggested. Therefore, further investigations and in vivo trials have to be done to determine the possible benefits for clinical applications.

Schabath, M. B., M. R. Spitz, et al. (2003). "Genetic instability in bladder cancer assessed by the comet assay." *J Natl Cancer Inst* **95**(7): 540-7.

Latent genetic instability has been associated with an increased risk for several cancers. We used the comet assay (single-cell gel electrophoresis) to assess whether genetic instability, as reflected by susceptibility to DNA damage, was associated with the risk of bladder cancer in a case-control study. Schabath et al used the comet assay to measure baseline and benzo[a]pyrene diol epoxide (BPDE)- and gamma-radiation-induced DNA damage in individual peripheral blood lymphocytes from 114 incident case patients with bladder cancer and 145 matched healthy control subjects. All subjects provided personal information, including smoking history. DNA damage was visualized with the comet assay and quantified by the Olive tail moment parameter, a relative measure. Multivariable analysis was used to assess relative risks for bladder cancer associated with DNA damage. All statistical tests were two-sided. Baseline levels of DNA damage were statistically significantly higher in case patients (tail moment = 1.40) than in control subjects (tail moment = 1.21) (difference = 0.19, 95% confidence interval [CI] = 0.04 to 0.32; $P = .015$), as were gamma-radiation-induced (tail moment = 4.76 versus 4.22; difference = 0.54, 95% CI = 0.11 to 0.96; $P = .013$) and BPDE-induced (tail moment = 4.06 versus 3.45; difference = 0.61, 95% CI = 0.23 to 0.99; $P = .002$) DNA damage. When data were dichotomized at the median value for DNA damage in control subjects and adjusted for age, sex, ethnicity, and smoking status, an increased estimated relative risk of bladder cancer was statistically significantly associated with DNA damage at baseline (odds ratio [OR] = 1.84, 95% CI = 1.07 to 3.15) and after gamma-radiation (OR = 1.81, 95% CI = 1.04 to 3.14) but not after BPDE treatment (OR = 1.69, 95% CI = 0.98 to 2.93). **CONCLUSION:** Latent genetic instability as measured by the comet assay is associated with an increased estimated relative risk of bladder cancer.

Sejima, T., T. Isoyama, et al. (2004). "Comparative study of apoptotic antitumor effect between angiogenesis inhibitor AGM-1470 and MVAC chemotherapy on rat urinary bladder cancer." *Urol Int* **73**(3): 226-33.

To investigate the antitumor apoptotic effect of AGM-1470 by comparing it to that induced by methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) therapy, currently the standard chemotherapy for bladder cancer, in a rat bladder cancer model. A total of 45 six-week-old female rats were divided into 3 equal groups: those receiving AGM-1470 treatment; those receiving MVAC

treatment, and controls. All rats were cystectomized to evaluate the therapeutic effect with regard to macroscopic tumor findings, hematoxylin and eosin pathology, apoptosis detection, and immunohistochemistry (IHC) for bcl-2. Our experimental protocol succeeded in producing invasive bladder tumors in the majority of rats. Tumor volume was significantly reduced in the AGM-1470 and MVAC treatment groups compared with that in the control group. The apoptotic indices of tumor cells was significantly higher in the AGM-1470 and MVAC treatment groups than in the control group. IHC for bcl-2 demonstrated no statistical difference in expression among the groups. However, the apoptotic indices of high-level bcl-2 expression were significantly lower than the indices of low-level expression in the AGM-1470 group. AGM-1470 and MVAC appear to exert a prominent mass reduction effect via tumor cell apoptosis in cases of invasive bladder tumor, although these therapies did not demonstrate any obvious modulation of bcl-2 protein expression status. Bcl-2 overexpression might be an obstacle to AGM-1470 therapy because of its significant inhibitory effect on apoptosis.

Sen, B., D. C. Wolf, et al. (2007). "Identification of interspecies concordance of mechanisms of arsenic-induced bladder cancer." *Toxicol In Vitro* **21**(8): 1513-29.

Exposure to arsenic causes cancer by inducing a variety of responses that affect the expression of genes associated with numerous biological pathways leading to altered cell growth and proliferation, signaling, apoptosis and oxidative stress response. Affymetrix GeneChip arrays were used to detect gene expression changes following dimethylarsinic acid (DMA) exposure to human bladder cells (UROtsa) or rat bladder cells (MYP3) and rat bladder epithelium in vivo at comparable doses. Using different experimental models coupled with transcriptional profiling allowed investigation of the correlation of mechanisms of DMA-induced toxicity between in vitro and in vivo treatment and across species. The observations suggest that DMA-induced gene expression in UROtsa cells is distinct from that observed in the MYP3 cells. Principal component analysis shows a more distinct separation by treatment and dose in MYP3 cells as compared to UROtsa cells. However, at the level of pathways and biological networks, DMA affects both common and unique processes in the bladder transitional cells of human and rats. Twelve pathways were found common between human in vitro, rat in vitro and rat in vivo systems. These included signaling pathways involved in adhesion, cellular growth and differentiation. Fifty-five genes found to be commonly

expressed between rat in vivo and rat in vitro systems were involved in diverse functions such as cell cycle regulation, lipid metabolism and protein degradation. Many of the genes, processes and pathways have previously been associated with arsenic-induced toxicity. Our finding reiterates and also identifies new biological processes that might provide more information regarding the mechanisms of DMA-induced toxicity. The results of our analysis further suggest that gene expression profiles can address pertinent issues of relevance to risk assessment, namely interspecies extrapolation of mechanistic information as well as comparison of in vitro to in vivo response.

Shariat, S. F., R. Ashfaq, et al. (2006). "Correlation of cyclin D1 and E1 expression with bladder cancer presence, invasion, progression, and metastasis." *Hum Pathol* **37**(12): 1568-76.

The objective of this study was to determine the correlation of the expression of cyclin D1 and E1 with the expression of commonly altered cell cycle regulators and bladder cancer presence, staging, and clinical outcomes. We performed immunohistochemical staining for cyclin D1, cyclin E1, p53, p21, p27, and retinoblastoma protein (pRB) on serial cuts from normal urothelium from 9 controls, radical cystectomy specimens from 226 consecutive patients with advanced transitional cell carcinoma, and lymph nodes with metastasis from 50 of the 226 cystectomy patients. Cyclin D1 and E1 immunoreactivity were considered low when samples demonstrated less than 10% and 30% nuclear reactivity, respectively. Normal bladder urothelium from all 9 control patients showed uniformly intense expression of cyclin D1 and E1. Cyclin D1 expression was low in 99 (43.8%) of 226 cystectomy specimens and 25 (50.0%) of 50 metastatic lymph node specimens. Cyclin D1 immunoreactivity was not associated with any pathologic characteristics or clinical outcomes. Cyclin E1 expression was low in 125 (55.3%) of 226 cystectomy specimens and 22 (44.0%) of 50 metastatic lymph node specimens. Low cyclin E1 expression was significantly associated with advanced pathologic stage, lymphovascular invasion, and lymph node metastases. In multivariate analyses, low cyclin E1 expression was significantly associated with bladder cancer-specific mortality ($P = .048$), but not disease recurrence ($P = .056$). Low cyclin E1 expression was significantly associated with altered expression of pRB, p27, and cyclin D1. Low cyclin D1 expression was significantly associated with altered expression of pRB, p21, and cyclin E1. Cyclin E1 expression stratifies patients with bladder transitional cell carcinoma into those with more

"indolent" behavior and those with features of biologically and clinically aggressive disease.

Shariat, S. F., J. A. Karam, et al. (2008). "Molecular markers in bladder cancer." *Curr Opin Urol* **18**(1): 1-8.

Bladder cancer is a diverse disease whose molecular phenotypes are being elucidated. In this review, we summarize currently known molecular pathways and associated markers in bladder cancer. RECENT FINDINGS: Genetic and epigenetic aberrations have been closely associated with tumor pathogenesis and prognosis. Cell cycle markers have been most extensively studied. More recently, apoptotic and angiogenic pathways are being investigated. Studying the role of multiple concurrent molecular alterations improves the prognostic ability of these markers. The use of tissue microarrays and high-throughput molecular profiling is accelerating the discovery of new markers. Molecular biology is paramount to our understanding of bladder cancer pathogenesis. The search for new markers, and elucidating cross-talk between markers in different pathways, is warranted. Molecular markers have the potential benefit of improving detection, prognosis and treatment of bladder cancer. In addition, understanding the molecular profile of the individual patient could usher us into a new era of improving prediction of the natural history of the disease and providing a more personalized and tailored treatment. Prospective trials are still needed, however, to objectively establish the true benefit of these markers in prognostic and therapeutic arenas.

Shariat, S. F., H. Tokunaga, et al. (2004). "p53, p21, pRB, and p16 expression predict clinical outcome in cystectomy with bladder cancer." *J Clin Oncol* **22**(6): 1014-24.

To determine whether p53, p21, pRB, and/or p16 expression is associated with bladder cancer stage, progression, and prognosis. Immunohistochemical staining for p53, p21, pRB, and p16 was carried out on serial sections from archival specimens of 80 patients who underwent bilateral pelvic lymphadenectomy and radical cystectomy for bladder cancer (median follow-up, 101 months). p53, p21, and pRB or p16 expression was altered in 45 (56%), 39 (49%), and 43 (54%) tumors, respectively. Sixty-six patients (83%) had at least one marker altered, and 21 patients (26%) had all three altered. Abnormal expressions of p53, p21, and pRB/p16 expression were associated with muscle-invasive disease ($P=.007$, $P=.003$, and $P=.003$, respectively). The alteration of each marker was independently associated with disease progression ($P< .038$) and disease-specific survival ($P< .039$). In multivariable models that included standard

pathologic features and p53 with p21 or p53 with pRB/p16, only p53 and lymph node metastases were associated with bladder cancer progression ($P < 0.026$) and death ($P < 0.028$). In models that included p21 and pRB/p16, only p21 and lymph node metastases were associated with bladder cancer progression ($P < 0.022$) and death ($P < 0.028$). In a model that included the combined variables p53/p21 and pRB/p16, only p53/p21 and lymph node status were associated with bladder cancer progression ($P < 0.047$) and death ($P < 0.036$). The incremental number of altered markers was independently associated with an increased risk of bladder cancer progression ($P = 0.005$) and mortality ($P = 0.007$). **CONCLUSION:** Although altered expression of each of the four cell cycle regulators is associated with bladder cancer outcome in patients undergoing radical cystectomy, p53 is the strongest predictor, followed by p21, suggesting a more pivotal role of the p53/p21 pathway in bladder cancer progression.

Shen, S. S., C. L. Smith, et al. (2006). "Expression of estrogen receptors-alpha and -beta in bladder cancer cell lines and human bladder tumor tissue." *Cancer* **106**(12): 2610-6.

Estrogen receptors (ERs) are known to mediate important physiologic responses as well as the growth of some tumors in response to estradiol stimulation. In a previous study the selective ER modulator raloxifene was shown to induce apoptosis in an ERbeta-positive bladder cancer cell line. However, the expression of ERbeta in human bladder cancer has not been thoroughly investigated. ERalpha and ERbeta expression in 224 bladder tumor samples was evaluated using tissue microarray and immunohistochemistry. Levels of ERalpha and ERbeta protein and mRNA expression were determined in several bladder cancer cell lines using quantitative reverse-transcriptase polymerase chain reaction (RT-PCR) and Western blot analysis. The effect of estradiol and antiestrogen treatments on RT4 bladder cancer cell growth was determined by cell proliferation assays. Analyses revealed that only 2 human bladder cancers weakly expressed ERalpha. In contrast, the expression of ERbeta was detected in 141 tumors (63%). ERbeta was expressed in 58% of WHO Grade 1 and 2 tumors, whereas 70% of Grade 3 tumors demonstrated expression ($P = 0.085$). Importantly, although only 53% and 55% of Ta and T1 tumors demonstrated ERbeta expression, 80% of T2, 81% of T3, and 75% of T4 tumors showed ERbeta expression. The differences in ERbeta expression between Ta/T1 and T2/T3/T4 tumors were found to be highly significant ($P < 0.001$). Metastatic transitional cell carcinomas had ERbeta expression (80%) comparable to that of muscle invasive bladder cancers.

Western blot analysis detected ERbeta protein expression in each of the 5 bladder cancer cell lines tested, whereas no or very low levels of ERalpha were found. Quantitative RT-PCR revealed that higher levels of ERbeta than ERalpha mRNA were present in 5637, T-24, TSU-Pr1, and TCC-Sup bladder cancer cells, whereas ER-alpha mRNA levels were greater than ERbeta in RT4 cells. Treatment with 17beta-estradiol modestly increased RT4 cell growth, whereas the antiestrogens, 4-hydroxytamoxifen, raloxifene, or ICI 182,780 inhibited the growth of RT4 cells. ERbeta is the dominant receptor expressed in bladder cancer cell lines and in the majority of human bladder tumors. Moreover, the degree of ERbeta expression increases with increasing stage and grade of differentiation. Antiestrogens have an inhibitory effect on the growth of bladder cancer cells in vitro.

Shiau, A. L., Y. P. Lin, et al. (2007). "Development of a conditionally replicating pseudorabies virus for HER-2/neu-overexpressing bladder cancer therapy." *Mol Ther* **15**(1): 131-8.

Overexpression of the HER-2/neu oncogene, a frequent molecular event in a variety of cancers including bladder cancer, is associated with tumor progression and poor prognosis. Therapeutic strategies to targeting HER-2/neu-overexpressing cancer cells have shown promise. Pseudorabies virus (PrV), a herpesvirus of swine, may be exploited as an oncolytic agent for human cancer. Herein, we generated a conditionally replicating glycoprotein E-defective PrV mutant carrying glycoprotein D and herpes simplex virus type 1 thymidine kinase genes, which are essential for viral entry and replication, under the transcriptional control of the HER-2/neu promoter. The recombinant PrV, designated YP2, selectively replicated in and lysed HER-2/neu-overexpressing human bladder, mouse bladder, and hamster oral cancer cells in vitro. Notably, YP2 retarded MBT-2 bladder tumor growth in mice by more than 50% and more than half of the mice survived for over 50 days, whereas all the control mice survived less than 30 days. Taken together, our results suggest that YP2 may have therapeutic potential for the treatment of invasive bladder cancer. Furthermore, because HER-2/neu is overexpressed in a broad spectrum of cancers, this conditionally replicating PrV may be broadly applicable.

Shibata, M. A., J. Morimoto, et al. (2004). "Experimental gene therapy in mammary and urinary bladder cancer using electrogene transfer." *Med Electron Microsc* **37**(4): 216-24.

Shibata et al investigated the effectiveness of in vivo electrogene transfer as a means of therapy in rat urinary bladder carcinoma and in mammary

carcinoma models in both athymic and syngeneic mice using the herpes simplex virus 1 thymidine kinase (HSVtk) or IL-12 genes in combination with ganciclovir (GCV). A significant increase in the levels of tissue apoptosis and necrosis was induced with a single injection of HSVtk vector directly into bladder and mammary tumors followed by in vivo transfection and a regimen of intraperitoneal GCV injection. This procedure induced significant selective tumor cell death, characterized by marked inflammation and peripheral macrophage influx. Active caspase-3 was also strongly expressed in areas of cell death, indicating the initiation of apoptosis. This result was confirmed in corollary in vitro studies on a mouse bladder carcinoma cell line in which elevated caspase-3, -8, and -9 activities and decreased mitochondrial membrane potential were observed as a result of transfection with HSVtk and addition of GCV to the medium. In the syngeneic mouse mammary cancer model, we additionally found both tumor volume and metastasis to lymph nodes and lungs to be significantly reduced throughout the 2-month experiment. However, in contrast to their syngeneic counterparts, HSVtk/GCV therapy did not effectively inhibit mammary tumor growth/metastasis in an athymic mouse model, leading us to believe that T-cell-mediated immune responses may participate via the bystander effect in HSVtk/GCV experimental therapy. We subsequently evaluated the antitumor activity of IL-12, which can activate T-cell-mediated immune responses involving macrophages, in the syngeneic mammary tumors and found that IL-12 also significantly suppressed mammary tumor growth and metastasis. We thus suggest that in vivo electrogene transfer is a useful transfection tool in cancer gene therapy and, in addition, we show that T-cell-mediated immune responses may be a critical factor in cancer gene therapy using HSVtk/GCV and IL-12.

Shinohara, A., S. Sakano, et al. (2009). "Association of TP53 and MDM2 polymorphisms with survival in bladder cancer patients treated with chemoradiotherapy." *Cancer Sci* **100**(12): 2376-82.

Platinum-based chemoradiotherapy (CRT) as bladder conservation therapy has shown promising results for muscle-invasive bladder cancer. However, CRT might diminish survival as a result of the delay in cystectomy for some patients with non-responding bladder tumors. Because the p53 tumor suppression pathway, including its MDM2 counterpart, is important in chemotherapy- and radiotherapy-associated effects, functional polymorphisms in the TP53 and MDM2 genes could influence the response to treatment and the prognosis following CRT. We investigated associations between two such polymorphisms, and p53 overexpression, and response

or survival in bladder cancer patients treated with CRT. The study group comprised 96 patients who underwent CRT for transitional cell carcinoma of the bladder. Single nucleotide polymorphisms (SNPs) in TP53 (codon 72, arginine > proline) and MDM2 (SNP309, T > G) were genotyped using PCR-RFLP, and nuclear expression levels of p53 were examined using immunohistochemistry. None of the genotypes or p53 overexpression was significantly associated with response to CRT. However, patients with MDM2 T / G + G / G genotypes had improved cancer-specific survival rates after CRT (P = 0.009). In multivariate analysis, the MDM2 T / G + G / G genotypes, and more than two of total variant alleles in TP53 and MDM2, were independently associated with improved cancer-specific survival (P = 0.031 and P = 0.015, respectively). In addition, MDM2 genotypes were significantly associated with cystectomy-free survival (P = 0.030). These results suggest that the TP53 and MDM2 genotypes might be useful prognostic factors following CRT in bladder cancer, helping patient selection for bladder conservation therapy.

Shinohara, N. and T. Koyanagi (2002). "Ras signal transduction in carcinogenesis and progression of bladder cancer: molecular target for treatment?" *Urol Res* **30**(5): 273-81.

Ras oncogenes are considered to play a key role in the carcinogenesis and progression of human bladder cancer. The oncogenes code for the Ras p21 proteins, which localize in the internal part of the cell membrane and act as molecular switches to mediate downstream signaling from a variety of extracellular stimuli. Activation of Ras proteins induces the constitutive activation of downstream kinase cascades, which results in continuous mitogenic signaling and transformation of immortalized cells in human bladder cancer. Therefore inactivation of the activated Ras function might be effective for the development of a novel treatment strategy against human bladder cancer. Recently several ways to suppress Ras activities, including inhibitors of Ras signal transduction and a ras suppressor mutant, have been reported. Here we review the current concepts of the basic mechanisms of the intracellular Ras signaling pathway and ras activation in the carcinogenesis and progression of human bladder cancer and discuss clinical potentials of their therapeutic interventions.

Shirakawa, T., K. Hamada, et al. (2004). "A cox-2 promoter-based replication-selective adenoviral vector to target the cox-2-expressing human bladder cancer cells." *Clin Cancer Res* **10**(13): 4342-8.

Cyclooxygenase-2 (Cox-2), an enzyme that catalyzes the synthesis of prostaglandins, is overexpressed in a variety of premalignant and

malignant conditions, including urinary bladder cancer. In the present study, we examined the feasibility of using Cox-2 promoter-based replication-selective adenovirus for targeting bladder cancer cells that express Cox-2 transcriptional activity. A series of human cancer cell lines, including three bladder cancer cell lines (KK47, T24, and 5637), were evaluated for their Cox-2 and CAR (the Coxsackievirus and adenovirus receptor) mRNA expression levels by quantitative real-time PCR. AdE3-cox2-327, a replication-selective adenovirus in which the expression of E1a is controlled by the Cox-2 promoter, was generated, and its tissue-specific activity was tested in vitro and in vivo. Three bladder cancer cell lines express higher levels of Cox-2 mRNA than does the human prostate cancer cell line PC3, the primary cultured human benign prostatic fibroblast, PF cells, and the human colon cancer cell line Colo320. Relatively higher expression of CAR mRNA was detected in the KK47, 5637, respectively, and Colo320 than in the T24, PC-3, and PF cells. In vitro assays revealed significant growth suppression of both Cox-2- and CAR-expressing bladder cancer cells KK47 and 5637 in comparison with the other cells that lack Cox-2 expression and/or CAR expression. The present study demonstrated both specificity and efficacy of AdE3-cox2-327, a selectively replicated adenovirus, toward the Cox-2-expressing bladder cancer cells in vitro and in vivo. We also found that CAR expression in the target cancer cells is an important factor for the efficacy of selectively replicated adenovirus-based gene therapy.

Siemens, D. R., S. Crist, et al. (2003). "Comparison of viral vectors: gene transfer efficiency and tissue specificity in a bladder cancer model." *J Urol* **170**(3): 979-84.

Gene transfer efficiency and specific cell targeting of vectors is a major obstacle in preclinical studies of gene therapy for malignant disease. Previous attempts at gene transfer in bladder cancer models have resulted in variable urothelial and tumor transgene expression after intravesical administration of recombinant viral vectors. In the current study we compared the gene transfer efficiencies of different viral vectors. We compared the gene transfer efficiencies of the viral vectors replication-deficient adenovirus, attenuated vaccinia virus (NYVAC) and canarypox virus (ALVAC) in vitro and in an orthotopic murine bladder cancer model. We used beta-galactosidase and firefly luciferase reporter gene expression to compare gene transfer efficiency. Significantly higher transgene expression was observed in vitro when these cells were infected with NYVAC or ALVAC compared with adenovirus vectors. Similarly the efficiency of adenovirus vectors

to transfer genetic material into bladder urothelium and orthotopic bladder tumors was inferior to that of ALVAC and NYVAC vectors, which interestingly appeared to have a predilection to infect the orthotopic tumor. Analysis of the expression of coxsackie-adenovirus receptor using reverse transcriptase-polymerase chain reaction revealed the bladder tumor cell lines were lacking this adenovirus receptor. While adenovirus transferred genes poorly to normal bladder, coxsackie-adenovirus receptor expression was high in bladder tissue. The viral vectors examined in these experiments resulted in significantly different gene transfer in the orthotopic bladder cancer model, underscoring the importance of vector selection in gene therapy protocols.

Slaton, J. W., R. Millikan, et al. (2004). "Correlation of metastasis related gene expression and relapse-free survival in patients with locally advanced bladder cancer treated with cystectomy and chemotherapy." *J Urol* **171**(2 Pt 1): 570-4.

Patients with locally advanced (ie clinically extravesical) transitional cell carcinoma are at high risk for recurrence after cystectomy. Although randomized trials have established an incremental benefit from the addition of chemotherapy in this setting, many patients still have disease relapse, and therefore it is necessary to determine patient and tumor characteristics that correlate with outcome in this setting. We investigated the tumor expression of several metastasis related genes and the association of gene expression with disease specific survival of patients with locally advanced transitional cell carcinoma treated randomized to either neoadjuvant or adjuvant chemotherapy and radical cystectomy. Archival paraffin embedded specimens were available for 64 patients enrolled in a clinical trial of the methotrexate, vinblastine, doxorubicin and cisplatin regimen and cystectomy. Only samples obtained before exposure to chemotherapy were studied. The expression of several metastasis related genes, including basic fibroblast growth factor, vascular endothelial growth factor (VEGF), interleukin-8, matrix metalloproteinase (MMP)-9, and E-cadherin were assayed on paraffin sections using a colorimetric in situ hybridization assay. Expression of basic fibroblast growth factor, interleukin-8 and MMP-9 did not correlate with outcome. Expression of VEGF and E-cadherin were strongly related to disease specific survival. In addition, the ratio of MMP-9-to-E-cadherin was strongly prognostic for disease specific survival. These data advance the hypotheses that VEGF expression and an "invasive phenotype" characterized by the ratio of MMP-9-to-E-cadherin expression are mechanistically relevant to clinically aggressive locally advanced bladder cancers that are

not cured by currently available combined modality treatment. Thus, in our view there is a compelling rationale to target these aspects of the malignant phenotype in this patient population.

Smith, N. D., J. N. Rubenstein, et al. (2003). "The p53 tumor suppressor gene and nuclear protein: basic science review and relevance in the management of bladder cancer." *J Urol* **169**(4): 1219-28.

An extensive body of literature regarding p53 has accumulated during the last 2 decades. The cellular mechanisms of p53 are complex yet well-defined, whereas its clinical usefulness in the management of bladder cancer remains controversial. We outline the basic constitutive functions of p53 and summarize its current role in the management of transitional cell carcinoma of the bladder. We conducted a MEDLINE based literature review concerning the fundamental mechanisms of p53 and its role in the management of bladder cancer. The p53 gene is a tumor suppressor gene that acts as "guardian of the genome." Many diverse cellular events, including DNA damage and hypoxia, activate the p53 gene. The p53 protein functions as a transcription factor, regulating downstream genes involved in cell cycle arrest, DNA repair and programmed cell death. Loss of p53 function confers genomic instability, impaired apoptosis and diminished cell cycle restraint. Therefore, p53 mutations select for certain critical features of malignancy. Alteration of P53 is the most common mutation in human cancer. Roughly half of all human malignancies, including many urological cancers, exhibit p53 mutations. In bladder cancer p53 mutations have been associated with higher tumor grade and advanced stage, as well as progression of superficial disease to muscle invasion. Moreover, p53 nuclear over expression appears to be an independent predictor of disease progression and decreased survival after cystectomy. The importance of p53 mutation in tumor cell biology is irrefutable. Wild-type p53 mediates imperative functions such as regulation of the cell cycle and programmed cell death. Deficiency of p53 function by mutation or inactivation abrogates normal cell cycle checkpoints and apoptosis, generating a favorable milieu for genomic instability and carcinogenesis. However, despite the manifest importance of p53 in human malignancy, its current role in the management of bladder cancer appears somewhat limited. A multitude of retrospective studies have associated p53 mutations with adverse outcomes in superficial and muscle invasive disease. Nonetheless, randomized prospective studies are needed to determine the potential clinical implications of p53 in bladder cancer.

Smith, S. C. and D. Theodorescu (2009). "The Ral GTPase pathway in metastatic bladder cancer: key mediator and therapeutic target." *Urol Oncol* **27**(1): 42-7.

Bladder cancer is a relatively common and strikingly costly malignancy. Here, we will focus on recent advances in our understanding of the molecular pathogenesis of metastatic bladder cancer, a stage of this disease curable in only a minority of patients. Our group has recently investigated the role of a class of small G-proteins known as the Ras-like or Ral GTPases and their role in this disease. These signaling proteins, regulated by the Ras pathway and other mechanisms, have been shown to be necessary for key cellular phenotypes associated with transformation or cancer progression in diverse cancer systems. In bladder cancer we have observed that these GTPases are overexpressed, are necessary for key phenotypes in models of bladder cancer progression, and finally, are essential for the regulation of expression of key molecules, including the prognostic marker and cell surface GPI-linked glycoprotein, CD24. These findings are reviewed here and suggest that Ral GTPases and their downstream pathways constitute key mediators of bladder cancer progression and may include targets for future therapeutic strategies.

So, A., P. Rocchi, et al. (2005). "Antisense oligonucleotide therapy in the management of bladder cancer." *Curr Opin Urol* **15**(5): 320-7.

In this paper, we will review the recent advances in antisense oligonucleotide therapy in the treatment of superficial bladder cancer. Bladder cancer has an exciting potential as a model to study antisense oligonucleotide therapy because of the ease of accessibility of treatment, ease of diagnosis through biopsy and urine cytology, and direct observation of treatment efficacy through cystoscopy and posttreatment biopsy. RECENT FINDINGS: We will elaborate on the recent developments in the delivery of antisense oligonucleotide and the implications of these results on the use of antisense oligonucleotide intravesically. We will also discuss recent preclinical in-vitro results of antisense oligonucleotide therapy in different bladder cancer cell lines. SUMMARY: Recent developments of the in-vitro and animal in-vivo effectiveness of antisense treatment in bladder cancer provide the foundation to pursue future phase I clinical trials. Antisense oligonucleotide technology is a promising tool that may become an effective method of treating bladder cancer.

Sorensen, B. S., N. Torring, et al. (2004). "The DNA damaging agent VP16 induces the expression of a subset of ligands from the EGF system in bladder

cancer cells, whereas none of the four EGF receptors are induced." *Mol Cell Biochem* **260**(1-2): 129-35.

Increased activity of the EGF system exerts a cell survival function in the presence of cytotoxic agents. The aim of our investigation was to identify the ligands and receptors from the EGF system, that are induced by the chemotherapeutic DNA damaging agent VP16 in bladder cancer cell lines. By use of real-time RT-PCR assays for all four receptors and six ligands from the EGF system we demonstrate that in HCV29 bladder cancer cells, amphiregulin, HB-EGF, and epiregulin mRNA levels are elevated (more than 100, 5, and 4 fold, respectively) by VP16. The remaining ligands (EGF, TGFalpha and betacellulin) are uninduced. The same was found for T24A bladder cancer cells, except that TGFalpha also was induced. The four receptors were reduced by VP16 in both cell lines. This demonstrates that the induction of the EGF system is mediated by an increased expression of a subset of the ligands, whereas the four receptors are reduced. For amphiregulin and HER1 we investigated with ELISA assays if the effects of VP16 also were observed at the protein level. We found that VP16 increase the amount of amphiregulin peptide both in the cell membrane and the culture medium. Similarly, the reduced EGF receptor mRNA expression correlated with reduced HER1 protein. Several investigations have shown that labile protein factors can be involved in the regulation of stress inducible growth factors and cytokines. We investigated if a labile protein regulates the expression of the subset of ligands that were induced with VP16. Blocking of protein neosynthesis with cycloheximide resulted in induced mRNA expression of exactly the same subset of ligands as observed with VP16 treatment of both HCV29 and T24A cells. This suggests that a labile protein factor regulates either the transcription or degradation of these mRNA's, and it can be speculated that VP16 also operate by inhibiting the activity of this factor. This is further stressed by the observation that combined treatment with cycloheximide and VP16 show no additive effect. In conclusion, we show that a subset of ligands from the EGF system is upregulated by VP16, whereas none of the four receptors are induced. This might represent a physiological response aimed at rescuing the cells.

Stravopodis, D. J., P. K. Karkoulis, et al. (2009). "Grade-dependent effects on cell cycle progression and apoptosis in response to doxorubicin in human bladder cancer cell lines." *Int J Oncol* **34**(1): 137-60.

Doxorubicin is an important component of combination therapy for muscle-invasive urinary bladder cancer. Treatment with this topoisomerase II poison is able to interfere with cell cycle progression and lead to cancer cell death. Using FACS analysis,

Western immunoblotting and semi-quantitative RT-PCR, we studied the effects of doxorubicin on cell cycle progression and apoptosis, and also explored the possibility of using groups of genes as biomarkers of prognosis and/or response to doxorubicin treatment in human urinary bladder cancer cells. Doxorubicin induced dose-dependent G2/M and/or G1/S cell cycle arrest, followed by grade- and dose-dependent reduction in the amount of the cytosolic trimeric form of FasL, activation of Caspase-8, Caspase-9, Caspase-3, cleavage of PARP, Lamin A/C, Bcl-XL/S and interestingly Hsp90, and finally cell death. Data presented here also suggest the use of the expression patterns of Cyclin-E2, Cyclin-F, p63, p73, FasL, TRAIL, Tweak, Tweak-R, XAF-1, OPG and Bok genes for identification of the differentiation grade, and Cyclin-B2, GADD45A, p73, FasL, Bik, Bim, TRAIL, Fas, Tweak-R, XAF-1, Bcl-2, Survivin, OPG, DcR2 and Bcl-XL genes for the detection of response to doxorubicin in human bladder cancer cells.

Su, C. C., G. W. Chen, et al. (2003). "Luteolin induces N-acetylation and DNA adduct of 2-aminofluorene accompanying N-acetyltransferase activity and gene expression in human bladder cancer T24 cell line." *Anticancer Res* **23**(1A): 355-62.

Arylamine N-acetyltransferase (NAT) plays an important role in the first step of arylamines metabolism. Luteolin has shown antibacterial, antioxidant and antineoplastic activity. To glean insights into the mechanism of action of luteolin, we assessed the effects of luteolin on NAT activity and gene expression and DNA-2-aminofluorene (AF) adduct formation in human bladder cancer T24 cells. By using high performance liquid chromatography, the amounts of N-acetylation of AF and DNA adducts were determined and quantitated. Gene expression was performed by using polymerase chain reaction and gel electrophoresis. The results indicated: 1) luteolin displayed a dose-dependent inhibition of NAT activity and gene expression (NAT1 mRNA) in T24 cells; 2) time-course experiments showed that N-acetylation of AF measured from intact T24 cells was inhibited by luteolin for up to 48 hours; 3) using standard steady-state kinetic analysis, it was demonstrated that luteolin was a possible uncompetitive inhibitor of NAT activity in T24 cells; and 4) the DNA-AF adduct formation in T24 cells was inhibited by luteolin. This report is the first finding which has shown luteolin to affect human T24 cell NAT activity and gene expression (NAT1 mRNA) and DNA-AF adduct formation.

Sudarshan, S., D. H. Holman, et al. (2005). "In vitro efficacy of Fas ligand gene therapy for the treatment of bladder cancer." *Cancer Gene Ther* **12**(1): 12-8.

Previous investigations have revealed that bladder cancer cells are generally resistant to Fas-mediated apoptosis by conventional Fas agonists. However, the ability of these cell lines to undergo Fas-mediated apoptosis may have been underappreciated. As a result, we investigated the in vitro efficacy of Fas ligand gene therapy for bladder cancer. Three human bladder cancer lines (T24, J82, and 5637) were treated with the conventional Fas agonist CH-11, a monoclonal antibody to the Fas receptor. Cells were also treated with a replication-deficient adenovirus containing a modified murine Fas ligand gene fused to green fluorescent protein (GFP), AdGFPFasL. A virus containing the GFP gene alone was used to control for viral toxicity (AdGFP). Cell death was quantified using a tetrazolium-based (MTS) assay. Cells were also evaluated by Western blotting to evaluate poly (ADP-ribose) polymerase, caspase 8, and caspase 9 cleavage and by flow cytometry to determine the presence of coxsackie/adenovirus receptor (CAR). These studies confirmed bladder cancer resistance to cell death by the anti-Fas monoclonal antibody CH-11. This resistance was overcome with AdGFPFasL at a multiplicity of infection (MOI) of 1000 achieving over 80% cell death in all cell lines. Furthermore, greater than 80% cell death was evident in 5637 cells treated with low-dose AdGFPFasL (MOI=10). 5637 cells expressed significantly higher levels of surface CAR than J82 or T24 cells ($P < 0.05$). AdGFPFasL is cytotoxic to bladder cancer cells that would otherwise be considered Fas resistant, supporting its in vivo potential. Enhanced sensitivity to AdGFPFasL may be in part due to increased cell surface CAR levels.

Sugimoto, S., N. Maass, et al. (2004). "Expression and regulation of tumor suppressor gene maspin in human bladder cancer." *Cancer Lett* **203**(2): 209-15.

Maspin is a member of serine protease inhibitor family with tumor suppressing activity for breast and prostate cancers, acting at the level of tumor invasion and metastasis. However, there have been no published data regarding the role of maspin in human bladder cancer. We evaluated maspin expression in 65 series of bladder cancer samples (22 transurethral resection (TUR) and 43 radical cystectomy) and studied the regulatory mechanism of maspin gene activation in bladder cancer cells. Maspin expression was immunohistochemically detected in four (18.2%) patients with TUR and 22 (51.2%) patients with radical cystectomy whereas no expression was observed in normal transitional cells located at tumor-free area in bladder. The maspin expression was significantly correlated with the development of muscle invasive bladder cancer ($P=0.00008$). Using a luciferase reporter system, maspin promoter activity was induced in the maspin-

positive bladder cancer cell lines as well as maspin-negative RT4 cells. Furthermore, treatment with the DNA methyltransferase inhibitor, 5-aza-2' deoxycytidine, and histone deacetylase inhibitor, trichostatin A, led to re-expression of maspin in RT4 cells. Our results indicate that maspin may contribute to bladder cancer development and that DNA methylation and histone deacetylation may be important for regulating maspin gene activation in bladder cancer cells.

Sweeney, P., T. Karashima, et al. (2003). "Efficient therapeutic gene delivery after systemic administration of a novel polyethylenimine/DNA vector in an orthotopic bladder cancer model." *Cancer Res* **63**(14): 4017-20.

Successful systemic gene therapy has been hindered by vector-related limitations, including toxicity and inefficient gene delivery to tumor cells after i.v. administration. To circumvent these problems, we developed a novel formulation between the polycation polyethyleneimine and DNA that mediates high-level tumor cell transduction in vitro and efficient i.v. gene delivery in that greater reporter gene expression occurred in tumor than in lung. Strikingly, administration of just 6 micro g of the polyethyleneimine/DNA-p53 vector every 3 days for 3 weeks indicated restoration of normal cell cycle regulation and apoptotic mechanisms as demonstrated by efficient p53 expression, increased apoptosis, and a 70% reduction in tumor size in an orthotopic bladder cancer model. This novel vector formulation represents a new method to increase i.v. delivery of genes to tumors.

Swellam, M., N. Abd-Elmaksoud, et al. (2004). "Incidence of Bcl-2 expression in bladder cancer: relation to schistosomiasis." *Clin Biochem* **37**(9): 798-802.

Apoptosis (programmed cell death) and the genes regulating this process (e.g., Bcl-2) have recently become a focus of interest in the study of cancer development and progression. The bcl-2 gene product plays a role as an inhibitor of apoptosis; it contributes to oncogenesis by suppressing signals that induce apoptotic cell death. The aim of this study was to determine the expression of bcl-2 in schistosomal bladder cancer and to compare it with the established clinicopathological factors. Tumor tissues from 118 patients with bladder cancer were examined [57 with squamous cell carcinoma (SCC) and the remaining 61 with transitional cell carcinoma (TCC)]. Of 118 patients, 60 had schistosomiasis associated with bladder cancer. Bcl-2 expression was determined by enzyme immunoassay and the results were confirmed by Western blot and immunodot blot techniques. Bcl-

2 was significantly expressed in SCC compared to those with TCC type in the presence of schistosomiasis. Moreover, bcl-2 was associated with clinical stages and lymph node involvement but not with histological grades. These observations detect a potential role for bcl-2 expression in schistosomal carcinogenesis, and hence selecting patients for future anti-bcl-2 therapy.

Swellam, M., A. A. El-Aal, et al. (2004). "Deletions of p15 and p16 in schistosomal bladder cancer correlate with transforming growth factor-alpha expression." *Clin Biochem* 37(12): 1098-104.

Cell proliferation is stimulated by growth factors and inhibited by p15 and p16 gene products. We compared cell regulators, TGF-alpha, p15, and p16, in schistosomal and non-schistosomal bladder cancer to explore possible differences in their alterations between the two subtypes and their correlations with proliferation pattern [synthetic phase fraction (SPF)], DNA ploidy, and clinicopathological factors. Tumor tissue samples were obtained from 120 patients. Expressions of p15 and p16 genes were investigated by the polymerase chain reaction, while TGF-alpha protein expression was measured by an enzyme immunoassay (EIA) method. Deletion of both p15 and p16 was observed in 62 and 46 bladder tumors, respectively. TGF-alpha was overexpressed in 64 bladder tumors. A highly significant association was observed between the two deleted genes and TGF-alpha positivity. Of the entire group, p15 and p16 alteration and positive TGF-alpha ($>$ or $=$ cutoff value) were significantly expressed in schistosomal bladder cancer (68.1%, 60.9%, and 65.2%), and squamous cell carcinoma type (SCC) (69.1%, 64.7% and 72.1%) compared to those with non-schistosomal bladder cancer (29.4%, 7.8%, and 37.3%) or transitional cell carcinoma (TCC) (28.8%, 3.8%, and 28.8), respectively. A significant association between p15 and p16 deletion and TGF-alpha positivity with high SPF, aneuploid DNA pattern, late stages, and high histological grades was also documented. **CONCLUSION:** Alteration of p15 and p16 genes and overexpression of TGF-alpha appears to be an event in bladder cancer that occurs more frequently in schistosomal bladder cancer and SCC, and may play an important role in their development. These observations may provide insight into treatment guided by molecular changes.

Tada, Y., M. Wada, et al. (2002). "Increased expression of multidrug resistance-associated proteins in bladder cancer during clinical course and drug resistance to doxorubicin." *Int J Cancer* 98(4): 630-5.

Overexpression of the P-glycoprotein/multidrug resistance 1 (MDR1) and

multidrug resistance protein 1 (MRP1) gene is closely associated with the clinical outcome of various malignancies, and it is involved in responses to some anticancer chemotherapeutic agents including doxorubicin. Six human MRP subfamily members (MRP2-7) with structural similarities to MRP1 have been identified. Recently, the relationships between MRP2 and MRP3 expression levels of some cancer cells and drug sensitivity to doxorubicin have been reported, but the relationship between the clinical samples and drug sensitivity remains unclear. We determined the expressions of the MDR1, MRP1, MRP2 and MRP3 gene in bladder cancer during the clinical course and sought to learn whether the expression was correlated with drug responses to doxorubicin. Doxorubicin, used in chemotherapeutic treatment including intravesical and systemic chemotherapy, is an important anticancer agent for the treatment of bladder cancer. We used quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis for our study, and the sensitivity to doxorubicin in bladder cancer was determined using the in vitro succinate dehydrogenase inhibition test. Using 47 clinical samples of bladder cancer, we confirmed the significant correlation of MDR1, MRP1 and MRP3 mRNA levels with resistance to doxorubicin. We showed that the expression of MDR1, MRP1, MRP2 and MRP3 in recurrent tumors and residual tumors after chemotherapeutic treatment was higher than that in untreated primary tumors. In particular, the MDR1 expression in residual tumors was 5.7-fold higher than that in untreated primary tumors.

Tanaka, M. and H. B. Grossman (2003). "In vivo gene therapy of human bladder cancer with PTEN suppresses tumor growth, downregulates phosphorylated Akt, and increases sensitivity to doxorubicin." *Gene Ther* 10(19): 1636-42.

The PTEN gene, located on chromosome 10, is a phosphatase in the phosphatidylinositol 3'-kinase (PI3K)-mediated signal transduction pathway. PTEN inhibits the activation of Akt, a serine-threonine kinase involved in proliferative metabolic and antiapoptotic pathways, and has tumor suppressive properties. We created a PTEN adenoviral vector, Ad-MMAC, to assess the role of PTEN in the treatment of bladder cancer. Direct injection of Ad-MMAC into established subcutaneous UM-UC-3 (PTEN deleted, upregulation of phosphorylated Akt) and UM-UC-6dox (wild-type PTEN, upregulation of phosphorylated Akt) tumors in nude mice resulted in PTEN expression, apoptosis, and significantly decreased growth compared to Ad-CTR- or Phosphate-buffered saline (PBS)-treated tumors. UM-UC-3 tumors completely disappeared in all of mice

treated with Ad-MMAC, but PBS- and Ad-CTR-treated UM-UC-3 tumors continued to grow rapidly. UM-UC-14 tumors (wild-type PTEN) were transiently suppressed by Ad-MMAC. Downregulation of vascular endothelial growth factor and decreased microvessel density were seen in tumors treated with Ad-MMAC in vivo. Combination therapy with Ad-MMAC and doxorubicin improved the in vivo efficacy of PTEN gene therapy in the doxorubicin-resistant cell line UM-UC-6dox. Treatment with Ad-MMAC and doxorubicin completely eradicated established UM-UC-6dox tumors in three of 10 mice. UM-UC-14 tumors were transiently suppressed by this combined treatment. These data demonstrate that PTEN gene therapy can effectively treat bladder cancers that have genomic alterations in PTEN. Furthermore, tumors that exhibit drug resistance associated with expression of phosphorylated Akt can be effectively treated with PTEN gene therapy and chemotherapy.

Tang, W., Y. He, et al. (2009). "A novel Bifidobacterium infantis-mediated TK/GCV suicide gene therapy system exhibits antitumor activity in a rat model of bladder cancer." *J Exp Clin Cancer Res* **28**: 155.

Bladder cancer is the ninth most common malignancy in the world. Successful clinical management remains a challenge. In order To search for novel targeted and efficacious treatment, we sought to investigate anti-tumor activity of BI-TK suicide gene therapy system in a rat model of bladder tumors. We first constructed and tested an anaerobic Bifidobacterium infantis-mediated thymidine kinase (BI-TK) suicide gene therapy system. To test the in vivo efficacy of this system, we established a rat model of bladder tumors, which was induced by N-methyl-nitrosourea perfusion. Bifidobacterium infantis containing the HSV-TK (i.e., BI-TK) were constructed by transformation of recombinant plasmid pGEX - TK. The engineered BI-TK was injected into tumor-bearing rats via tail vein, followed by intraperitoneal injection of ganciclovir (GCV). Using the rat model of bladder tumors, we found that bladder tumor burdens were significantly lower in the rats treated with BI-TK/GCV group than that treated with normal saline control group ($p < 0.05$). While various degrees of apoptosis of the tumor cells were detected in all groups using in situ TUNEL assay, apoptosis was mostly notable in the BI-TK/GCV treatment group. Immunohistochemical staining further demonstrated that the BI-TK/GCV treatment group had the highest level of caspase3 protein expression than that of the empty plasmid group and normal saline group ($p < 0.05$). Thus, our results demonstrate that the Bifidobacterium infantis-mediated TK/GCV

suicide gene therapy system can effectively inhibit rat bladder tumor growth, possibly through increasing caspase 3 expression and inducing apoptosis.

Tang, Y., A. R. Simoneau, et al. (2008). "Effects of the kava chalcone flavokawain A differ in bladder cancer cells with wild-type versus mutant p53." *Cancer Prev Res (Phila)* **1**(6): 439-51.

Flavokawain A is the predominant chalcone from kava extract. We have assessed the mechanisms of flavokawain A's action on cell cycle regulation. In a p53 wild-type, low-grade, and papillary bladder cancer cell line (RT4), flavokawain A increased p21/WAF1 and p27/KIP1, which resulted in a decrease in cyclin-dependent kinase-2 (CDK2) kinase activity and subsequent G(1) arrest. The increase of p21/WAF1 protein corresponded to an increased mRNA level, whereas p27/KIP1 accumulation was associated with the down-regulation of SKP2, which then increased the stability of the p27/KIP1 protein. The accumulation of p21/WAF1 and p27/KIP1 was independent of cell cycle position and thus not a result of the cell cycle arrest. In contrast, flavokawain A induced a G(2)-M arrest in six p53 mutant-type, high-grade bladder cancer cell lines (T24, UMUC3, TCCSUP, 5637, HT1376, and HT1197). Flavokawain A significantly reduced the expression of CDK1-inhibitory kinases, Myt1 and Wee1, and caused cyclin B1 protein accumulation leading to CDK1 activation in T24 cells. Suppression of p53 expression by small interfering RNA in RT4 cells restored Cdc25C expression and down-regulated p21/WAF1 expression, which allowed Cdc25C and CDK1 activation, which then led to a G(2)-M arrest and an enhanced growth-inhibitory effect by flavokawain A. Consistently, flavokawain A also caused a pronounced CDK1 activation and G(2)-M arrest in p53 knockout but not in p53 wild-type HCT116 cells. This selectivity of flavokawain A for inducing a G(2)-M arrest in p53-defective cells deserves further investigation as a new mechanism for the prevention and treatment of bladder cancer.

Taylor, J. A., 3rd, G. A. Kuchel, et al. (2007). "Null mutation for macrophage migration inhibitory factor (MIF) is associated with less aggressive bladder cancer in mice." *BMC Cancer* **7**: 135.

Inflammatory cytokines may promote tumorigenesis. Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine with regulatory properties over tumor suppressor proteins involved in bladder cancer. We studied the development of bladder cancer in wild type (WT) and MIF knockout (KO) mice given N-butyl-N-(4-hydroxybutyl)-nitrosamine (BBN), a known carcinogen, to determine the role of MIF in bladder

cancer initiation and progression. 5-month old male C57Bl/6 MIF WT and KO mice were treated with and without BBN. Animals were sacrificed at intervals up to 23 weeks of treatment. Bladder tumor stage and grade were evaluated by H&E. Immunohistochemical (IHC) analysis was performed for MIF and platelet/endothelial cell adhesion molecule 1 (PECAM-1), a measure of vascularization. MIF mRNA was analyzed by quantitative real-time polymerase chain reaction. Poorly differentiated carcinoma developed in all BBN treated mice by week 20. MIF WT animals developed T2 disease, while KO animals developed only T1 disease. MIF IHC revealed predominantly urothelial cytoplasmic staining in the WT control animals and a shift toward nuclear staining in WT BBN treated animals. MIF mRNA levels were 3-fold higher in BBN treated animals relative to controls when invasive cancer was present. PECAM-1 staining revealed significantly more stromal vessels in the tumors in WT animals when compared to KOs. CONCLUSION: Muscle invasive bladder cancer with increased stromal vascularity was associated with increased MIF mRNA levels and nuclear redistribution. Consistently lower stage tumors were seen in MIF KO compared to WT mice. These data suggest that MIF may play a role in the progression to invasive bladder cancer.

Terao, S., T. Shirakawa, et al. (2005). "Recombinant interleukin-2 enhanced the antitumor effect of ADV/RSV-HSV-tk/ACV therapy in a murine bladder cancer model." *Anticancer Res* **25**(4): 2757-60.

Previous studies demonstrated the antitumor effects of IL-2 and ADV/RSV-HSV-tk in bladder tumor models. In our study, we employed the intramuscular injection of recombinant IL-2 combined with ADV/RSV-HSV-tk gene therapy in the MBT-2 murine bladder tumor model. In the in vitro study, after adenoviral gene transduction efficiency had been assessed, the cytotoxicity of ADV/RSV-HSV-tk/ACV was examined. In the in vivo study, ADV/RSV-HSV-tk was injected into MBT-2 subcutaneous tumors, ACV was injected intraperitoneally daily for 13 days and recombinant IL-2 was injected intramuscularly daily for 10 days. The X-gal staining of MBT-2 cells infected with 125 multiplicity of injection (MOI) indicated > 20% adenoviral gene transduction efficiency. The cell growth of MBT-2 infected with 125 MOI was significantly inhibited by 40 microM of ACV. In the in vivo study, the combination therapy significantly inhibited tumor growth in the MBT-2 tumor model. CONCLUSION: The systemic administration of recombinant IL-2 in combination with HSV-tk gene therapy exhibited an enhanced antitumor effect.

Terao, S., T. Shirakawa, et al. (2007). "Midkine promoter-based conditionally replicative adenovirus for targeting midkine-expressing human bladder cancer model." *Urology* **70**(5): 1009-13.

To develop a novel therapeutic strategy against human bladder cancer using Ad-MK-E1a-a midkine (MK) promoter-regulated, conditionally replicating, adenovirus. We tested several human cancer cell lines in vitro, including those of bladder cancer (KK47, 5637, and T24), lung cancer (A549), and head and neck cancer (H891). In each cell line, we examined MK mRNA expression by TaqMan real-time quantitative polymerase chain reaction, MK promoter activity, after plasmid transfection, using a luciferase assay, and the transduction efficiency by co-transfection with the cytomegalovirus-beta-gal plasmid. In these cells, we assessed the cell type-specific replication of Ad-MK-E1a virus by measuring the E1a DNA copy number by real-time polymerase chain reaction and the cell growth inhibition due to this virus using the Alamar blue assay. In animal studies, nude mice were subcutaneously inoculated with KK47 cells and later intratumorally injected with phosphate-buffered saline or Ad5-CMV-LacZ or Ad-MK-E1a. The MK mRNA expression level and MK promoter-driven luciferase activity were relatively greater and markedly increased, respectively, in the 5637, A549, and KK47 cells than in the T24 and H891 cells. After Ad-MK-E1a infection, the E1a DNA copy number increased more significantly in the KK47, 5637, and A549 cells than in the T24 and H891 cells. At a multiplicity of infection of 0.01, Ad-MK-E1a significantly inhibited KK47 and 5637 cell growth. In vivo, Ad-MK-E1a injection markedly inhibited KK47 tumor growth. We have demonstrated the antitumor effect of Ad-MK-E1a in a human bladder cancer model overexpressing MK mRNA.

Theodorescu, D., L. M. Sapinoso, et al. (2004). "Reduced expression of metastasis suppressor RhoGDI2 is associated with decreased survival for patients with bladder cancer." *Clin Cancer Res* **10**(11): 3800-6.

RhoGDI2 was recently shown to be a metastasis suppressor gene in models of bladder cancer. We sought to further understand its importance in human cancer by determining the level of its expression and the distribution of its encoded protein in normal human tissues and cell lines and to evaluate whether its protein expression is a determinant of human bladder cancer progression. RhoGDI2 mRNA and protein expression was evaluated in cell lines and human tissues using Affymetrix and tissue microarrays, respectively. Tissue microarrays represented most human normal adult tissues and material from 51 patients that had

undergone radical cystectomy for bladder cancer. In these 51 patients, the chi(2) test was used to test for associations between RhoGDI2 and stage, grade of urothelial carcinoma, histological type, and disease-specific survival status. Cox proportional hazards regression analyses were used to estimate the effect of RhoGDI2 expression level on time to development of metastatic disease and disease-specific survival time, adjusting for grade, stage, and histological type. In normal tissues, there was strong RhoGDI2 protein expression in WBCs, endothelial cells, and transitional epithelium. RhoGDI2 mRNA expression was inversely related to the invasive and metastatic phenotype in human bladder cancer cell lines. In patients with bladder cancer, univariate analysis indicated that reduced tumor RhoGDI2 protein expression was associated with a lower actuarial 5-year disease-free and disease-specific survival ($P = 0.01$). In addition, patients with tumors that had low or absent RhoGDI2 had a shorter time to disease-specific death ($P \leq 0.01$). When tumor grade, stage, histological type, and RhoGDI2 staining level were examined using multivariate analysis, RhoGDI2 expression was found to be an independent predictive factor for disease-specific death ($P = 0.03$). These results suggest that RhoGDI2 is an independent predictor of prognosis for patients with bladder cancer and provide clinical evidence in support of its involvement in cancer metastasis.

Tomlinson, D. C., O. Baldo, et al. (2007). "FGFR3 protein expression and its relationship to mutation status and prognostic variables in bladder cancer." *J Pathol* **213**(1): 91-8.

FGFR3 is frequently activated by mutation in urothelial carcinoma (UC) and represents a potential target for therapy. In multiple myeloma, both over-expression and mutation of FGFR3 contribute to tumour development. To define the population of UC patients who may benefit from FGFR-targeted therapy, we assessed both mutation and receptor over-expression in primary UCs from a population of new patients. Manual or laser capture microdissection was used to isolate pure tumour cell populations. Where present, non-invasive and invasive components in the same section were microdissected. A screen of the region of the highest tumour stage in each sample yielded a mutation frequency of 42%. Mutations comprised 61 single and five double mutations, all in hotspot codons previously identified in UC. There was a significant association of mutation with low tumour grade and stage. Subsequently, non-invasive areas from the 43 tumours with both non-invasive and invasive components were analysed separately; 18 of these had mutation in at least one region, including nine with mutation in all regions examined, eight with

mutation in only the non-invasive component and one with different mutations in different regions. Of the eight with mutation in only the non-invasive component, six were predicted to represent a single tumour and two showed morphological dissimilarity of fragments within the block, indicating the possible presence of distinct tumour clones. Immunohistochemistry showed over-expression of FGFR3 protein in many tumours compared to normal bladder and ureteric controls. Increased expression was associated with mutation (85% of mutant tumours showed high-level expression). Overall, 42% of tumours with no detectable mutation showed over-expression, including many muscle-invasive tumours. This may represent a non-mutant subset of tumours in which FGFR3 signalling contributes to the transformed phenotype and which may benefit from FGFR-targeted therapies.

Tomlinson, D. C., C. D. Hurst, et al. (2007). "Knockdown by shRNA identifies S249C mutant FGFR3 as a potential therapeutic target in bladder cancer." *Oncogene* **26**(40): 5889-99.

More than 60% of low-grade non-invasive papillary urothelial cell carcinomas contain activating point mutations of fibroblast growth factor receptor 3 (FGFR3). The phenotypic consequences of constitutive activation of FGFR3 in bladder cancer have not been elucidated and further studies are required to confirm the consequences of inhibiting receptor activity in urothelial cells. We measured FGFR3 transcript levels and demonstrated that transcript levels were significantly more abundant in low-stage and grade tumours. We identified a tumour cell line, 97-7, expressing the most common FGFR3 mutation (S249C) at similar FGFR3 transcript levels to low-stage and grade tumours. In these cells, S249C FGFR3 protein formed stable homodimers and was constitutively phosphorylated. We used retrovirus-mediated delivery of shRNA to knockdown S249C FGFR3. This induced cell flattening, decreased cell proliferation and reduced clonogenicity on plastic and in soft agar. However, no effects of knockdown of wild-type FGFR3 were observed in telomerase immortalized normal human urothelial cells, indicating possible dependence of the tumour cell line on mutant FGFR3. Re-expression of S249C FGFR3 in shRNA-expressing 97-7 cells resulted in a reversal of phenotypic changes, confirming the specificity of the shRNA. These results indicate that targeted inhibition of S249C FGFR3 may represent a useful therapeutic approach in superficial bladder cancer.

Tong, Q., F. Zeng, et al. (2003). "Growth inhibiting effects of antisense eukaryotic expression vector of proliferating cell nuclear antigen gene on human

bladder cancer cells." *Chin Med J (Engl)* **116**(8): 1203-6.

To explore the growth inhibiting effects on human bladder cancer by antisense RNA targeting the proliferating cell nuclear antigen (PCNA) gene. The eukaryotic expression vector for antisense PCNA cDNA was constructed and transferred into a bladder cancer EJ cell line. The PCNA expression in the cancer cells was detected by RT-PCR and Western blotting assays. The in vitro proliferation activities of the transferred cells were observed by growth curve, tetrazolium bromide (MTT) colorimetry, tritiated thymidine ((3)H-TdR) incorporation, flow cytometry and clone formation testing, while its in vivo anti-tumor effects were detected on nude mice allograft models. After the antisense vector, pLAPSN, was transferred, cellular PCNA expression was inhibited at both protein and mRNA levels. The growth rates of EJ cells were reduced from 27.91% to 62.07% ($P < 0.01$), with an inhibition of DNA synthesis rate by 52.31% ($P < 0.01$). Transferred cells were blocked at G(0)/G(1) phases in cell-cycle assay, with the clone formation ability decreased by 50.81% ($P < 0.01$). The in vivo carcinogenic abilities of the transferred cancer cells were decreased by 54.23% ($P < 0.05$). Antisense PCNA gene transfer could inhibit the growth of bladder cancer cells in vitro and in vivo, which provided an ideal strategy for gene therapy of human cancers.

Tong, Q., F. Zeng, et al. (2002). "Construction of the antisense eukaryotic vector for proliferating cell nuclear antigen gene and its expression in bladder cancer EJ cell line." *J Huazhong Univ Sci Technolog Med Sci* **22**(4): 327-30.

To explore a novel strategy for antisense gene therapy of cancer, the coding sequence of human proliferating cell nuclear antigen (PCNA) cDNA was reversely inserted into the eukaryotic vector pLXSN by molecular cloning techniques and transferred into bladder cancer EJ cells with liposome. The PCNA expression in transferred cells was dynamically detected by immunofluorescence and RT-PCR techniques. Changes of proliferation activities of cancer cells were assayed by MTT colorimetric and cloning formation methods. In the experiment, the antisense eukaryotic vector was successfully constructed and named as pLAPSN. After transfection with it for 1-7 days, PCNA protein and mRNA levels in cancer cells were blocked by 16.74%-84.21% ($P < 0.05$) and 23.27%-86.15% ($P < 0.05$) respectively. The proliferation activities of transferred cells were inhibited by 27.91%-62.07% ($P < 0.01$), with cloning formation abilities being decreased by 50.81% ($P < 0.01$). It was concluded that the in vitro proliferation activities of cancer cells could be effectively inhibited

by blocking PCNA expression with antisense technique, which could serve as an ideal strategy for gene therapy of bladder cancer.

Torabi-Pour, N., A. M. Nouri, et al. (2002). "Comparative study of peptides isolated from class I antigen groove of urological specimens. a further step toward the future of peptide therapy in bladder cancer patients." *Urol Int* **68**(3): 183-8.

The major histocompatibility (MHC) class I antigens act as associative molecules for interaction amongst immuno-competent cells. The grooves of class I antigens are normally loaded with self peptides of between 8 and 11 amino acids. However, when the cells transform to malignant state they may carry peptide(s) of non-self origin within these grooves. Using immuno-bead purification followed by high-performance liquid chromatography (HPLC), this study attempted to isolate peptides from class I antigens of various biological specimens. The combination of immuno-bead purification (BP) and HPLC was reliable for peptide isolation. Class I antigens and associated peptides could be isolated from normal peripheral blood leukocyte (PBL). Under the same conditions, the PBL yielded almost twice as much peptide as that of tumour cell lines. The HPLC profile of peptides (range of 8-10 amino acid residues) isolated from a bladder and a cervical tumour cell line showed unique features. In the case of the bladder line there were at least 22 peptides. In addition, the class I-associated peptides could also be isolated from kidney tumour fragments of three individuals. In each the isolated peptides showed a unique HPLC peak profile with some similarities as well as differences. These data indicated a variation in the nature of peptides isolated from different specimens. The approach showed the feasibility of preparing peptide(s) from a relatively small number of cells. The data also showed that peptide isolation could also be carried out from tumour tissue biopsies paving the way for the future of peptide vaccination in cancer patients following the identification of putative tumour-specific replicate(s).

Tseng, Y. S., C. C. Tzeng, et al. (2006). "Aurora-A overexpression associates with Ha-ras codon-12 mutation and blackfoot disease endemic area in bladder cancer." *Cancer Lett* **241**(1): 93-101.

Our data revealed that 59.4% of the bladder cancer specimens showed Aurora-A overexpression, of which 31.8% also had Ha-ras codon-12 mutation; 45.5% were from blackfoot-disease endemic areas in which arsenic exposure is a major environment factor associated with various cancer formation. We further demonstrated that arsenic treatment of the immortalized bladder cell line, E7, increased Aurora-A expression. All together, co-existence of Aurora-A

overexpression and Ha-ras mutation suggests a possible additive effect on the tumorigenesis of bladder cancer. In addition, Aurora-A overexpression and up-regulated by arsenic exposure opens a new direction for exploring the occurrence of bladder cancer occurrence in Taiwan.

Tsui, K. H., H. H. Juang, et al. (2008). "Association of nucleophosmin/B23 with bladder cancer recurrence based on immunohistochemical assessment in clinical samples." *Acta Pharmacol Sin* 29(3): 364-70.

To investigate the possible correlation of nucleophosmin/B23 expression with bladder carcinoma recurrence. Surgically-resected bladder tumors staged pT_a to pT₄ were examined for nucleophosmin/B23 expression by immunohistochemistry. The study group consisted of 132 consecutive patients surgically treated at Chang Gung Memorial Hospital between December 1998 and November 1999. The mean follow up was 72 months (range: 48-84 months). Nuclear nucleophosmin/B23 staining was detected in 96% of advanced stage and poorly-differentiated tumors. Higher nucleophosmin/B23 levels were linked to more advanced tumor stages, grades, poor prognosis, and likelihood of recurrence (P<0.05). The Cox multivariate analysis indicated the nucleophosmin/B23 expression as an independent indicator for tumor recurrence (P=0.009). **CONCLUSION:** The results suggest that nucleophosmin/B23 is a favorable prognostic indicator for bladder cancer. Nucleophosmin/B23 could be a useful molecular tumor marker for predicting bladder cancer recurrence.

Urakami, S., H. Shiina, et al. (2006). "Epigenetic inactivation of Wnt inhibitory factor-1 plays an important role in bladder cancer through aberrant canonical Wnt/beta-catenin signaling pathway." *Clin Cancer Res* 12(2): 383-91.

Aberrant activation of the Wingless-type (Wnt) pathway plays a significant role in the pathogenesis of several human cancers. Wnt inhibitory factor-1 (Wif-1) was identified as one of the secreted antagonists that can bind Wnt protein. We hypothesize that Wif-1 plays an important role in bladder cancer pathogenesis. To test this hypothesis, epigenetic and genetic pathways involved in the Wif-1 gene modulation and expression of Wnt/beta-catenin-related genes were analyzed in 4 bladder tumor cell lines and 54 bladder tumor and matched normal bladder mucosa. Wif-1 mRNA expression was significantly enhanced after 5-aza-2'-deoxycytidine treatment in bladder tumor cell lines. Wif-1 promoter methylation level was significantly higher and Wif-1 mRNA expression was significantly lower in bladder

tumor samples than in bladder mucosa samples. In the total bladder tumor and bladder mucosa samples, an inverse correlation was found between promoter methylation and Wif-1 mRNA transcript levels. However, loss-of-heterozygosity at chromosome 12q14.3 close to the Wif-1 gene loci was a rare event (3.7%). Nuclear accumulation of beta-catenin was significantly more frequent in bladder tumor than in bladder mucosa and inversely correlated with Wif-1 expression. In addition, known targets of the canonical Wnt/beta-catenin signaling pathway, such as c-myc and cyclin D1, were up-regulated in bladder tumor compared with bladder mucosa, and this up-regulation was associated with reduced Wif-1 expression at both mRNA and protein levels. Furthermore, transfection of Wif-1 small interfering RNA into bladder tumor cells expressing Wif-1 mRNA transcripts had increased levels of c-myc and cyclin D1 and accelerated cell growth. **CONCLUSION:** This is the first report showing that CpG hypermethylation of the Wif-1 promoter is a frequent event in bladder tumor and may contribute to pathogenesis of bladder cancer through aberrant canonical Wnt/beta-catenin signaling pathway. The present study elucidates novel pathways that are involved in the pathogenesis of bladder cancer.

van der Poel, H. G., B. Molenaar, et al. (2002). "Epidermal growth factor receptor targeting of replication competent adenovirus enhances cytotoxicity in bladder cancer." *J Urol* 168(1): 266-72.

We evaluated the delivery and oncolytic potential of targeted replication competent adenoviruses in bladder cancer lines. Seven established human bladder cancer tumor lines (5637, SW800, TCCsup, J82, Scaber, T24 and 253J) were studied for the expression of integrins alpha(v)beta3, alpha(v)beta5, Cocksackievirus and adenovirus receptor, epidermal growth factor receptor (EGF-R) and epithelial cell adhesion molecule antigens using flow cytometry analysis. Bispecific single chain Fv fragments were used to target replication deficient luciferase reporter adenovirus to EGF-R (425-s11) or to epithelial cell adhesion molecule (C28-s11) antigens. Moreover, a fiber modified adenovirus targeting alpha(v)-integrins was studied. Replication competent serotype-5 adenoviruses attenuated to replicate specifically in retinoblastoma pRb (Ad5-d24) or p53 deficient (Ad5-d55K) cells were tested in vitro for oncolytic properties. Low to absent Cocksackievirus and adenovirus receptor expression was found in 5 of the 7 tumor lines (SW800, J82, T24, 5637 and Scaber). EGF-R expression was found in all cell lines, whereas elevated epithelial cell adhesion molecule expression was seen in 3 (5637, Scaber and TCCsup), alpha(v)beta3-integrin was found in 1

(Scaber) and alpha(v)beta5-integrin was found in 3 (TCCsup, 253J and T24). EGF-R targeting using 425-s11 improved transgene expression in all cell lines from 2.1 to 12.5 times over nontargeted viruses. Epithelial cell adhesion molecule and integrin targeting was inferior to EGF-R targeting with a maximal increase in transgene expression of 2 times for epithelial cell adhesion molecule in 5637 cells and 1.6 times for integrin targeting in T24 cells. Comparison of the wild-type replication competent virus with conditionally replicating adenoviruses (Ad5-d55K and Ad5-d24) showed superior oncolytic activity for the latter 2 in all lines. Furthermore, improved cytotoxicity (29% to 33%) was obtained in 4 of the 7 lines after pre-incubation of Ad5-d24 with 425-s11. EGF-R directed bispecific single chain antibodies enhance adenovirus mediated transgene expression and oncolysis in bladder cancer lines.

Vecchione, A., C. Seignani, et al. (2004). "Inactivation of the FHIT gene favors bladder cancer development." *Clin Cancer Res* **10**(22): 7607-12.

The fragile histidine triad (FHIT) gene located on chromosome 3p14.2 is frequently deleted in human tumors. We have previously reported deletions at the FHIT locus in 50% of bladder carcinoma derived cell lines and reduced expression in 61% of primary transitional carcinomas of the urinary bladder. To additionally investigate the role of FHIT alterations in the development of bladder cancer, we used heterozygous and nullizygous Fhit-deficient mice in a chemically induced carcinogenesis model. Results showed that 8 of 28 (28%) and 6 of 13 (46%) of the Fhit -/- and +/-, respectively, versus 2 of 25 (8%) Fhit +/+ mice developed invasive carcinoma after treatment with N-butyl-N-(4-hydroxybutyl) nitrosamine. To explore the possibility of a FHIT-based gene therapy for bladder cancer, we studied the effects of restored Fhit protein expression on cell proliferation, cell kinetics, and tumorigenicity in BALB/c nude mice, with human SW780 Fhit-null transitional carcinoma derived cells. In vitro transduction of SW780 Fhit-negative cells with adenoviral-FHIT inhibited cell growth, increased apoptotic cell population, and suppressed s.c. tumor growth in nude mice. These findings suggest the important role of Fhit in bladder cancer development and support the effort to additionally investigate a FHIT-based gene therapy.

Veerla, S., I. Panagopoulos, et al. (2008). "Promoter analysis of epigenetically controlled genes in bladder cancer." *Genes Chromosomes Cancer* **47**(5): 368-78.

DNA methylation is an important epigenetic modification that regulates several genes crucial for tumor development. To identify epigenetically

regulated genes in bladder cancer, we performed genome wide expression analyses of eight-bladder cancer cell lines treated with the demethylating agents 5-aza-2'-cytidine and zebularine. To identify methylated C-residues, we sequenced cloned DNA fragments from bisulfite-treated genomic DNA. We identified a total of 1092 genes that showed > or =2-fold altered expression in at least one cell line; 710 showed up-regulation and 382 down-regulation. Extensive sequencing of promoters from 25 genes in eight cell lines showed an association between methylation pattern and expression in 13 genes, including both CpG island and non-CpG island genes. Overall, the methylation patterns showed a patchy appearance with short segments showing high level of methylation separated by larger segments with no methylation. This pattern was not associated with MeCP2 binding sites or with evolutionarily conserved sequences. The genes UBXD2, AQP11, and TIMP1 showed particular patchy methylation patterns. We found several high-scoring and evolutionarily conserved transcription factor binding sites affected by methylated C residues. Two of the genes, FGF18 and MMP11, that were down-regulated as response to 5-aza-2'-cytidine and zebularine treatment showed methylation at specific sites in the untreated cells indicating an activating result of methylation. Apart from identifying epigenetically regulated genes, including TGFBR1, NUPR1, FGF18, TIMP1, and MMP11, that may be of importance for bladder cancer development the presented data also highlight the organization of the modified segments in methylated promoters. This article contains supplementary material available via the Internet at <http://www.interscience.wiley.com/jpages/1045-2257/suppmat>.

Velicescu, M., D. J. Weisenberger, et al. (2002). "Cell division is required for de novo methylation of CpG islands in bladder cancer cells." *Cancer Res* **62**(8): 2378-84.

Cell division is essential for tumor development and progression. Methylation-mediated silencing caused by aberrant de novo methylation of CpG islands located in the promoter regions of growth regulatory genes occurs frequently in human cancers. We investigated the relationship between cell division and de novo methylation to determine whether de novo methylation can occur in the absence of cell division in cancer cells. We treated T24 bladder carcinoma cells with 5-Aza-2'-deoxycytidine to induce a transient demethylation and then compared the timing and kinetics of remethylation of the p16 gene locus under conditions of either G(0)-G(1) growth arrest induced by serum starvation and confluence or continuous cell proliferation in complete medium.

Variable levels of remethylation were detected in CpG poor regions of DNA, as well as repetitive DNA elements in the absence of cell division, yet no remethylation occurred at CpG islands under these conditions. This correlated with continuous expression of p16 protein in these cells. DNA methyltransferase (DNMT)1 and DNMT3b3 proteins were undetectable in 5-Aza-2'-deoxycytidine-treated and untreated nondividing cells, and their mRNA transcripts were down-regulated in these cells. Although DNMT3a mRNA levels were also reduced, they recovered to original levels in nondividing cells after drug treatment. Our results suggest that cell division is required for de novo methylation of CpG islands and that DNMT3a may play a role in methylating CpG poor regions or repetitive DNA elements outside of the S phase of the cell cycle.

Villares, G. J., M. Zigler, et al. (2007). "Targeting EGFR in bladder cancer." *World J Urol* **25**(6): 573-9.

Expression and overexpression of the epidermal growth factor receptor (EGFR) have been described in several solid tumors including bladder, breast, colorectal, NSCLC, prostate, and ovarian cancers. In addition to gene amplification, point mutations within the kinase domain also occur. Previous reports indicate that the patient's response to gefitinib depends on either the presence of mutations within the kinase domain of EGFR or the expression of the most frequent alteration, the truncated EGFR variant III (EGFRvIII). Therefore, it is important to determine if these EGFR alterations are present in urothelial carcinoma. The kinase domain of EGFR (exons 18-21) from 11 bladder cancer cell lines as well as from 75 patient tumors was analyzed by automated sequencing. No mutations were detected in all samples tested. Furthermore, analysis of EGFRvIII by immunohistochemistry revealed that almost half of all the patient samples expressed this truncation in a urothelial carcinoma tissue microarray. However, there have been previous reports of inconsistencies in detecting EGFRvIII by immunohistochemistry owing to the specificity of the antibodies and the methodologies utilized. Therefore, these results were validated by reverse transcription PCR, real-time PCR and western blot analysis. In these assays, none of the samples tested positive for EGFRvIII. Taken together, these results indicate that mutations within the tyrosine kinase domain of EGFR and expression of EGFRvIII are rare events in bladder cancer and therefore do not contribute to the malignant phenotype of this tumor. These results have clinical implications in selecting tyrosine kinase inhibitors for the therapy of urothelial carcinoma.

Wallerand, H., R. R. Reiter, et al. (2008). "Molecular targeting in the treatment of either advanced or metastatic bladder cancer or both according to the signalling pathways." *Curr Opin Urol* **18**(5): 524-32.

An estimated 300,000 new cases of bladder cancer worldwide are diagnosed annually. Although new cytotoxic chemotherapeutic agents for either advanced or metastatic bladder cancer or both are used, no improvement in survival has been observed. Indeed, the 5-year survival rate of metastatic bladder cancer is very low (6%). The target-directed approach is an attractive challenge for treating specific genetic alterations involved in progression and metastasis development. This article aims to describe the new targeted therapies available to cure advanced cancer or metastatic bladder cancer or both according to the signalling pathways potentially involved. The rapidly expanding understanding of the pathogenesis of bladder cancer at the molecular level has led to the identification of signalling pathways involved in this disease and provided molecular targets for new biological agents directed against tumorigenesis and progression. The recent results of clinical trials have not only highlighted the need to select patients who could benefit from such a therapy but also the fact that oncology has completely entered into a new era. SUMMARY: Toxic chemotherapeutic agents are slowly being supplemented by a new generation of drugs that recognize specific targets in or on cancer cells. Recent technological advances in pharmacogenomics and proteomics have led to an improvement in identifying biomarkers predictive of response and thereby to identify patients who would be more likely to respond to such a therapy. There is a real hope to improve both the efficiency and the tolerability of bladder cancer treatment.

Wang, L., Z. Fuqing, et al. (2006). "Smac/DIABLO promotes mitomycin C-induced apoptosis of bladder cancer T24 cells." *J Huazhong Univ Sci Technolog Med Sci* **26**(3): 317-8, 328.

The enhancing effects of Smac gene on the mitomycin C-induced apoptosis of the bladder cancer cell line T24 were investigated. The Smac gene was transfected into bladder cancer cell line T24 under the induction of liposome. The intrinsic Smac level was detected by using immunohistochemistry and RT-PCR. The in vitro cellular growth activities were assayed by MTT colorimetry. Apoptosis was assayed by the flow cytometry. The results showed that as compared with the control cells, the apoptosis rate of T24 cells induced by mitomycin C was enhanced by transfected Smac gene. Flow cytometry revealed that, the apoptosis rate was 18.84% and 33.52%, and 10.72% and 26.24% respectively in blank and transfected cells treated with 0.05 or 0.005 mg/mL

mitomycin C ($P < 0.05$). It was concluded that Smac could enhance the apoptosis of T24 by mitomycin C, which could provide a useful experimental evidence for bladder cancer therapy.

Watanabe, R., C. Ohshima, et al. (2002). "Ganglioside G(M3) overexpression induces apoptosis and reduces malignant potential in murine bladder cancer." *Cancer Res* **62**(13): 3850-4.

We demonstrated previously (S. Kawamura et al., *Int. J. Cancer*, 94: 343-347, 2001) that large amounts of ganglioside G(M3) accumulate in superficial bladder tumor, compared with invasive bladder tumors and that exogenous G(M3) inhibits the invasive potential of bladder tumor cells. To apply the G(M3) overexpression system to bladder tumor therapy, direct evidence for the important role of G(M3) in bladder tumor invasion must be obtained through transfer of the gene responsible for G(M3) overexpression. To determine the most appropriate cancer cell line for elucidating the antitumor effect of ganglioside G(M3) overexpression, the present study examined glycolipid composition, enzyme activity, and mRNA expression of the glycosyltransferases responsible for G(M3) synthesis in the bladder tumor cell lines KK-47, J82, MGH-UI, YTS-1, and MBT-2. A murine bladder carcinoma cell line (MBT-2) was transfected with a G(M3) synthase [(lactosylceramide alpha2,3-N-acetyl sialic acid transferase); sialyltransferase-I; SAT-I] cDNA, because this line does not naturally express G(M3). Stable transfectants (MBT-2-SAT-I) that overexpressed G(M3) were characterized by a reduced potential for cell proliferation, motility, invasion, and xenograft tumor growth, and an increase in the number of apoptotic cells. In the proportion of synthetic S phase, cells did not differ between MBT-2-SAT-I and mock-transfectant cells. These results suggest that the decreased proliferative potential related to G(M3) overexpression was attributable to the increased number of apoptotic cells. Although details of the mechanism of apoptosis remain unclear, the overexpression of G(M3) by gene transfer of SAT-I may present a novel therapeutic modality.

Wild, P. J., J. W. Catto, et al. (2007). "Artificial intelligence and bladder cancer arrays." *Verh Dtsch Ges Pathol* **91**: 308-19.

Non-muscle invasive bladder cancer is a heterogeneous disease whose management is dependent upon the risk of progression to muscle invasion. Although the recurrence rate is high, the majority of tumors are indolent and can be managed by endoscopic means alone. The prognosis of muscle invasion is poor and radical treatment is required if cure is to be obtained. Progression risk in non-

invasive tumors is hard to determine at tumor diagnosis using current clinicopathological means. To improve the accuracy of progression prediction various biomarkers have been evaluated. To discover novel biomarkers several authors have used gene expression microarrays. Various statistical methods have been described to interpret array data, but to date no biomarkers have entered clinical practice. Here, we describe a new method of microarray analysis using neurofuzzy modeling (NFM), a form of artificial intelligence, and integrate it with artificial neural networks (ANN) to investigate non-muscle invasive bladder cancer array data (n=66 tumors). We develop a predictive panel of 11 genes, from 2800 expressed genes, that can significantly identify tumor progression (average Logrank $p = 0.0288$) in the analyzed cancers. In comparison, this panel appears superior to those genes chosen using traditional analyses (average Logrank $p = 0.3455$) and tumor grade (Logrank, $p = 0.2475$) in this non-muscle invasive cohort. We then analyze panel members in a new non-muscle invasive bladder cancer cohort (n=199) using immunohistochemistry with six commercially available antibodies. The combination of 6 genes (LIG3, TNFRSF6, KRT18, ICAM1, DSG2 and BRCA2) significantly stratifies tumor progression (Logrank $p = 0.0096$) in the new cohort. We discuss the benefits of the transparent NFM approach with respect to other reported methods.

Wolf, S., D. Mertens, et al. (2006). "Ala228 variant of trail receptor 1 affecting the ligand binding site is associated with chronic lymphocytic leukemia, mantle cell lymphoma, prostate cancer, head and neck squamous cell carcinoma and bladder cancer." *Int J Cancer* **118**(7): 1831-5.

Allelic loss of chromosome 8p21-22 is a frequent event in various human cancers including mantle cell lymphoma (MCL), prostate cancer, head and neck squamous cell carcinoma (HNSCC) and bladder cancer. The tumor necrosis factor-related apoptosis inducing ligand (TRAIL) receptors, including TNFRSF10A and TNFRSF10B, are located within this chromosomal region. Since recent studies demonstrate that chronic lymphocytic leukemia (CLL) and prostate cells are TRAIL induced apoptosis, TRAIL-receptors are strong tumor suppressor candidate genes in human cancers exhibiting loss of chromosomal material in 8p21.3. However, no mutation of the TRAIL receptor genes has been reported in CLL, MCL, prostate cancer, HNSCC so far. In this study we analyzed the complete coding region of TNFRSF10A and TNFRSF10B in a series of 32 MCL and 101 CLL samples and detected a single nucleotide polymorphism (SNP) in TNFRSF10A (A683C) with tumor specific allele distribution. We

examined allele distribution in 395 samples of different tumor entities (prostate cancer, n = 43; HNSCC, n = 40; bladder cancer, n = 179) and compared them to 137 samples from healthy probands. We found the rare allele of TNFRSF10A is more frequent in CLL, MCL, prostate cancer, bladder cancer and HNSCC. The A683C polymorphism did not cosegregate with other TNFRSF10A polymorphisms previously described. Thus screening for 683A-->C nucleotide exchanges may become important in diagnosis and/or treatment of these malignancies.

Wu, C. L., G. S. Shieh, et al. (2008). "Tumor-selective replication of an oncolytic adenovirus carrying oct-3/4 response elements in murine metastatic bladder cancer models." *Clin Cancer Res* **14**(4): 1228-38.

Oncolytic adenoviruses are attractive therapeutics for cancer because they selectively replicate in tumors. However, targeting tumor metastasis remains a major challenge for current virotherapy for cancer. Oct-3/4 is specifically expressed in embryonic stem cells and tumor cells. Oct-3/4 highly expressed in cancer cells may be a potential target for cancer therapy. We developed an E1B-55 kDa-deleted adenovirus, designated Ad.9OC, driven by nine copies of Oct-3/4 response element for treating Oct-3/4-expressing metastatic bladder cancer. We examined the expression of Oct-3/4 in human bladder tumor tissues and bladder cancer cell lines. We also evaluated the cytolytic and antitumor effects of Ad.9OC on bladder cancer cells in vitro and in vivo. Oct-3/4 expression was detected in bladder cancer cell lines, as well as in human bladder tumor tissues. Notably, Oct-3/4 expression was higher in metastatic compared with nonmetastatic bladder cancer cells. Ad.9OC induced higher cytolytic activity in metastatic bladder cancer cells than in their nonmetastatic counterparts, whereas it did not cause cytotoxicity in normal cells. Pharmacologic and short hairpin RNA-mediated Oct-3/4 inhibition rendered bladder cancer cells more resistant to Ad.9OC-induced cytolysis. Replication of Ad.9OC was detected in murine bladder cancer cells and bladder tumor tissues. We also showed the effectiveness of Ad.9OC for treating bladder cancer in subcutaneous, as well as metastatic, bladder tumor models. Ad.9OC may have therapeutic potential for treating Oct-3/4-expressing tumors. Especially, metastatic bladder tumors are good target for Ad.9OC treatment. Because Oct-3/4 is expressed in a broad spectrum of cancers, Ad.9OC may be broadly applicable.

Wu, F., Y. Chen, et al. (2008). "RNA-interference-mediated Cdc42 silencing down-regulates phosphorylation of STAT3 and suppresses growth in

human bladder-cancer cells." *Biotechnol Appl Biochem* **49**(Pt 2): 121-8.

Cdc42 (cell division cycle 42), a member of Rho GTPases, is involved in cell transformation, proliferation, survival, invasion and metastasis of human cancer cells. Here, RNAi (RNA interference)-mediated gene silencing was used to investigate the roles of Cdc42 and to assess its therapeutic potential in human bladder cancer. The results showed that Cdc42 silencing resulted in a marked reduction of Cdc42 mRNA and protein expression and a significant inhibition of cell proliferation from G(0)/G(1)- to S-phase in two (EJ and T24) human bladder-cancer cell lines. Moreover, RNAi-mediated inhibition of Cdc42 induced apoptosis of EJ cells 96 h after transfection. In addition, we found that silencing of Cdc42 could down-regulate the level of phosphorylated STAT3 (signal transducer and activator of transcription 3), but did not influence the level of total STAT3 in the two bladder-cancer cell lines. These results suggest that RNAi-mediated Cdc42 silencing may be a novel approach for gene therapy of bladder cancer.

Wu, Q., R. Mahendran, et al. (2003). "Nonviral cytokine gene therapy on an orthotopic bladder cancer model." *Clin Cancer Res* **9**(12): 4522-8.

The purpose is to assess cytokine gene transfection in tumor cells and its therapeutic efficacy in an orthotopic mouse bladder cancer model after liposome-mediated gene transfer. A total of 1×10^5 MB49 cells was instilled into the bladder of C57BL/6 mice after electrocautery to establish the tumor model. The plasmids were constructed by inserting the coding sequences for murine IFN- α 1 and granulocyte macrophage colony-stimulating factor into a plasmid vector pBudCE4.1. Transient transfection was performed using a cationic lipid N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammoniummethyl sulfate and methyl-beta-cyclodextrin-solubilized cholesterol. The in vitro expression of cytokines was checked by ELISA. The expression of the transgene in situ was confirmed by immunohistochemistry and 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside staining. Mice bearing orthotopic tumors were treated with plasmid DNA/liposome complex by intravesical instillation twice a week for 3 weeks. Superficial bladder tumors were established by intravesical instillation of MB49 into cauterized bladders. The expression level of cytokines in transfected cell lines was increased significantly. In situ gene transfer to bladder tumors was accomplished via intravesical instillation of plasmid DNA/N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammoniummethyl sulfate/methyl-beta-cyclodextrin-solubilized cholesterol after a single 2 h in situ transfection. The tumor incidence in the

treatment groups was dramatically decreased from 76.9% in the control group to 15.4-30.8% in the treatment groups. We demonstrated in the orthotopic mouse bladder cancer model that successful inhibition of tumor cell growth could be obtained with cytokine gene therapy. The results suggest that our liposome transfection system appears to be a promising method for gene therapy of bladder cancer in vivo.

Wu, Z. X., T. B. Song, et al. (2007). "Overexpression of PTEN suppresses growth and induces apoptosis by inhibiting the expression of survivin in bladder cancer cells." *Tumour Biol* **28**(1): 9-15.

The tumor suppressor gene PTEN, which encodes a multifunctional phosphatase protein, is mutated in a variety of human cancers. Several reports have indicated that it has growth-suppressive and proapoptosis properties and displayed an altered expression pattern during human oncogenesis. Overexpression of PTEN leads to decreasing cell growth and tumorigenicity in vitro and in vivo. In the present study, we further demonstrated that overexpression of PTEN mediated by adenovirus suppressed bladder cancer cell growth and significantly induced apoptosis, through downregulating of survivin and activating of caspase cascades. Our results indicate that Ad-PTEN exerts its tumor suppressive effect on bladder cancer cells through inhibiting survivin and upregulating caspase-related proteins. Thus Ad-PTEN may be potentially therapeutic for the treatment of bladder cancers.

Xia, G., S. R. Kumar, et al. (2006). "EphB4 receptor tyrosine kinase is expressed in bladder cancer and provides signals for cell survival." *Oncogene* **25**(5): 769-80.

We sought to evaluate the biological function of the receptor tyrosine kinase EphB4 in bladder cancer. All of the nine bladder cancer cell lines examined express EphB4 and the receptor could be phosphorylated following stimulation with its cognate ligand, EphrinB2. Out of the 15 fresh bladder cancer specimens examined, 14 expressed EphB4 with a mean sevenfold higher level of expression compared to adjacent normal urothelium. EphB4 expression was regulated by several mechanisms: EPHB4 gene locus was amplified in 27% tumor specimens and 33% cell lines studied; inhibition of EGFR signaling downregulated EphB4 levels; and forced expression of wild-type p53 reduced EphB4 expression. EphB4 knockdown using specific siRNA and antisense oligodeoxynucleotides molecules led to a profound inhibition in cell viability associated with apoptosis via activation of caspase-8 pathway and downregulation of antiapoptotic factor, bcl-xl. Furthermore, EphB4 knockdown significantly

inhibited tumor cell migration and invasion. EphB4 knockdown in an in vivo murine tumor xenograft model led to a nearly 80% reduction in tumor volume associated with reduced tumor proliferation, increased apoptosis and reduced tumor microvasculature. EphB4 is thus a potential candidate as a predictor of disease outcome in bladder cancer and as target for novel therapy.

Xing, J., Y. Zhu, et al. (2007). "Differential induction in telomerase activity among bladder cancer patients and controls on gamma-radiation." *Cancer Epidemiol Biomarkers Prev* **16**(3): 606-9.

Expression of telomerase is one of the hallmarks of tumor cells and has been used as a diagnostic biomarker and a therapeutic target in cancer. Novel findings have shown that telomerase activation in normal human epithelial cells may affect expression of several cancer-related genes, such as growth-related genes and c-myc gene, suggesting a possible role of telomerase in tumor initiation. Therefore, we hypothesized that individuals who are sensitive to mutagen challenge in terms of induced telomerase activity might have increased cancer risk. We tested this hypothesis in a bladder cancer case-control study (51 cases and 51 matched controls) by measuring baseline and gamma-radiation-induced telomerase activities in peripheral blood lymphocytes. We found a significantly higher gamma-radiation-induced telomerase activity in bladder cancer cases compared with the controls (1.34 versus 1.23; $P = 0.044$). A similar finding was also observed using the normalized telomerase activity (ratio of gamma-radiation induced versus baseline; 1.49 versus 1.19; $P < 0.001$). In further categorizing the telomerase activity using 75% of the normalized value in the controls as a cutoff point, we found a significantly increased risk for bladder cancer associated with higher induced telomerase activity (adjusted odds ratio, 3.62; 95% confidence interval, 1.38-9.51). In quartile analysis, a dose-response association was noted between the induced telomerase activity and increased bladder cancer risk ($P(\text{trend}) = 0.005$). Our findings provide the first evidence linking the mutagen-induced telomerase activity in peripheral blood lymphocytes to the risk of bladder cancer, which warrants further investigation in large-sized studies and other cancer types.

Yamada, Y., K. Naruse, et al. (2007). "Potential for molecular targeted therapy of HER-2/neu for invasive bladder cancer: examination of gene amplification by fluorescence in situ hybridization." *Oncol Rep* **18**(5): 1183-7.

Analysis of HER-2/neu gene amplification by fluorescence in situ hybridization was performed in 40

patients with invasive bladder cancer in order to evaluate the potential for molecular targeted therapy of HER-2 as a tailor-made treatment for patients with invasive bladder cancer. This study included 40 patients seen at the Aichi Medical University Hospital from January 2001 to December 2004 and were pathologically diagnosed with invasive transitional cell carcinoma of the bladder (pT2-pT4). The PathVysion kit was used to evaluate the status of HER-2/neu gene amplification, and a signal ratio $>$ or $=2.0$ was considered positive for HER-2/neu gene amplification. In primary foci 5 patients (12.5%) were positive for HER-2/neu gene amplification. According to the classification of grade and stage, no statistically significant difference was observed. Lymph node metastasis was found in 10 patients, and 3 patients (30%) were positive for HER-2/neu gene amplification. In the patients with HER-2/neu gene-amplified metastatic lymph nodes, primary foci were also positive for gene amplification, showing a statistically significant difference. This study indicates that 12.5% of patients with invasive bladder cancer may benefit from molecular targeted therapy of HER-2, and that molecular targeted therapy can be expected to be effective even for patients with lymph node metastases as long as their primary foci are positive for HER-2/neu gene amplification.

Yamanaka, K., M. Gleave, et al. (2005). "Enhanced radiosensitivity by inhibition of the anti-apoptotic gene clusterin using antisense oligodeoxynucleotide in a human bladder cancer model." *Oncol Rep* **13**(5): 885-90.

Clusterin has been shown to be implicated in the acquisition of resistant phenotype to various kinds of apoptotic stimuli, including radiation. In bladder cancer, our previous study demonstrated that overexpression of clusterin is closely associated with disease progression and recurrence. The objective of this study was to investigate whether radiation sensitivity was enhanced by suppressing clusterin gene expression with antisense (AS) oligodeoxynucleotide (ODN) in the human bladder cancer KoTCC-1 model. Clusterin mRNA in KoTCC-1 cells after radiation was up-regulated in a dose-dependent manner; however, AS clusterin ODN treatment resulted in a marked inhibition of clusterin mRNA even after irradiation. Combined treatment of KoTCC-1 cells with radiation and AS clusterin ODN synergistically decreased plating efficacy and induced apoptotic cell death compared with either radiation or AS clusterin ODN treatment alone. In vivo systemic administration of AS clusterin ODN enhanced radiation sensitivity, significantly reducing subcutaneous KoTCC-1 tumor volume in nude mice, compared with that of mismatch control ODN.

Moreover, additional administration of cisplatin to this combined regimen further achieved potential antitumor effects on subcutaneous KoTCC-1 tumor growth in nude mice. Collectively, these findings suggest that clusterin acts as a cell survival protein mediating radioresistance through the inhibition of apoptosis, and that inactivation of clusterin using AS technology might offer a novel strategy to improve the outcome of radiation therapy for patients with bladder cancer.

Yamashita, M., C. J. Rosser, et al. (2002). "Syn3 provides high levels of intravesical adenoviral-mediated gene transfer for gene therapy of genetically altered urothelium and superficial bladder cancer." *Cancer Gene Ther* **9**(8): 687-91.

Using our model to grow superficial human bladder cancer in the mouse bladder, we have found that the polyamide compound, Syn3, when injected intravesically for 1 hour at 1 mg/mL on two consecutive days, markedly increases rAd-beta-gal intravesical gene transfer and expression. This enhanced transgene expression was much greater than obtain by the use of 22% ethanol, which had previously been shown to increase intravesical adenoviral gene transfer, whereas little or no gene expression was seen with exposure to only rAd-beta-gal. beta-Galactosidase staining was seen in virtually every normal urothelial and superficial tumor cell present, including tumors that express little or no coxsackie-adenovirus receptors when Syn3 was present. High adenoviral-mediated gene transfer was also documented in the pig bladder using Syn3 in a similar protocol. Therefore, Syn3 may overcome the limitations of adequate intravesical adenoviral-mediated gene transfer and, when combined with an appropriate adenoviral-mediated gene, could offer an effective approach to the treatment of superficial bladder cancer and perhaps even genetically altered precursor lesions.

Yang, C. C., J. H. Yang, et al. (2004). "N-acetyltransferase activity is involved in paclitaxel-induced N-acetylation of 2-aminofluorene in human bladder cancer cells (T24)." *Anticancer Res* **24**(3a): 1501-6.

Arylamine N-acetyltransferase (NAT) plays an important role in the metabolism of 2-aminofluorene (AF) and some types of arylamine drugs and carcinogens. Our previous studies have demonstrated that paclitaxel decreases NAT activity in human bladder, blood, colon and lung cancer cells. In this study, paclitaxel was selected to test the inhibition of NAT activity (N-acetylation of AF) and NAT gene expression in a human bladder cancer cell line (T24). The NAT activity was determined by high

performance liquid chromatography for measuring the levels of N-acetylation of AF. The data showed that a 24-hour paclitaxel treatment decreased the amount of N-acetylation of AF in T24 cells. The NAT enzymes were stained and analyzed by Western blotting and flow cytometry. The tests indicated that paclitaxel decreased the levels of NAT in T24 cells. The expression of the NAT gene (mRNAT NAT) was determined by polymerase chain reaction (PCR) and cDNA microarray and it was found that paclitaxel induced the down-regulation of mRNA NAT expression in T24 cells.

Yang, K., X. Y. Zheng, et al. (2008). "Up-regulation of p21WAF1/Cip1 by saRNA induces G1-phase arrest and apoptosis in T24 human bladder cancer cells." *Cancer Lett* **265**(2): 206-14.

Very recent studies have reported that chemically synthesized small duplex RNAs complementary to the promoters of target genes can activate gene expression in different cancer cell lines. Such dsRNA have been referred to as saRNA for small activating RNA. The present study was conducted to evaluate the potential of p21(WAF1/Cip1) (p21) induction by small activating RNA targeting the p21 promoter in the treatment of bladder cancer. Using T24 human bladder cancer cells, we found that p21 saRNA caused dose- and time-dependent inhibition of cell proliferation and viability which was associated with induced G1-phase cell cycle arrest and apoptosis. The decreased anti-apoptotic protein Bcl-xL and activation of caspase-3 and PARP also supported the efficacy of the treatment. These data suggest that up-regulation of p21 by saRNA may be an effective way for treating human bladder and other types of cancers.

Yang, Y. M. and J. W. Chang (2008). "Bladder cancer initiating cells (BCICs) are among EMA-CD44v6+ subset: novel methods for isolating undetermined cancer stem (initiating) cells." *Cancer Invest* **26**(7): 725-33.

Bladder cancer stem (initiating) cell has not been isolated now, and no one verified its persistence experimentally. The aim of this study was to conclude the persistence of bladder cancer stem (initiating) cell in human primary bladder cancer and investigate the possibility of EMA(-) CD44v6(+) as markers of bladder cancer stem (initiating) cell. Genes differentially expressed between normal urothelium and low malignant bladder cancer were identified by DNA array assay. Overpressed stem cell related genes, Bmi-1 and EZH2, were verified by immunohistochemistry. Side population cells in bladder cancer were found under fluorescence microscope. The value of 28 potential surface markers

of bladder cancer stem (initiating) cell for isolating them were judged by immunohistochemistry. Both EMA(-) and CD44v6(+) cells located in basal layer (potential location of stem cells). After gathering the CD44v6(+) cells and EMA(-) cells by magnetic cell sorting, their ability for colony-forming, self-renewal and extensive proliferation were assayed by cells culture. Both EMA(-) cells and CD44v6(+) cells possess the ability for colony-forming, self-renewal and proliferation. We conclude the persistence of bladder cancer stem (initiating) cell. Bladder cancer stem (initiating) cell might be among EMA(-) CD44v6(+) subset. Our strategies for isolating bladder cancer stem (initiating) cell might be useful for isolating other undetermined epithelial cancer stem cell, especially those in well-differentiated cancers.

Yeh, C. C., L. T. Wu, et al. (2004). "The inhibition of N-acetyltransferase activity and gene expression in human bladder cancer cells (T24) by shikonin." *In Vivo* **18**(1): 21-31.

Shikonin has the potential to prevent, or be used in the treatment of, bladder transitional cell carcinoma induced by arylamines. We evaluated its effectiveness by measuring the amount of acetylated 2-aminofluorene (AF), AF-DNA adducts, changes of NAT mRNA and the amount of NAT enzyme. T24 human bladder cancer cells were incubated with 30 microM AF with different concentrations of shikonin for various times. T24 cells treated with shikonin (16 microM) were then harvested and used in 2 experiments: 1). T24 cells were incubated with 22.5 microM AF and shikonin (0, 16 microM) (co-treatment) for 6, 12, 18, 24 and 48 h. 2). T24 cells were incubated with various concentrations of AF and shikonin (0, 16 microM) for 24 h. AF and AAF were measured by HPLC. Then in the prepared human T24 cell cytosols different concentrations of AF and shikonin were added to measure the kinetic constants of NAT. Next, AF-DNA adducts in human T24 cells with or without treatment with shikonin were detected and measured. The final two steps included measuring the NAT Ag-Ab complex after treatment with and without shikonin and evaluating the effect of shikonin on the NAT genes. Higher concentrations of shikonin induced decreasing AF acetylation. We found that the longer the culture period, the greater the difference in AF acetylation in the same shikonin concentrations. It was also noted that increase in AAF was proportional to incubation time. In the presence of 16 microM of shikonin, N-acetylation of AF decreased by up to 72-84%. Shikonin decreased the amount of AAF production in human T24 cells in all examined AF doses. Both Km and Vmax values in the cytosolic NAT decreased after the addition of shikonin to the cytosol. Finally, shikonin decreased the amount of

AAF production and AF-DNA adducts formation in human 724 cells in all examined AF doses. The percentage of cells stained by antibody was significantly different after treatment with shikonin, especially with the higher shikonin concentrations. The NAT1 mRNA level and the NAT1/beta-actin ratio decreased significantly with higher concentrations (16-24 microM) of shikonin. Shikonin affected NAT activity, gene expression (NAT1 mRNA), AF-DNA adducts formation and formation of NAT Ag-Ab in human bladder tumor T24 cells. Therefore, shikonin should be considered as a candidate agent for the prevention or treatment of transitional cell carcinoma.

Yu, D. S., C. F. Lee, et al. (2007). "Immunotherapy for orthotopic murine bladder cancer using bacillus Calmette-Guerin recombinant protein Mpt-64." *J Urol* **177**(2): 738-42.

We investigated the efficacy of the recombinant bacillus Calmette-Guerin subunit protein vaccine Mpt-64 for inducing cytokine production and suppressing orthotopic bladder tumor growth in mice. One mycobacteria candidate gene (Mpt-64) was cloned and ligated into eukaryotic expression vectors. The induction and efficiency of Mpt-64 protein expression were detected using Western blotting. Various doses of Mpt-64 proteins were instilled intravesically 6 times in 2 weeks after intravesical implantation of MBT-2 tumor cells in chemical injured urothelium. Systemic cytokine responses, tumor growth and cumulative survival rates were monitored. In vitro expression of recombinant Mpt-64 subunit protein was efficient in our system. Mice treated with 100 and 200 microg Mpt-64 subunit proteins significantly inhibited orthotopic MBT-2 tumor growth in C3H/HeN mice compared with that in control and 50 microg treatment groups in terms of the tumor taking rate, bladder tumor burden and mortality rate. Meanwhile, marked increased serum interferon-gamma with a limited but significant increase in serum interleukin-2 was observed in mice treated with 100 and 200 microg Mpt-64 proteins compared with control and 50 microg treated groups. A highly immunopotent recombinant Mpt-64 subunit protein of bacillus Calmette-Guerin was produced and it elicited immune responses with a high serum interferon-gamma level, inhibited orthotopic tumor growth and prolonged survival in tumor bearing mice. Thus, intravesical immunogenic therapy using recombinant Mpt-64 protein may be an alternative bacillus Calmette-Guerin regimen for superficial bladder cancer.

Yu, D. S., C. F. Lee, et al. (2004). "Antitumor effects of recombinant BCG and interleukin-12 DNA

vaccines on xenografted murine bladder cancer." *Urology* **63**(3): 596-601.

To evaluate the antitumor effects of recombinant bacille Calmette-Guerin (BCG) DNA (multi-rBCG) and murine interleukin-12 DNA (mIL-12) vaccines on xenografted MBT-2 murine bladder tumors. Treatment with combined multi-rBCG and mIL-12 was examined in syngeneic C3H/HeN mice and athymic nude mice. The delivery efficiency of multi-rBCG expression was detected by flow cytometry. Inhibition of tumor growth was monitored, and antitumor effects were evaluated after one dose of electroporation immunogenetherapy, with measurement of cytokines and phenotyping of infiltrating lymphocytes in tumors. In vivo expression of multi-rBCG was efficient and reached a maximum on day 7 after electroporation. Treatment with multi-rBCG plus mIL-12 significantly inhibited tumor growth in C3H/HeN mice, with increased production of Th1-type cytokines, including interferon-gamma and IL-12. Treatment with multi-rBCG and/or mIL-12 in C3H/HeN mice induced infiltration of CD4+/CD8+ T cells and expansion of natural killer cells within tumors. By contrast, however, athymic nude mice treated in the same way showed no significant immune cells within tumors and died of the fast growing tumors. Electroporation using multi-rBCG plus mIL-12 could be effective immunotherapy for existing bladder cancer. The antitumor effects correlated with the elicitation of Th1 lymphocytes and natural killer cell-mediated cytotoxic immune responses.

Yu, J., T. Zhu, et al. (2007). "A novel set of DNA methylation markers in urine sediments for sensitive/specific detection of bladder cancer." *Clin Cancer Res* **13**(24): 7296-304.

This study aims to provide a better set of DNA methylation markers in urine sediments for sensitive and specific detection of bladder cancer. Fifty-nine tumor-associated genes were profiled in three bladder cancer cell lines, a small cohort of cancer biopsies and urine sediments by methylation-specific PCR. Twenty-one candidate genes were then profiled in urine sediments from 132 bladder cancer patients (8 cases for stage 0a; 68 cases for stage I; 50 cases for stage II; 4 cases for stages III; and 2 cases for stage IV), 23 age-matched patients with noncancerous urinary lesions, 6 neurologic diseases, and 7 healthy volunteers. Despite six incidences of four genes reported in 3 of 23 noncancerous urinary lesion patients analyzed, cancer-specific hypermethylation in urine sediments were reported for 15 genes ($P < 0.05$). Methylation assessment of an 11-gene set (SALL3, CFTR, ABCC6, HPR1, RASSF1A, MT1A, RUNX3, ITGA4, BCL2, ALX4, MYOD1,

DRM, CDH13, BMP3B, CCNA1, RPRM, MINT1, and BRCA1) confirmed the existing diagnosis of 121 among 132 bladder cancer cases (sensitivity, 91.7%) with 87% accuracy. Significantly, more than 75% of stage 0a and 88% of stage I disease were detected, indicating its value in the early diagnosis of bladder cancer. Interestingly, the cluster of reported methylation markers used in the U.S. bladder cancers is distinctly different from that identified in this study, suggesting a possible epigenetic disparity between the American and Chinese cases. Methylation profiling of an 11-gene set in urine sediments provides a sensitive and specific detection of bladder cancer.

Yuan, S. S., H. L. Chang, et al. (2006). "Selective cytotoxicity of squamocin on T24 bladder cancer cells at the S-phase via a Bax-, Bad-, and caspase-3-related pathways." *Life Sci* **78**(8): 869-74.

Annonaceous acetogenins are a group of potential anti-neoplastic agents isolated from Annonaceae plants. We purified squamocin, a cytotoxic bis-tetrahydrofuran acetogenin, from the seeds of *Annona reticulata* and analyzed its biologic effects on cancer cells. We showed that squamocin was cytotoxic to all the cancer lines tested. Furthermore, squamocin arrested T24 bladder cancer cells at the G1 phase and caused a selective cytotoxicity on S-phase-enriched T24 cells. It induced the expression of Bax and Bad pro-apoptotic genes, enhanced caspase-3 activity, cleaved the functional protein of PARP and caused cell apoptosis. These results suggest that squamocin is a potentially promising anticancer compound.

Zhang, X., L. Dong, et al. (2008). "Conditioned medium from Ad-IFN- α -infected bladder cancer and normal urothelial cells is cytotoxic to cancer cells but not normal cells: further evidence for a strong bystander effect." *Cancer Gene Ther* **15**(12): 817-22.

We have reported earlier that a bystander effect is seen in cancer cells that are resistant to high concentrations of the interferon- α protein (Intron A) when treated with adenoviral-mediated interferon- α (Ad-IFN- α). We now provide further evidence for this bystander effect using conditioned medium (CM) collected from Ad-IFN- α -infected cancer and normal urothelial cells. The CMs collected from UC-9 and KU7 bladder cancer cells as well as normal urothelial cells following transfection with Ad-IFN produce cell death when added to various cancer cell types in culture but not to normal urothelial cells. The CM could be filtered, frozen and thawed, and diluted to at least one part Ad-IFN CM to five parts fresh control medium and the diluted CM still shows a similar cytotoxicity as a 100% concentration of Ad-IFN CM. This cytotoxicity was observed by both flow

cytometry and MTT assays as well as by phase microscopy, and a significant sub-G1 population was seen whether the CM was collected 48, 72 or 96 h after initial Ad-IFN treatment. In addition, the CM could be partially inactivated by exposure to 65 degrees C for 30 min and totally inactivated by placement at 92 degrees C for 3 min, whereas Intron A was not inactivated under the same conditions. Importantly, although significant caspase 8 and caspase 9 cleavage occurred in Ad-IFN-treated cells as a direct effect of Ad-IFN transfection, the Ad-IFN CM produced no activation of caspase 8 and caspase 9, indicating that a different mechanism of cell death was produced by the bystander factor(s) than the direct effect of Ad-IFN. This bystander effect in turn may play an important role in the efficacy of the current Ad-IFN clinical trial for superficial bladder cancer now underway.

Zhang, X., A. S. Multani, et al. (2003). "Adenoviral-mediated retinoblastoma 94 produces rapid telomere erosion, chromosomal crisis, and caspase-dependent apoptosis in bladder cancer and immortalized human urothelial cells but not in normal urothelial cells." *Cancer Res* **63**(4): 760-5.

Retinoblastoma (RB)94, which lacks the NH(2)-terminal 112 amino acid residues of the full-length RB protein (RB110), is a more potent tumor and growth suppressor than RB110. In this study, Ad-RB94, but not Ad-RB110, produced marked growth inhibition, cytotoxicity, caspase-dependent apoptosis, and G(2)-M block in the human RB-negative, telomerase-positive bladder cancer cell line UM-UC14. This effect was completely inhibited by pretreatment with caspase inhibitors ($P < 0.0001$). Similar results were seen in RB-positive and other RB-negative bladder cancer cell lines. Ad-RB94 produced rapid telomere length shortening and loss of telomere signal, which was associated with polyploidy and chromosomal aberrations ($P < 0.001$). Ad-RB94, however, showed no cytotoxicity to telomerase-negative human normal urothelium cells but was highly cytotoxic to telomerase-positive human E6 and E7 immortalized urothelial cells ($P < 0.0001$). In addition, telomerase-negative cells, which maintain their telomere length through an alternative lengthening of telomeres DNA recombination pathway, showed no cytotoxicity to RB94. These results suggest that the induction of rapid telomere erosion and chromosomal crisis by RB94 in telomerase-positive cancer and in telomerase-expressing immortalized human cells is a major factor in its selective and potent tumor suppression and cytotoxic activity. The lack of cytotoxicity to normal cells should also provide a high therapeutic index

when used in gene therapy protocols for the treatment of bladder and other cancers.

Zhang, Y., R. Mahendran, et al. (2002). "The signalling pathway for BCG-induced interleukin-6 production in human bladder cancer cells." *Biochem Pharmacol* **63**(2): 273-82.

Intravesical bacillus Calmette-Guerin (BCG) is currently the therapy of choice for superficial bladder cancer with a 60-70% response rate. Induction of cytokine production (e.g. IL-6, etc.) by BCG has been found in patient's urine in vivo as well as bladder cancer cell lines. However, the signalling mechanisms are still unclear. In this study, we investigated the effect of BCG on cAMP production and its role in regulating interleukin-6 expression in the human bladder cancer cell line, MGH. After 1 hr exposure to BCG, IL-6 gene expression in MGH cells increased by 2.5-3-fold and cAMP production increased by 8-10-fold in a time- and dose-dependent manner. BCG-induced cAMP production was inhibited by both antifibronectin antibody and an adenylate cyclase inhibitor, SQ22536 in a dose-dependent way. In the presence of SQ22536, IL-6 expression in MGH cells was also greatly reduced. Furthermore, cAMP-dependent kinase inhibitors H7 and HA1004 also inhibited BCG-induced IL-6 expression in MGH, with HA1004 being much less effective than H7. Thus, BCG induces cAMP production and may regulate interleukin-6 expression partially via a cAMP-dependent pathway in human bladder cancer cells.

Zhang, Z., J. Karam, et al. (2006). "The application of epigenetic modifiers on the treatment of prostate and bladder cancer." *Urol Oncol* **24**(2): 152-60.

Prostate cancer and transitional cell carcinoma (TCC) of bladder are the 2 most common malignancies in the male adult urogenital system. Epigenetic gene silencing, particularly tumor suppressor genes, has become a new area of cancer research. Agents such as deoxyribonucleic acid methyltransferase inhibitors or histone deacetylase inhibitors are epigenetic modifiers that can restore gene expression and alter the malignant phenotype of cancer. They provide a new therapeutic avenue for prostate cancer and TCC. It is also likely that combination regimens using epigenetic modifiers with other classes of agents may have higher therapeutic efficacy for prostate cancer and TCC, especially metastatic and/or refractory cases. We review current knowledge of epigenetic event in prostate cancer and TCC, and discuss the possible clinical implications for these 2 diseases.

Zhang, Z., T. Shirakawa, et al. (2003). "Combination with CD/5-FC gene therapy enhances killing of

human bladder-cancer cells by radiation." *J Gene Med* **5**(10): 860-7.

Resistance to radiation and chemotherapy is a significant obstacle to the treatment of advanced bladder cancer. Gene therapy combined with radiation represents a new approach to cancer treatment. In the present study, we investigated whether adenovirally directed, cytosine deaminase (CD)/5-fluorocytosine (5-FC) gene therapy could induce cell toxicity and radiosensitization through the intracellular production of 5-fluorouracil (5-FU) in bladder-cancer cells. Three human bladder-cancer cell lines, KK47 (wild-type p53+), T24 (p53 mutated) and 5637 (p53 mutated), were investigated. A recombinant adenovirus vector containing the CD gene (Ad-RSV-CD) was used. Cells were infected with Ad-RSV-CD and treated with 5-FC. Forty-eight hours after infection, the cells were irradiated and cytotoxicity assays performed to determine the extent of increase in in vitro cytotoxicity. A KK47 subcutaneous tumor-xenografts model was used in an animal study to examine the tumor growth inhibitory effect of this combination therapy. Ad-RSV-CD was directly injected into the tumor and daily 5-FC was intraperitoneally injected. Forty-eight hours after injection of Ad-RSV-CD, the tumor was irradiated. The tumor volume was measured every day. In all three cell lines, the combination treatment enhanced the cell killing of human bladder-cancer cells in vitro. It also enhanced the tumor-growth inhibition in the KK47 tumor model. In the present study, we demonstrated that CD/5-FC gene therapy combined with radiation therapy enhances cell killing of human bladder-cancer cells in in vitro and in vivo animal models.

Zhou, J. H., C. J. Rosser, et al. (2002). "Visualizing superficial human bladder cancer cell growth in vivo by green fluorescent protein expression." *Cancer Gene Ther* **9**(8): 681-6.

There has been no reliable orthotopic model available to visualize the growth of human superficial bladder cancer over time and to evaluate the efficacy of intravesical therapies. We have developed a novel approach to accomplish this task by generating human superficial bladder tumor cells to stably express high levels of green fluorescent protein (GFP) in vivo. Superficial bladder tumors were produced in athymic mice by intravesical instillation. In our initial studies tumors were quantitated by image analysis at a single time point, and the results compared to the estimation of the percentage of GFP cells present using flow cytometry after obtaining single cell suspensions of normal and tumor cells in the same bladder. A high correlation between the two methods was seen. Therefore, in subsequent studies, approximately 1 week after the intravesical instillation of the GFP

expressing cancer cells a small incision was made to expose the bladder. The anterior, posterior, and lateral images of each bladder were captured to visualize GFP-expressing tumors. The ratio of green fluorescence pixel area, which represented the tumor burden, to the total area of the bladder was then calculated. A similar procedure was performed at 2, 3, and 4 weeks after instillation of the tumor cells. Using this procedure tumor progression over time could be measured in each mouse. By using this approach, it will now be possible to monitor the initial tumor sizes in the bladder of each mouse and then to evaluate the efficacy of various intravesical therapy protocols including intravesical gene therapy alone or in combination with other treatment modalities.

Zhu, H. J., Z. Q. Zhang, et al. (2004). "Cloning and analysis of human UroplakinII promoter and its application for gene therapy in bladder cancer." *Cancer Gene Ther* **11**(4): 263-72.

The differential expression of the desired gene product in the target tissue is central for gene therapy. One approach is to use a tissue-specific promoter to drive therapeutic gene expression. UroplakinII (UPII) is a urothelium-specific membrane protein. To investigate the feasibility of targeting gene therapy for bladder cancer, a DNA fragment of 2542-bp upstream of the UPII gene was amplified by PCR and linked to a promoterless firefly luciferase reporter gene. The transient transfection showed that the DNA fragment resulted in preferential expression in bladder carcinoma cells, with negligible expression in nonurothelium cells. Furthermore, the DNA segment located between -2545 and -1608 decided the tissue-specificity of the UPII promoter, the segment located between -328 and -4 being the core promoter of UPII. We generated two recombinant adenoviruses under the control of the UPII promoter: Ad-hUPII-GFP, carrying green fluorescence protein (GFP), and Ad-hUPII-TNF, carrying the tumor necrosis factor alpha (TNFalpha). ELISA revealed that the secretion of TNFalpha by Ad-hUPII-TNF-infected bladder cancer cells was significantly higher than Ad-hUPII-TNF-infected nonurothelium cells. The conditioned medium from Ad-hUPII-TNF-infected bladder cancer cells apparently inhibited the proliferation of L929 cells, a TNFalpha-sensitive cell line, comparing to Ad-hUPII-TNF-infected nonurothelium cells. Intravesical inoculation with Ad-hUPII-TNF inhibited tumor growth in the orthotopic human bladder cancer model. The sustained high level of TNFalpha in urine was identified with ELISA. Taken together, these data suggest that most of the cis elements that confer the bladder-specificity and differentiation-dependent expression of the human UPII gene reside in the 2542-bp sequence, and TNFalpha driven by the human UPII

(hUPII) promoter is effective in the specific inhibition of bladder cancer growth both in vivo and in vitro. These results may yield a new therapeutic approach for bladder cancer and provide information on the molecular regulation of urothelial growth, differentiation, and disease.

Zhu, Z., S. Xing, et al. (2006). "Modification of alternative splicing of Bcl-x pre-mRNA in bladder cancer cells." *J Huazhong Univ Sci Technolog Med Sci* **26**(2): 213-6.

To modify the splicing pattern of Bcl-x and compare the effect of this approach with that of the antisense gene therapy in BIU-87 cell line of bladder cancer, by using 5'-Bcl-x AS to target downstream alternative 5'-Bcl-x splice site to shift splicing from Bcl-xL to Bcl-xS and 3'-Bcl-x AS antisense to the 3'-splice site of exon III in Bcl-x pre-mRNA to down regulation of Bcl-xL expression, the inhibitory effects on cancer cells by modification of alternative splicing and antisense gene therapy were observed and compared by microscopy, MTT Assay, RT-PCR, FACS, Western blotting and clone formation. The growth of cells BIU-87 was inhibited in a dose- and time-dependent manner. Its inhibitory effect began 12 h after the exposure, reaching a maximum value after 72h. The number of cells decreased in S phase and the number increased in G1 phase. The ability to form foci was reduced and the antisense gene therapy was approximately half as efficient as modification of alternative splicing in inducing apoptosis. It is concluded that modification of splicing pattern of Bcl-x pre-mRNA in bladder cancer cell BIU-87 is better than antisense gene therapy in terms of tumor inhibition.

Zhu, Z., S. Xing, et al. (2003). "Effect of Ad-p16 combined with CDDP or As2O3 on human bladder cancer cells." *J Huazhong Univ Sci Technolog Med Sci* **23**(2): 169-72.

To evaluate the therapeutic efficiency of combined use of p16-expressing adenovirus and chemotherapeutic agents CDDP or As2O3 on human bladder cancer cell line EJ, the human bladder cancer cell line EJ were transfected with adenovirus-mediated p16 gene (Ad-p16), with administration of cisplatin (CDDP) or arsenic trioxide (As2O3). The cell growth, morphological changes, cell cycle, apoptosis and molecular changes were measured using cell counting, reverse microscopy, flow cytometry, cloning formation, immunocytochemical assays and in vivo therapy experiments to evaluate the therapeutic efficacy of such combined regimen. Ad-p16 transfer and CDDP or As2O3 administration to EJ cells could exert substantially stronger therapeutic effects than the single agent treatment. Especially in in vivo

experiments, combined administration of p16 and CDDP or As2O3 induced almost tumor diminish compared to the partial tumor diminish induced by single agent. Moreover, delivery of Ad-p16, or administration of minimal-dose CDDP or As2O3 or combined regimen could induce massive apoptosis of EJ cell. Cell cycle analysis demonstrated that administration of CDDP or As2O3 remarkably arrested EJ cell in G1 prior to apoptotic cell death. When treated with combined regimen, cells were arrested in G1 to a greater extent prior to apoptotic cell death. It is concluded that after introduction into EJ cell, Ad-p16 shows enhanced therapeutic efficacy for EJ cell when used in combination with CDDP or As2O3.

Zhu, Z., S. Xing, et al. (2002). "Adenovirus-mediated transfer of p53 and p16 inhibiting proliferating activity of human bladder cancer cell EJ in vitro and in vivo." *J Huazhong Univ Sci Technolog Med Sci* **22**(4): 324-6.

To evaluate the effects of adenovirus (Ad)-mediated transfer of p53 and p16 on human bladder cancer cells EJ, EJ were transfected with Ad-p53 and Ad-p16. Cell growth, morphological change, cell cycle, apoptosis were measured using MTT assay, flow cytometry, cloning formation, immunocytochemical assays. Ad-p16 or Ad-p53 alone could inhibit the proliferating activity of EJ cells in vitro. Ad-p53 could induce apoptosis of partial EJ cells. G1 arrest was observed 72 h after infection with Ad-p16, but apoptosis was not obvious. The transfer of Ad-p16 and Ad-p53 could significantly inhibit the growth of EJ cells, decrease the cloning formation rate and induce apoptosis of large number of EJ cells. The occurrence time of subcutaneous tumor was delayed and the tumor volume in 4 weeks was diminished by using Ad-p53 combined with Ad-p16 and the difference was significant compared with using Ad-p53 or Ad-p16 alone. It was suggested that the transfer of wild-type p53 and p16 could significantly inhibit the growth of human bladder cancer in vitro and in vivo.

Zieger, K. (2008). "High throughput molecular diagnostics in bladder cancer - on the brink of clinical utility." *Mol Oncol* **1**(4): 384-94.

An enormous body of high-throughput genome-wide data, in particular gene expression data, has been gathered from roughly all human cancer forms in the past 10 years. This has widely increased our understanding of the cancer disease and its molecular changes and pathways, with a large contribution from studies of cancer cell lines and functional genomics. In the last three years, the focus has been moved to clinical outcome parameters as

recurrence, progression, metastasis and treatment response. The huge variability of molecular changes and poor availability of samples have hampered progress in the field of epithelial cancer (carcinoma). However, independent validation of molecular profiles across high-throughput platforms, methods, laboratories and cancer populations has recently been successfully performed for several carcinomas, including bladder cancer. Application of advanced bioinformatics to identify interrelated pathways has revealed common signatures predictive of molecular subgroups, improving histopathological diagnosis, and ultimately outcome prediction. With breast cancer leading the field, colorectal, bladder and renal cell carcinomas well on their way, and many others soon to join, the era of clinical applications of high-throughput molecular methods in cancer lies closely ahead. This review illustrates in detail the perspectives for the management of bladder cancer.

Zou, C., Y. Guan, et al. (2002). "N-(4-hydroxyphenyl)retinamide (4-HPR) modulates GADD45 expression in radiosensitive bladder cancer cell lines." *Cancer Lett* **180**(2): 131-7.

We previously demonstrated that N-(4-hydroxyphenyl)retinamide (4-HPR) and gamma-irradiation, when used in combination, had a synergistic effect in inducing apoptosis in bladder cancer cells, suggesting that 4-HPR may increase radiosensitivity in bladder cancer cells. To unravel molecular correlates in this radiosensitizing effect of 4-HPR, we examined the baseline and 4-HPR-induced expression of GADD45 to elucidate possible mechanisms by which 4-HPR enhanced the effect of gamma-irradiation in three bladder cancer cell lines. To investigate the role of p53 in mediating the radiosensitizing effect of 4-HPR, we also examined mutations in exons 5-9 by using direct sequencing and the levels of p53 expression by using RT-PCR and Western blot, before and after treatment with 4-HPR in these bladder cancer cell lines. Two cell lines had low expression of GADD45, and a dose-dependent increase in GADD45 expression induced by 4-HPR was found in bladder cancer cell lines without p53 mutations in exons 5-9. A combination of gamma-irradiation and 4-HPR showed a significantly greater effect in enhancing GADD45 expression than either agent used alone. The results indicate that the combined treatment with 4-HPR and gamma-irradiation has a stronger effect on GADD45 expression than the treatment with either agent alone, which suggests that the two agents may have an additive/synergistic effect. However, a normal p53 function appears to be necessary for the dose-dependent induction of GADD45 by 4-HPR. Once our results are verified and replicated by other

investigators, 4-HPR may have a potential clinical implication in effectively treating bladder cancer in combination with low-gamma-irradiation therapy.

Zou, L., P. Zhang, et al. (2006). "shRNA-targeted hTERT suppress cell proliferation of bladder cancer by inhibiting telomerase activity." *Cancer Chemother Pharmacol* **57**(3): 328-34.

RNA interference (RNAi) has demonstrated profound prospect in human gene research. hTERT, the rate-limiting component of telomerase activity, is highly expressed in bladder cancer cells. Here, we investigated the anti-proliferation effects of small hairpin interfering RNA (shRNA)-targeted hTERT gene on bladder cancer in vitro and in vivo. The results showed that ph2-shRNA, the most-effective vector carrying shRNA-targeted hTERT, could significantly inhibit the cell proliferation by down-regulating hTERT expression, decreasing telomerase activity, decreasing cell number of S phase, increasing the cell number of G0/G1 phase in T24 cells and xenograft tumor tissues, and attenuate the tumor growth of xenograft mice model compared with controls. Our results demonstrate that hTERT-directed shRNAs are potent inhibitors of bladder cancer.

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