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Current progress in adenovirus mediated gene therapy for patients with prostate carcinoma

Review Article

Ahter D. Sanlioglu[1,3], Turker Koksal[2,3], Mehmet Baykara[2,3], Guven Luleci[1,3], Bahri Karacay[4] and Salih Sanlioglu[1,3,*]

[1]Departments of Medical Biology and Genetics, [2]Department of Urology and [3]The Human Gene Therapy Unit of Akdeniz University, Faculty of Medicine, Antalya, Turkey, 07070; [4]Department of Pediatrics, University of Iowa, College of Medicine, Iowa City, IA, 52240, USA

***Correspondence**: Salih Sanlioglu V.M.D., Ph.D., Director of The Human Gene Therapy Unit of Akdeniz University, Faculty of Medicine, B- Block, 1st floor, Campus, Antalya, 07070 Turkey; Phone: (90) 242-227-4343/ext: 44359, Fax: (90) 242-227-4482; e-mail: sanlioglu@akdeniz.edu.tr

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Summary

Prostate cancer is the most frequently diagnosed male cancer in the world. Like all cancers, prostate cancer is a disease of uncontrolled cell growth. In some cases tumors are slow growing and remain local, but in others they may spread rapidly to the lymph nodes, other organs and especially bone. Although surgery and radiation can cure early stages of organ confined prostate carcinoma (stages I and II), there is no curative therapy at this time for locally advanced or metastatic disease (stages III and IV). The likelihood of postsurgical local recurrence increases with capsular penetration as detected in 30 % of the patients at the time of radical prostatectomy. Moreover, 10-15 % of patients have metastatic cancer at the time of diagnosis. Considering the fact that 60 % local recurrence is observed in patients receiving radiation therapy with or without adjuvant hormonal ablation therapy, it is generally believed that androgen ablation therapy simply delays the progression of prostate carcinoma to a more advanced stage. In addition, the overall ten-year survival rate of patients with locally recurrent prostate cancer is only around 35 %; thus; the ultimate progression into androgen independent prostate carcinoma appears to be inevitable. Gene therapy arose as a novel treatment modality with the potential to decrease the morbidity associated with conventional therapies. Therefore, gene therapy is expected to lower the incidence of tumor recurrence and finally improve the outcome of patients with recurrent and androgen independent prostate carcinoma. Viral vectors are most commonly used for the purpose of gene therapy. Currently, there are a total of 40 clinical trials being conducted using viral vectors for the treatment of prostate carcinoma. 22 out of 40 clinical protocols (55 %) approved for the treatment of prostate cancer utilize adenovirus vectors. Most of these adenovirus mediated therapeutic approaches employ either selectively replicating adenoviruses or suicide gene therapy approaches. In this review, we mainly concentrated on the progress in adenovirus mediated gene therapy approaches for prostate cancer. Analysis of the death ligand mediated gene therapy approach was also discussed in detail, while our novel findings were incorporated as an example for up-to-date approaches used for adenovirus mediated gene therapy against prostate carcinoma.

I. Introduction

Prostate cancer is the second leading cause of death in men from cancer following lung carcinoma with an annual mortality rate of 38,000 (Yeung and Chung, 2002). There are 200,000 newly diagnosed cases of prostate carcinoma every year in the United States alone (Boring et al, 1994; Greenlee et al, 2001). As a result, prostate carcinoma is claimed to be the most frequently diagnosed male cancer in the United States (Powell et al, 2002). Despite the fact that there has been a considerable effort for screening and early detection of prostate cancer in recent years, the lifetime risk of being diagnosed with prostate cancer is still reported to be 1 in 5 (Grumet and Bruner, 2000). Several hundred clinical studies using experimental or approved chemotherapeutics failed to improve survival rates of patients with prostate cancer (Devi, 2002). Because prostate cancer is a heterogeneous

disease, treating patients with prostate cancer still remains a formidable task. In addition, the molecular mechanism responsible for the onset of the disease is poorly understood. However, earlier detection of prostate cancer has been associated with an improved outcome (Perrotti et al, 1998). Thus, the detection of prostate cancer at an earlier stage remains to be the most realistic chance for therapy.

For this purpose, different molecular screening methods (Ross et al, 2002a, 2002b) have been employed, but the most effective method is yet to be established. The most commonly used screening assays are based on the detection of up-regulated prostate specific markers such as prostate specific antigen (PSA). Currently, prostate specific antigen, (Farkas et al, 1998) when it is used in conjunction with other markers such as Gleason Scoring (Koksal et al, 2000) and TNM grading (Schroder et al, 1992), is considered to be a valuable tool to evaluate the histological grade of prostate carcinomas (Xess et al, 2001). As a result, patients were provided with various treatment options based on the results obtained with these parameters. These treatment options included but were not limited to operation, (Klotz, 2000b) radiotherapy, (Do et al, 2002) chemotherapy (Wang and Waxman, 2001) and hormone therapy (Klotz, 2000a; Smith et al, 2002). Regrettably, these conventional treatment modalities could not decrease the casualties from prostate cancer (Hsieh and Chung, 2001). Hence, there is a great need for development of novel treatment modalities to fight against prostate cancer. These remorseful facts ignited the initiation of gene therapy trials for prostate carcinoma (Sanda, 1997). So far, various viral vectors including lentivirus (Yu et al, 2001a), herpes simplex virus (Jorgensen et al, 2001), adeno-associated virus (Vieweg et al, 1995) and adenovirus (Loimas et al, 2001) were tested as carriers for therapeutic genes against prostate cancer. Other types of viruses such as Semliki Forest virus and Sindbis virus were also tested for gene delivery to prostate cancer cells (Loimas et al, 2001), but these viruses were unable to transduce prostate cells efficiently. Due to its antigenic properties and tissue transduction characteristics, adenovirus arose as a favored transporter vector. The exploitation of the tissue specific promoter in gene therapy especially eased adenovirus use in clinical trials (Lu and Steiner, 2000). In this review, we mainly highlighted the progress in adenovirus mediated prostate cancer gene therapy within the last three years with a particular emphasis in death ligand mediated gene therapy approach.

II. Immunomodulation

Tumors exhibit some degree of immunogenicity and the human immune system responds to these tumor specific antigens by mounting humoral and cellular responses, which are essential for the eradication of tumors. Adenovirus is commonly used for the delivery of genes encoding tumor-associated antigens in order to augment tumor-specific immune responses. However, antiviral immunity against adenovirus is a big concern, challenging its application in gene therapy. Various methods were employed in order to get around the antiviral immunity barrier to increase the efficacy of adenovirus mediated gene delivery. One of these methods involves the testing of a collagen-based matrix (Gelfoam) (Siemens et al, 2001). Coinjection of Gelfoam with adenovirus vectors carrying prostate-specific antigen (Ad5-PSA) into mice naive to PSA but immune to adenovirus, relinquished the inhibitory effects of adenoviral immunity on CTL activation. Viral vectors are also being tested to deliver tumor specific peptides into dendritic cells (DCs) to evoke an immune response. The degree of immune response generated relies on the functionality of DCs following viral transduction. To prove this, adenovirus and retrovirus vectors were compared on the basis of their influence on the functionality of DCs (Lundqvist et al, 2002a). Adenovirus-transduced monocyte-derived DCs (MO-DCs) stimulated allogenic lymphocytes and produced high levels of TNF and IL12. In addition, the expression of NF-κB and antiapoptotic molecules such as Bcl-X(L) and Bcl-2 (Lundqvist et al, 2002b) were also increased in adenovirus-transduced MO-DCs. Consequently, these cells became more resistant to spontaneous as well as Fas-mediated cell death. In contrast, retroviruses failed even to transduce MO-DCs. Although CD34(+) cell-derived DCs were transducable with retroviruses to a lesser extent, they were less potent in their ability to stimulate allogenic lymphocytes in comparison to nontransduced DCs. These results suggest that adenovirus transduction of DCs increased the survival and the potency of DC mediated activation of the immune system. This might be important for prolonging the antigen presentation to generate a greater degree of immune response.

Cytokine stimulated tumor infiltrating macrophages also play a major role in the generation of the cellular immune response against the tumor. The role of tumor-infiltrating macrophages in IFN-β-induced host defense against prostate cancer was revealed using xenograft mice models injected with adenovirus carrying IFN-β gene (Zhang et al, 2002a). Injection of an adenoviral vector encoding murine IFN-β (AdIFN-β) directly into the tumor suppressed the growth of PC-3MM2 tumors as well as prevented metastasis and prolonged the survival of tumor-bearing mice. Based on immunohistochemical staining, AdIFN-β infection resulted in the reduction of microvessel density of the tumor and increased apoptotic cell death (Cao et al, 2001). On the contrary, macrophage-selective anti-Mac-1 and anti-Mac-2 antibodies significantly reduced the antitumor effect of AdIFN-β induced therapy. Therefore, it was concluded that tumor-infiltrating macrophages must be involved in IFN-β induced suppression of tumor growth and metastasis.

III. Suicide Gene Therapy

Suicide strategy is a combined treatment modality involving chemotherapy and the gene transfer technology. The underlying principle is to limit the cytotoxicity of a drug to the local area of the tumor. To achieve this, the cDNA of a prodrug-converting enzyme is delivered into the tumor using viral vectors followed by regional or systemic application of the corresponding prodrug. As

soon as the prodrug reaches the tumor, it is taken up and converted to a cytotoxic drug by tumor cells expressing the prodrug-converting enzyme. For example, 5-Fluorouracil (5-FU) is widely used as a chemotherapeutic agent for the treatment of various malignancies. Although clinical trials have been conducted, so far 5-FU manifested a poor therapeutic index, which drastically limited its clinical use for cancer therapy. It is still not known whether the lack of success was due to problems associated with drug delivery or inherent insensitivity of cancer cells to this metabolite. However, adenovirus (Ad) vector-mediated cytosine deaminase (CD)/5-fluorocytosine (5-FC) gene therapy had the potential to overcome pharmacokinetic issues associated with systemic 5-FU administration. Escherichia coli cytosine deaminase converts the prodrug 5-FC to the cytotoxic product 5-FU. Adenovirus encoding cytosine deaminase (AdCD) gene was injected into the prostate cancer cells transplanted orthotopically on mice followed by the systemic use of 5-FC in order to investigate the antitumor and anti-metastatic effects of this approach (Zhang et al, 2002c). An effective inhibition on tumor growth and metastasis was observed through in situ injection of AdCD followed by systemic use of 5-FC in the xenograft mouse model of prostate cancer. The use of E. coli uracil phosphoribosyltransferase (UPRT), a pyrimidine salvage enzyme, which modifies 5-FU into 5-fluorouridine monophosphate, improved the activity of AdCD through enhancing the anti-tumoral effect of 5-FU. In order to assess the efficacy of the combined suicide gene therapy approach, two separate adenovirus constructs expressing either the E. coli CD or E. coli UPRT genes were infected into androgen refractory prostate cancer cell line DU145 bearing mice. This combined gene therapy approach drastically regressed the growth of tumors in these animals better than what was achieved with AdCD alone (Miyagi et al, 2003).

The most commonly used prodrug-converting enzyme for clinical approaches is the herpes simplex virus thymidine kinase gene (HSV-tk). The enzyme thymidine kinase phosphorylates the prodrug ganciclovir (GCV) to ganciclovir monophosphate, which is then further phosphorylated by cellular enzymes to ganciclovir triphosphate, a toxic metabolite and inhibitor of DNA polymerase. The efficacy of this approach was evaluated in an extended phase I/II study involving 36 prostate cancer patients with local recurrence after radiotherapy. These patients received single or repeated cycles of replication-deficient adenoviral mediated HSV-tk plus GCV in situ gene therapy (Miles et al, 2001). The study concluded that the repeated cycles of in situ HSV-tk plus GCV gene therapy can safely be administered to patients with prostate cancer who failed radiotherapy and have a localized recurrence. The therapeutic parameters such as PSA doubling time (PSADT), the mean PSA reduction (PSAR), and return to initial PSA (TR-PSA) values were all increased as a response to the treatment, indicating a therapeutic effect. A combined gene therapy approach using a recombinant adenovirus containing a fusion gene of CD and HSV-tk controlled by a cytomegalovirus (CMV) enhancer-promoter was designed to explore new frontiers in prostate cancer gene therapy (Lee et al, 2002b). Both of the prostate carcinoma cell lines tested (DU-145 or PC-3 cells) were effectively transduced and killed by this replication-incompetent adenovirus encoding CD-TK fusion protein in the presence of prodrugs. The effect of radiation and heat treatment was also tested using this vector system. Interestingly, heat treatment not only increased the expression of CD-TK but sensitized prostate cancer cells to radiation as well. These results suggested that combining heat treatment with radiation therapy improved the efficacy of the adenovirus mediated suicide gene therapy approach for prostate carcinoma. The CD-TK fusion fragment was also cloned into a lytic, replication-competent adenovirus (Ad5-CD/TKrep) and administered into patients with prostate carcinoma in a Phase I trial. This was the first gene therapy study in which a replication-competent virus was used to deliver a therapeutic gene to humans (Freytag et al, 2002a). This study demonstrated that intraprostatic administration of the replication-competent Ad5-CD/TKrep virus followed by 2 weeks of 5-fluorocytosine and ganciclovir prodrug therapy led to the destruction of tumor cells in patients without safety concerns. In addition, the efficacy and the toxicity of replication-competent adenovirus-mediated double suicide gene therapy (AdCD-TK) combined with an external beam radiation therapy (EBRT) approach was tested as a trimodal treatment modality in a preclinical study (Freytag et al, 2002b). Animals bearing prostate tumors were first injected with the lytic, replication-competent Ad5-CD/TKrep virus, then received 1 week of 5-fluorocytosine + ganciclovir (GCV) prodrug therapy supplemented with EBRT. The results from this study suggested that replication-competent adenovirus-mediated double suicide gene therapy combined with EBRT is very effective in eliminating tumors and reducing metastasis in an orthotropic mouse model of prostate carcinoma.

The efficacy of another gene-directed enzyme pro-drug therapy based on the Escherichia coli enzyme purine nucleoside phosphorylase (PNP) was tested in androgen-independent prostate cancer cells. PNP modifies the prodrug fludarabine to 2-fluoroadenine (Voeks et al, 2002). In this study, a recombinant ovine adenovirus vector (OAdV220) with a different receptor choice than that of human adenovirus type 5 carrying the PNP gene under the control of RSV promoter was used for functional studies. OAdV220 manifested a higher transgene expression compared to human Ad5 vector in infected murine RM1 prostate cancer cells during in vitro studies. Furthermore, the OAdV220 construct dramatically inhibited subcutaneous tumor growth when fludarabine phosphate was administered systemically in immunocompetent mice. Similar results were obtained using human PC3 xenografts in mice. PNP is also known to convert the prodrug 6MPDR to a toxic purine (6MP) causing cell death. In order to assess the efficacy of this approach for prostate cancer, replication-deficient human type-5 adenovirus (Ad5) carrying the PNP gene (Ad5-SVPb-PNP) was directly injected into PC3 tumors (Martiniello-Wilks et al, 2002). The specificity and the level of transgene expression from this recombinant adenoviral vector were controlled by the promoter from

the androgen-dependent, prostate-specific rat probasin (Pb) gene hooked up to the SV40 enhancer (SVPb). Unexpectedly, the SVPb element confirmed substantial prostate specificity even in the absence of androgens. Intratumoral delivery of Ad5-SVPb-PNP followed by 6MPDR administration significantly suppressed the growth of human prostate tumors in nude mice. These results suggested that Ad5-SVPb-PNP has therapeutic potential even in the absence of androgens for the treatment of prostate carcinoma.

Another non-toxic prodrug, CB1954, which is converted to a toxic metabolite by the Escherichia coli nitroreductase gene (NTR), was tested as a suicide gene therapy approach for prostate cancer. Adenovirus vector expressing NTR (CTL102) was injected into subcutaneous prostate cancer xenografts followed by systemic CB1954 administration (Djeha et al, 2001). A clear anti-tumor effect of the approach was observed. In addition to all the methods mentioned above, a novel approach inspired from radioiodine therapy for thyroid cancer was developed using sodium iodide symporter (NIS). NIS is normally exclusively expressed in thyroid glands. Adenovirus carrying the NIS gene (AdCMVNIS) was constructed and tested for the treatment of prostate cancer following ^{131}I administration (Spitzweg et al, 2001). Injection of AdCMVNIS construct to prostate cancer xenografts manifested highly active radioiodine uptake resulting in a drastic reduction in the tumor size following ^{131}I administration in nude mice. This new approach represented an effective and potentially curative modality leading to the accumulation of therapeutically effective radioiodine in prostate.

Diphtheria toxin (DT) is known to be a potent inhibitor of protein synthesis. The fact that a single molecule of DT can result in cell death complicated the utilization of DT as a suicide gene for cancer therapy. Thus, the feasibility of using DT gene therapy would greatly be influenced by tissue specific gene expression. Adenovirus vector carrying the catalytic domain (A chain) of DT under the control of the prostate-specific antigen (PSA) promoter (Ad5PSE-DT-A) induced apoptosis in PSA-positive prostate cancer cells in the presence of exogenous androgen (R1881) (Li et al, 2002a). In addition, Ad5PSE-DT-A injection regressed the growth of a PSA-positive LNCaP xenograft in nu/nu mice. Non-PSA-secreting DU-145 cells did not manifest the same effect due to the lack of activation of PSA promoter in these cells. Therefore, the Ad5PSE-DT-A viral gene therapy approach might be a viable alternative in the treatment of PSA-secreting androgen-dependent prostate carcinoma.

IV. Joint approaches involving immunomodulation-hormonal or radiation therapy in combination with suicide gene approach

AdHSV-tk suicide gene therapy was coupled to adenovirus-mediated IL-12 delivery as a combined gene therapy approach in order to enhance NK activity induced by HSV-tk gene expression and ganciclovir (GCV) treatment (Hall et al, 2002). This dual treatment generated radical local and systemic growth suppression in a metastatic model of mouse prostate cancer (RM-1). The unification of AdHSV-tk/GCV + Ad.mIL-12 gene therapy approaches resulted in the induction of apoptosis due to increased expression of Fas and FasL and improved anti-metastatic activity secondary to a strong NK effect. Intra-tumoral injection of AdHSV-tk vector followed by systemic ganciclovir or local radiation therapy or the combination of gene and radiation therapy was administered to subcutaneously transplanted mouse prostate tumors (Chhikara et al, 2001). The combined treatment reduced tumor growth by 61% compared to 38% obtained by single therapy modalities. Combined therapy also increased the mean survival time. In order to analyze systemic anti-tumor activity, lung metastases were generated by tail vein injection of RM-1 prostate cancer cells. While radiotherapy alone had no effect on the metastatic growth, the number of lung nodules was reduced by 37% following treatment with AdHSV-tk. The combinational therapy led to an additional 50% reduction in lung colonization. This was the first study demonstrating a significant systemic effect of AdHSV-tk administration combined with radiation. A Phase I/II study of radiotherapy and in situ gene therapy (adenovirus/herpes simplex virus thymidine kinase gene/valacyclovir) in combination with or without hormonal therapy in the treatment of prostate cancer was conducted recently (Teh et al, 2001). Based on the preliminary results, no serious side effect of the combined therapy was observed. This was reported as the first trial of its kind in the field of prostate cancer, and is expected to enlarge the curative index of radiotherapy by merging in situ gene therapy.

V. Molecular signaling pathways modulating the efficacy of adenovirus mediated therapeutic gene delivery

Expression of certain hormone and growth factor receptors as well as cytokines and related downstream molecules can affect the efficacy of adenovirus-mediated gene therapy for prostate cancer. For example, gonadotrophin-releasing hormone (GnRH) restrains cell growth of reproductive tissue via gonadotrophin-releasing hormone receptors (GnRH-Rs) expressed in most cancers of reproductive tissues like that of prostate. Unfortunately, endogenous GnRH-R expression was not detected in PC3 cells, indicating that the cells are insensitive to GnRH. Exogenous expression of high affinity GnRH-R using adenovirus vectors (AdGnRH-R) facilitated antiproliferative effects of GnRH agonists in prostate cancer cells (Franklin et al, 2003). In addition, most of the prostate cancer cell lines overexpress fibroblast growth factors (FGFs). FGF signaling controls cell proliferation and inhibits cell death. A recombinant adenovirus expressing a dominant-negative FGF receptor (AdDNFGFR-1) was created in order to determine the biological significance of altered FGF signaling in human

prostate cancer (Ozen et al, 2001). AdDNFGFR-1 infection of LNCaP and DU145 prostate cancer cells induced extensive cell death within 48 hours. Some of the prostate cancer cell lines are androgen dependent (LNCaP) whereas some are androgen independent (DU145 or PC3). Androgen ablation therapy, surgery, and radiation therapy are relatively effective in treating androgen dependent prostate carcinoma. However these treatments were ineffective for androgen-insensitive prostate carcinoma. Upregulation of IL6 cytokine induced by the constitutive NF-κB and Jun D activation is one of the distinctive parameters of androgen independent cell lines (Giri et al, 2001). IL6 is known to function as a proliferation and differentiation factor for prostate carcinoma. The infection with adenovirus vectors encoding either the dominant negative form of IκBα gene or Jun D reduced IL6 gene expression, leading to growth suppression of prostate cancer cells (Zerbini et al, 2003). Some but not all prostate cancer cells respond to vitamin D treatment. 1α, 25-Dihydroxyvitamin D(3) (1α, 25-(OH)(2)D(3)) is known to have significant antiproliferative effects on certain prostatic carcinoma (PC) cell lines. 1α, 25-(OH)(2)D(3) inhibited cell growth and upregulated p21 expression in PC cell lines such as ALVA-31 and LNCaP (Moffatt et al, 2001). Stable transfection with a p21 antisense construct abolished the growth inhibition of ALVA-31 cells without altering vitamin D receptor expression. On the contrary, adenovirus-mediated expression of a sense p21 cDNA significantly reduced the proliferation of 1α, 25-(OH)(2)D(3) unresponsive TSU-Pr1 and JCA-1 prostate cancer cell lines. Therefore, Adp21 gene therapy may be useful even for prostate cancer patients not responding to vitamin D treatment.

Molecular signaling pathways are also altered in cancer cells. For instance, highly metastatic tumor cell lines display increased activity for focal adhesion kinase (FAK). The role of FAK in regulating migration of prostate carcinoma cell lines with increasing metastatic potential was studied in detail (Slack et al, 2001). Highly tumorigenic PC3 and DU145 cells displayed intrinsic migratory capacity correlating with an increased FAK expression and activity. On the contrary, poorly tumorigenic LNCaP cells required a stimulus to migrate. Inhibiting the FAK/Src signal transduction pathway by overexpressing FRNK (Focal adhesion kinase-Related Non-Kinase), an inhibitor of FAK activation, significantly inhibited migration of prostate carcinoma cells. Modulation of phosphatidylinositol 3'-kinase (PI3'-kinase), leading to Akt activation, frequently occurs in prostate cancer and disrupts apoptotic signaling induced by various cytokines such as tumor necrosis factor TNF and TNF-related apoptosis-inducing ligand (TRAIL). Two prostate cancer cell lines with constitutively activated PI3'-kinase cascades (LNCaP and PC-3) were examined in order to study the role of PI3' phosphorylation in cellular response to TNF or TRAIL alone. Both TNF and TRAIL failed to activate apoptosis in either LNCaP or PC-3 cells. Interestingly, downregulation of PI3'-kinase/Akt signaling significantly enhanced the apoptotic activity of both TNF and TRAIL in LNCaP cells but not in PC-3 cells. Infection with adenovirus delivered PTEN/MMAC1 (phosphatase and tensin homologue/mutated in multiple advanced cancers) reduced Akt activation, activated apoptosis and sensitized cells to TNF but not to TRAIL in LNCaP cell line (Beresford et al, 2001). Therefore, it was concluded that although PI3'-kinase signaling inhibits both TNF and TRAIL mediated apoptosis, this may only represent one of the several apoptotic resistance mechanisms in signaling pathways.

Selenium compounds are known to be potential chemotherapeutic agents for prostate cancer. NF-κB has been categorized as the key antiapoptotic signaling molecule often activated in transformed cells. Testing of selenium compounds on DU145 and JCA1 prostate carcinoma cells revealed that these compounds induced apoptosis through the inhibition of NF-κB pathways in these cell lines (Gasparian et al, 2002b). Increased IKK activity was blamed for constitutive NF-κB activation responsible for survival of androgen independent prostate carcinoma cell lines (Gasparian et al, 2002a).

60-80 % of prostate cancers acquire the PTEN mutation during tumorigenesis. This results in the constitutive activation of the PI3'-kinase pathway and prostatic cell proliferation. The loss of PTEN activity is also correlated with the loss of activity of the FOXO family of forkhead transcription factors such as FKHRL1 and FKHR. Interestingly, these transcription factors are shown to control the expression of apoptosis inducing ligand TRAIL. Not surprisingly, the expression of TRAIL was also reduced in PTEN-lacking prostate cancer cells, leading to decreased apoptosis. Restoration of TRAIL expression using adenovirus-mediated overexpression of these transcription factors in LAPC4 prostate cancer cell line induced apoptosis (Modur et al, 2002).

VI. Apoptosis Modulators

A. The exploitation of death ligands to induce apoptosis in cancer cells

Apoptosis, known as programmed cell death (Reed, 2000) is defined as cell's preferred form of death under hectic conditions (Sears and Nevins, 2002). In reality, it is also a key mechanism for homeostasis throughout embryonic and adult life. Genetic aberrations disrupting programmed cell death underpin tumorigenesis and drug resistance. Therefore, the specific activation of apoptosis within tumor cells could be a highly effective therapeutic intervention for prostate cancer. Currently, chemotherapy (Stein et al, 2002) and radiotherapy (Wang et al, 2002) are among the most commonly used treatment modalities against prostate cancer. The tumor suppressor gene, p53, is required in order for both of these treatment methods to work as anti-tumor agents (Levine, 1997). However, more than half of the human tumors acquire p53 mutations during tumorigenesis (Horowitz, 1999; Zeimet et al, 2000). As a result, tumors lacking p53 display resistance to both chemotherapy and radiotherapy (Obata et al, 2000). Intriguingly, death ligands induce apoptosis independent of p53 status of the cells (Ehlert and Kubbutat, 2001; Norris et al, 2001). Thus, these methods constitute somewhat of a complementary treatment modality to currently employed conventional treatments.

At present, death ligands are being evaluated as potential cancer therapeutic agents (Herr and Debatin, 2001). Previously, several studies using external Fas agonists, anti-Fas antibodies and membrane-bound FasL failed to induce Fas L mediated apoptosis in prostate cancer cells. Although the down regulation of c-FLIP expression through the use of anti-sense oligonucleotides sensitized DU145 cells to an anti-Fas monoclonal antibody (Hyer et al, 2002), efficient cell killing was not observed by this approach. However, intracellular expression of FasL using adenoviruses efficiently killed 70-90% of various human prostate cancer cell lines tested (Hyer et al, 2000). Furthermore, part of this cell killing was attributed to the bystander effect mediated by FasL carried within the apoptotic bodies and cellular debris (Hyer et al, 2003). Despite the fact that human prostate cancer cells express apoptotic FasL, some of the cell lines, such as LNCaP, are resistant to Fas L mediated cell death. Even so, prior exposure to IFNγ sensitized orthotropic prostate primary tumors to recombinant adenovirus mediated FasL delivery (Selleck et al, 2003). Despite the fact that tumor necrosis factor (TNF) (Terlikowski, 2001) and FasL (Nagata, 1997) have been studied extensively and were shown to effectively induce apoptosis in cancer cells, their systemic use in cancer gene therapy is not recommended due to the systemic toxicity.

With the discovery of a novel death ligand, TRAIL/Apo2L, (Wiley et al, 1995; Pitti et al, 1996) a new era emerged for the deployment of death ligands for cancer gene therapy (Nagane et al, 2001). The fact that TRAIL does not cause any harm to normal cells but can selectively induce apoptosis in cancer cells brought up the possibility of TRAIL testing for systemic use (Griffith and Lynch, 1998). Five different receptors were identified to interact with TRAIL; TRAIL-R1, TRAIL-R2, TRAIL-R3, TRAIL-R4 and osteoprotegrin (Abe et al, 2000; Sheikh and Fornace, 2000). TRAIL-R1 and TRAIL-R2 function as authentic death receptors inducing apoptosis while TRAIL-R3 and TRAIL-R4 are unable to induce such signaling but can serve as decoy receptors (Meng et al, 2000). However even today, no single mechanism has been found to account for TRAIL resistance observed in normal cells. The soluble form of TRAIL has successfully been tested and no toxicity due to systemic use was observed in animal models. However, large quantities of TRAIL were needed in order to suppress the tumor growth. A replication-deficient adenovirus encoding human TRAIL (TNFSF10; Ad5-TRAIL) was generated as an alternative to recombinant, soluble TRAIL protein (Griffith and Broghammer, 2001). Ad5-TRAIL infection into TRAIL-sensitive prostate tumor cells induced apoptosis through the activation of Caspase 8 pathways. Normal prostate epithelial cells were not harmed by Ad5-TRAIL infection. Moreover, in vivo Ad5-TRAIL administration suppressed the outgrowth of human prostate tumor xenografts in SCID mice. Eight prostate cancer cell lines (CWR22Rv1, Du145, DuPro, JCA-1, LNCaP, PC-3, PPC-1, and TsuPr1) and primary cultures of normal prostate epithelial cells (PrEC) were tested for sensitivity to soluble TRAIL induced cell death in another study (Voelkel-Johnson et al, 2002). 100 ng/mL of soluble TRAIL administration did not induce apoptosis in Du145, DuPro, LNCaP, TsuPr1, and PrEC. Interestingly, treatment with the chemotherapeutic agent doxorubicin sensitized almost all prostate cancer cells to TRAIL-induced cell death. On the other hand, an adenoviral vector expressing full-length TRAIL (AdTRAIL-IRES-GFP) killed prostate cancer cell lines and, unexpectedly, PrEC as well, independent of doxorubicin cotreatment. This study suggested that the AdTRAIL-IRES-GFP gene therapy approach, complemented with tissue-specific promoters, would be useful for the treatment of prostate carcinoma. However, the mechanism of TRAIL resistance in normal cells is not understood and some prostate cancer cells appeared to be TRAIL-resistant (Nesterov et al, 2001). In one study, ALVA-31, PC-3, and DU 145 cell lines were highly sensitive to apoptosis induced by TRAIL, while TSU-Pr1 and JCA-1 cell lines were moderately sensitive, and the LNCaP cell line was resistant (Nesterov et al, 2001). Due to the lack of active lipid phosphatase PTEN, LNCaP cells demonstrated a constitutive Akt activity. Akt is a negative regulator of the phosphatidylinositol (PI)3-kinase/Akt pathway. PI3-kinase inhibitors sensitized LNCaP prostate cancer cells to TRAIL. In addition, adenovirus expressing a constitutively active Akt reversed the ability of wortmannin to potentiate TRAIL-induced BID cleavage. This suggested that constitutive Akt activity inhibits TRAIL-mediated apoptosis (Nesterov et al, 2001).

B. NF-κB inhibiting approaches used to breakdown TRAIL resistance in prostate cancer cells

The mechanism of TRAIL induced apoptosis and resistance is outlined in **Figure 1**. So far, at least two different hypotheses that may partly explain TRAIL resistance are asserted. The first hypothesis advocates that normal cells carry decoy receptors (TRAIL-R3, TRAIL-R4), which compete with apoptosis inducing TRAIL receptors (TRAIL-R1, TRAIL-R2) for binding to TRAIL (Pan et al, 1997; Sheridan et al, 1997). In this hypothesis, it is believed that decoy receptors either function to dilute out TRAIL ligands (like TRAIL-R3) or supply anti-apoptotic signals (like TRAIL-R4) to cells. As reported previously, TRAIL-R4 binding activates the anti-apoptotic NF-κB signaling pathway, leading to the blockade of TRAIL induced apoptosis (Degli-Esposti et al, 1997). In addition, the expression of decoy receptors is down-regulated in cancer cells through promoter hypermethylation leading to differential sensitivity to TRAIL (van Noesel et al, 2002). However, the link between TRAIL resistance and the expression of decoy receptors has not been clearly established in human cells (Griffith and Lynch, 1998). Interestingly, activation of death receptors such as TRAIL-R1 and TRAIL-R2 also stimulated the NF-κB pathway (Chaudhary et al, 1997; Schneider et al, 1997). Under these circumstances, the reason(s) for cells undergoing apoptosis despite the induction of anti-apoptotic pathways through the same death receptors is not fully understood.

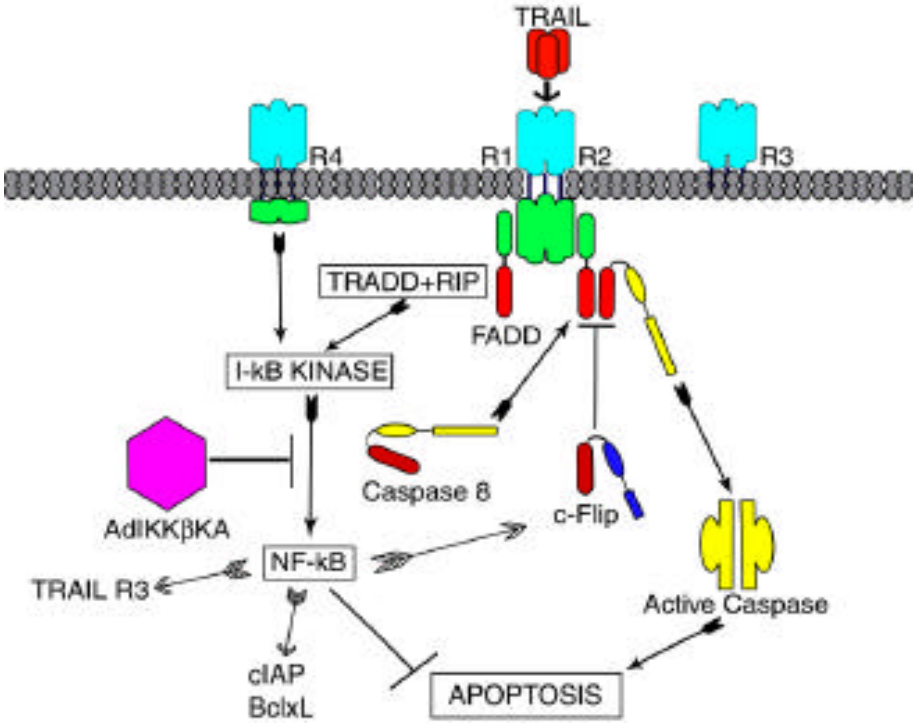

Figure 1: A gene therapy strategy to block anti-apoptotic NF-κB signaling pathway to induce TRAIL sensitivity in prostate cancer cells. Activation of TRAIL receptor 1 (R1) or 2 (R2) by trimeric TRAIL ligands leads to the recruitment of Fas associated death domain protein (FADD) to the membrane. Then, FADD recruits procaspase 8 to form death inducing signaling complex (DISC). DISC induced signaling activates caspase pathway inducing cells into apoptosis. TRAIL receptor 3 (R1) and 4 (R4) serve as decoy receptors. R4 activates NF-κB signaling pathways as well. In addition, NF-κB pathway is also activated by R1 and R2 via TNFR-associated death domain protein (TRADD) and receptor interacting protein (RIP). Consequently, NF-κB activation augments expressions of various anti-apoptotic genes such as cIAP, BclxL and cFlip in addition to R3. c-Flip, a procaspase 8 homologue, competes with procaspase 8 for binding to FADD. Thereby it inhibits apoptotic signaling. The expression of adenovirus delivered IKKβKA mutant prevented the activation of anti-apoptotic NF-κB signaling. This method sensitized prostate cancer cells to TRAIL.

The second hypothesis claims the presence of apoptosis inhibitory substances in these cells. Such a molecule, cFLIP (FLICE Inhibitory Protein), a caspase 8 homologue, has been shown to obstruct death ligand induced apoptosis (Irmler et al, 1997; Griffith et al, 1998). Intriguingly, NF-κB activating agents up-regulated cFLIP synthesis (Kreuz et al, 2001). Furthermore, the NF-κB pathway has been proven to increase TRAIL-R3 synthesis, a decoy receptor for TRAIL, (Bernard et al, 2001) and the expression of apoptosis inhibitor Bcl-xL (Hatano and Brenner, 2001; Ravi et al, 2001) resulting in the obstruction of TRAIL mediated apoptosis. Apoptosis inhibitors such as cIAP are also activated by NF-κB pathways (Mitsiades et al, 2002). Based on these results, we can clearly state that the active NF-κB signaling pathway may provide cells with TRAIL resistance by at least four different ways (**Figure 1**). Additionally, it has been reported that a novel tumor suppressor gene, PTEN/MMAC1 (Steck et al, 1997; Simpson and Parsons, 2001) negatively regulated TNF induced NF-κB activity (Ozes et al, 1999; Mayo et al, 2002) through the IKK complex (Gustin et al, 2001). The observation in which IKK activity is required for PI3K-Akt induced NF-κB activation (Burow et al, 2000; Demarchi et al, 2001) confirmed this report (Madrid et al, 2001; Sizemore et al, 2002). Due to a negative correlation between the expression of PTEN and the progression of prostate cancer, advanced prostate cancer cells might have intrinsically higher NF-κB activity due to the progressive loss of PTEN. Absence of PTEN function may result in increased Akt activity induced by PI3K. Since NF-κB is a downstream target for Akt, (Kane et al, 1999; Romashkova and Makarov, 1999; Andjelic et al, 2000; Jones et al, 2000) TRAIL resistance would ultimately be ensured in cells by way of the NF-κB pathway. In agreement with this hypothesis, PTEN sensitized prostate cancer cells to TRAIL induced apoptosis (Yuan and Whang, 2002). Thus, these possible scenarios make NF-κB inhibiting vectors such as Ad.IKKβKA (Sanlioglu et al, 2001a) or Ad.IκBαSR (Batra et al, 1999; Sanlioglu and Engelhardt, 1999) ideal candidates for overcoming the TRAIL resistance in PTEN mutant prostate cancer cells. In a similar manner, TNF induced apoptosis can also be prevented by NF-κB activation as reported (Beg and Baltimore, 1996; Van Antwerp et al, 1996). Previously, NF-κB inhibiting approaches such as adenovirus mediated transfer of IKKβ (Ad.IKKβKA) (Sanlioglu et al, 2001a, 2001b) or IκBα (Ad.IκBαSR) (Batra et al, 1999; Sanlioglu and Engelhardt, 1999) dominant negative mutants were successfully deployed in order to sensitize lung cancer cells to TNF. Since some tumor cells have intrinsically high NF-κB activity, which might be responsible for TRAIL resistance, NF-κB blocking agents can potentially be useful to overcome TRAIL resistance. For example, a constitutive NF-κB activation was observed in renal carcinoma (Oya et al, 2001). Not surprisingly, melanoma cells having a constitutive NF-κB

activity exhibit TRAIL resistance (Franco et al, 2001). Resistant melanoma cells were sensitized to TRAIL either with proteasome inhibitors or transfections with plasmids encoding degradation resistant IκBα protein (Franco et al, 2001). In accordance with these studies, we have tested if adenovirus mediated NF-κB inhibiting approach would sensitize prostate cancer cells to TRAIL. Consequently, adenovirus mediated delivery of IKKβKA mutant (Ad.IKKβKA) sensitized PTEN mutant prostate cancer cells (PC3) to TRAIL as shown in **Figure 2**. At first, PC3 cells appeared to be relatively resistant to pro-apoptotic effects of TRAIL when cells were infected with adenovirus vector encoding hTRAIL (Ad.hTRAIL) even at an MOI of 1000 DNA particles/cell (**Figure 2 Panel A**). Infection with Ad.IKKβKA vector alone did not yield any cell death either (**Figure 2, Panel B**). However, when the dose of Ad.hTRAIL vector was kept constant at an MOI of 1000 DNA particles/cell, increasing the amount of Ad.IKKβKA construct sensitized PC3 cells to TRAIL mediated apoptosis (**Figure 2, Panel C**).

C. Intracellular proapoptotic regulators

Although caspases are the effector mediators of apoptosis, the expression of proapoptotic molecules such as procaspase 3 or 7 using adenovirus constructs did not induce apoptosis in prostate cancer cells due to the inability of these caspases to undergo autocatalytic activation (Li et al, 2001). A novel suicide gene therapy approach was developed using chemically inducible effector caspases to trigger apoptosis in prostate cancer cells. Cell death was mediated by replication-deficient adenoviral vector expressing conditional caspase-1 (Ad-G/iCasp1) or caspase-3 (Ad-G/iCasp3) and the caspase activation was achieved by nontoxic, lipid-permeable, chemical inducers of dimerization (CID) (Shariat et al, 2001). Aggregation and activation of these recombinant caspases occurred, leading to rapid apoptosis only after vector transduction followed by CID administration in both human (LNCaP and PC-3) and murine (TRAMP-C2 and TRAMP-C2G) prostate cancer cell lines. Subcutaneous TRAMP-C2 tumors displayed focal but extensive apoptosis following direct injection of Ad-G/iCasp1 in vivo. In order to express caspase 9 exclusively in prostate, a recombinant adenovirus carrying iCaspase-9 was constructed with two copies of the androgen response region (ARR) placed upstream of the probasin promoter elements (ADV.ARR(2)PB-iCasp9) (Xie et al, 2001b). AP20187 is a chemical dimeric ligand, which causes dimerization and thereby activation of iCaspase-9 leading to rapid apoptosis in both dividing and nondividing cells. Testing of ADV.ARR(2)PB-iCasp9 construct in LNCaP tumor xenografts demonstrated that this construct induces apoptosis in prostate cancer cells only in the presence of AP20187.

The proapoptotic members of Bcl- 2 protein family including Bax, Bak, Bad, and Bik also mediate apoptosis. Apoptosis-inducing proteins were cloned into adenovirus constructs and shown to induce apoptosis in prostate cancer cell lines previously.

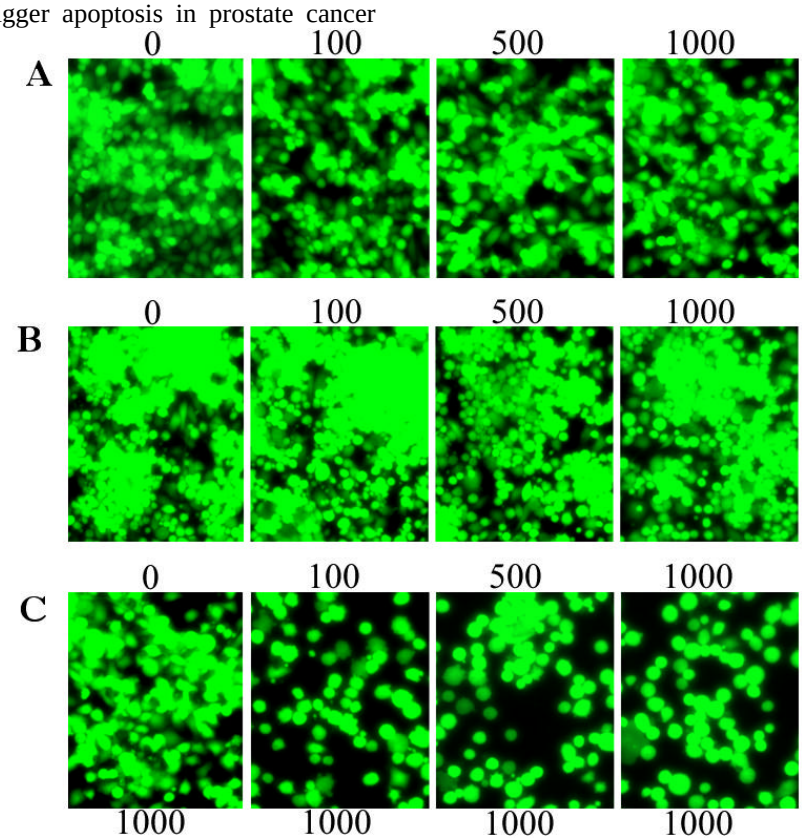

Figure 2. Adenovirus mediated IKKβKA expression sensitized PC3 cells to TRAIL mediated apoptosis. PC3 cells were infected with increasing MOIs of either Ad5hTRAIL (Panel A) or Ad.IKKβKA (Panel B). In panel C, the dose of Ad.IKKβKA vector was increased gradually (stated just above each panel) while the amount of Ad5hTRAIL was kept constant (as indicated under the panel). Cell death was detected using molecular probe's Live and Death Cellular viability and toxicity kit 48 hours following infection. Numbers indicate viral doses as MOI values of DNA particles/cell.

However, overexpression of proapoptotic genes without the use of tissue specific promoters could result in unwanted apoptosis even in normal cells. In order to provide tissue specificity, an adenoviral construct was generated containing Bax cDNA under control of the probasin promoter that included two androgen response elements (Av-ARR2PB-Bax). Av-ARR2PB-Bax construct drove Bax overexpression in an androgen-dependent way in androgen receptor (AR)-positive cell lines of prostatic origin but not in others. The androgen dihydrotestosterone activated apoptosis in LNCaP cells infected with Av-ARR2PB-Bax but not in those infected with control vectors. These results demonstrated that Av-ARR2PB-Bax induced apoptosis was androgen dependent and limited to AR positive cells of prostatic epithelium. On the other hand, using a binary co-transfection strategy involving Ad/GT Bax and Ad/PGK-GV16; overexpression of proapoptotic Bax protein induced apoptosis both in androgen-insensitive (DU145 and PC3), and androgen-sensitive (LNCaP) cell lines (Honda et al, 2002). The same binary approach was tested to assess the consequences of Bcl-2 overexpression in the progression of prostate carcinoma leading to apoptosis-resistant and androgen-independent phenotype in DU145, PC3 and LNCaP cell lines which represent models of advanced prostate carcinoma. Bax expression generated by the adenoviral co-transfection system induced apoptosis even in these Bcl-2 overexpressing cell lines. These results suggest that the Ad/GT Bax and Ad/PGK-GV16 combined expression system might represent a powerful gene therapy strategy for the treatment of androgen-independent and apoptosis-resistant prostate carcinoma. Moreover, monogene and polygene approaches were compared in an experimental prostate cancer model using apoptotic genes bad and bax driven by a prostate specific promoter (ARR(2)PB) in an adenovirus construct (Zhang et al, 2002b). The ARR(2)PB is a dihydrotestosterone (DHT)-inducible third-generation probasin-derived promoter. In this study, animals bearing tumors of prostatic origin responded better to combined bad and bax therapy than either of the vectors alone. Therefore, it was concluded that polygene therapy involving more than one apoptotic molecule is more effective in xenograft models of androgen-dependent or independent prostate cancer than monogene therapy alone. It is also known that overexpression of anti-apoptotic genes such as Bcl-2 in prostate carcinoma provides resistance to radiation therapy and androgen ablation. A second-generation adenoviral vector (ARR2PB.Bax.GFP) was constructed with the modified prostate-specific probasin promoter (ARR2PB) directing the expression of a HA-tagged Bax gene in order to restore the balance of Bcl-2 family members to induce apoptosis in prostate cancer cells (Lowe et al, 2001). ARR2PB.Bax.GFP vector induced significant levels of apoptosis in LNCaP cells 48 hours following infection even in the presence of high levels of Bcl-2 protein. No toxicity in liver, lung, kidney, and spleen was detected by systemic administration of ARR2PB.Bax.GFP in nude mice. Therefore, a second-generation adenovirus-mediated, prostate-specific Bax gene therapy appeared to be a very safe and efficient approach for the treatment of prostate cancer. Another member of the proapoptotic Bcl-2 family, namely "Bik", was cloned into adenovirus vectors to explore its therapeutic potential. AdBik infection also induced apoptosis and suppressed the growth of PC-3 xenografts established in nude mice (Tong et al, 2001).

Several other genes were also tested for their ability to induce apoptosis in prostate tumor cell lines as well as in xenograft models. The antiapoptotic protein CLN3 negatively regulates endogenous ceramide production, an inducer of apoptotic cell death. CLN3 protein is overexpressed in most of the cancer cell lines tested including those of prostate (Du145, PC-3, and LNCaP). An adenovirus-expressing antisense CLN3 (Ad-AS-CLN3) blocked CLN3 protein expression in prostate cancer cell lines as demonstrated by Western Blotting (Rylova et al, 2002). Ad-AS-CLN3 infection resulted in the inhibition of cell growth and reduction in cell viability of cancer cells through elevation of endogenous ceramide production. This study revealed CLN3 as a novel target to induce apoptosis in prostate cancer cells. A recombinant adenovirus containing pHyde cDNA gene (AdpHyde), a novel gene cloned from Dunning rat prostate cancer cells, was constructed in order to study its function (Zhang et al, 2001). Surprisingly, the AdpHyde construct inhibited the growth of human prostate cancer cells and induced apoptosis involving the caspase-3 pathway in human prostate cancer tumor xenografts in nude mice. Ionic movement also influences apoptosis. For instance, K^+ efflux is an early event in apoptosis, which is regulated by K^+ channel-associated protein (KChAP). A recombinant adenovirus encoding KChAP (Ad/KChAP) was constructed in order to determine if KChAP expression could induce apoptosis in prostate cancer cells (Wible et al, 2002). The LNCaP cell line displayed a reduction in cell size upon infection with Ad/KChAP. The Ad/KChAP construct also induced apoptosis in DU145 cells in a p53 independent manner. In addition, infection with Ad/KChAP prevented growth of DU145 and LNCaP tumor xenografts in nude mice.

VII. Tumor suppressor genes

Aberrations in the expression of tumor suppressor genes have been one of the key factors affecting the outcome of cancer therapy. Several studies examined the possible use of tumor suppressor genes as therapeutic agents for prostate cancer. Doxorubicin (Dx) is a commonly used chemotherapeutic agent in recurrent prostate cancer and is a strong inducer of p53 expression leading to p21(CIP1/WAF1) transactivation. As suggested by previous reports, p21 plays a role in the modulation of chemotherapy-induced apoptosis, prostate cancer progression and androgen regulation. Two androgen-regulated human prostate cancer cell lines (MDA PCa 2b and LNCaP) were exposed to Dx and growth factor withdrawal in order to investigate if p21 plays a role in the survival of prostate cancer cells under stress (Martinez et al, 2002). Infection with adenovirus vectors encoding the antisense strand of p21 reduced p21 levels, sensitized prostate cancer cells to Dx and facilitated apoptosis in response to growth factor withdrawal. These results suggest that modulation of p21 pro-survival gene

expression via adenovirus constructs sensitizes prostate cancer cells to chemotherapeutics and androgen withdrawal. Another tumor suppressor protein, p27, also known as cyclin-dependent kinase inhibitor (CDKI), is normally expressed in human prostate. However, the majority of human prostate cancers have reduced levels of p27. The down regulation of this putative tumor suppressor gene through proteolysis is mediated by SCFSKP2 ubiquitin ligase complex. Adenovirus-mediated overexpression of SKP2 induced ectopic down-regulation of p27 in LNCaP prostate carcinoma cells (Lu et al, 2002). This observation confirmed that SKP2 activity was the major determinant of p27 levels in human prostate cancer cells. Based on in vitro studies, it is believed that the overexpression of SKP2 might be one of the mechanisms allowing prostate cancer cells to escape growth control mediated by p27. Therefore, knocking out SKP2 function would be a logical novel approach to fight prostate cancer. In another study, an adenovirus construct carrying p27 coding sequences Adp27(Kip1) was generated to assess whether the overexpression of p27 has any affect on the prostatic tumor growth in vivo (Katner et al, 2002). Injection of Adp27(Kip1) vector reduced the growth of LNCaP tumor xenografts in mice. This study supported the idea that Adp27(Kip1) can serve as a potential therapeutic vector for the treatment of prostate carcinoma.

p14(ARF), encoded by the human INK4a gene locus, is another tumor suppressor protein which is frequently inactivated in human cancer. p14(ARF) has recently been implicated in p53-independent cell cycle regulation and apoptosis. A replication-deficient adenoviral construct carrying p14(ARF) coding sequence (Ad-p14(ARF)) was generated in order to explore the pro-apoptotic function of p14(ARF) in relationship to p53 function (Hemmati et al, 2002). Ad-p14(ARF) construct induced apoptosis in p53/Bax-mutated DU145 prostate cancer cells and HCT116 cells lacking functional Bax expression. This study demonstrated that overexpression of p14 through adenovirus vectors is sufficient to induce apoptosis in p53- and bax-deficient prostate cancer cells. Prostate carcinoma with p53 mutant phenotype represents a clear obstacle for irradiation therapy. Ionizing radiation (IR) and adenoviral p53 gene therapy (Ad5CMV-p53) were utilized individually as well as in combination in order to assess the effectiveness of combined therapy for prostate cancer (Sasaki et al, 2001). In this study, IR alone did not induce significant levels of apoptotic cell death in DU145 and PC-3 cells. However, after combined therapy, the proportion of apoptotic cells was greatly amplified in both of the cell lines tested. Therefore, it was concluded that the observed synergistic effect might be useful for the treatment of radio-resistant prostate carcinoma.

The loss of MMAC/PTEN tumor suppressor gene expression is frequently detected in human tumors. Survival signaling through the phosphatidylinositol-3 kinase/Akt pathway is constitutively activated in cells lacking functional PTEN expression. Therefore, the functional effect of MMAC/PTEN expression was examined in LNCaP cells, which are devoid of a functional PTEN product (Davies et al, 1999). Infection with an adenovirus construct driving the expression of MMAC/PTEN resulted in a specific inhibition of Akt/PKB activation. This is consistent with the phosphatidylinositol phosphatase activity of MMAC/PTEN. Compared to adenovirus delivered p53 expression, MMAC/PTEN expression induced apoptosis in LNCaP cells to a lesser extent. Interestingly, the growth suppression properties of MMAC/PTEN were significantly greater than those accomplished with p53. Moreover, Bcl-2 overexpression in LNCaP cells blocked both the adenovirus mediated MMAC/PTEN- and p53-induced apoptosis, but it did not affect the growth-suppressive properties of MMAC/ PTEN. This is consistent with the fact that MMAC/PTEN may play multiple roles in the cell. Prostate cells were infected with adenovirus vector carrying PTEN coding sequence in order to determine if supplying PTEN function would sensitize these cells to various apoptotic stimuli (Yuan and Whang, 2002). As predicted, adenovirus-mediated PTEN delivery sensitized LNCaP prostate cancer cells to apoptosis through the inhibition of constitutive Akt activation. Since PTEN G129E mutant lacking lipid phosphatase activity was unable to sensitize cells to apoptosis, it was concluded that the lipid phosphatase activity of PTEN was required for apoptosis. The therapeutic effect of adenoviral delivery of MMAC/PTEN was tested on both the in vitro and in vivo growth of PC3 human prostate cancer cells (Davies et al, 2002). The in vitro growth of PC3 cells was repressed by adenovirus expression of MMAC/PTEN via blocking of cell cycle progression. Although this approach did not inhibit the tumor progression of orthotopically implanted PC3 cells, a significant reduction was observed in the tumor size in vivo, in addition to complete inhibition of metastases. Therefore, it was suggested that MMAC/PTEN might play a role mostly in the regulation of the metastatic potential of prostate cancer.

A considerable fraction of prostate tumors display an alteration of Mxi1 expression, an antagonist to c-Myc. This was confirmed by transgenic approaches in which prostatic hyperplasia was observed in mice deficient for Mxi1. Mxi1-expressing adenovirus (AdMxi1) was generated to study the ability of Mxi1 to act as a growth suppressor in prostate tumor cells (Taj et al, 2001). Overexpression of Mxi1 using adenovirus vectors in the DU145 prostate carcinoma cell line resulted in growth arrest and decreased colony formation on soft agar. All these studies emphasize that the modulation of tumor suppressor gene function might be necessary for an optimum therapeutic response to fight against prostate cancer.

VIII. Cell adhesion molecules and anti-angiogenic approaches

Cell adhesion molecules play major roles especially in metastasis of cancer cells. Therefore, aberrant expression patterns of cell adhesion molecules are frequently associated with poor prognosis. For instance, the expression of a well-known cell adhesion molecule, C-CAM1, is downregulated during the early stages of prostate carcinoma in an animal model (TRAMP) (Pu et al, 1999). C-CAM1 was cloned into an adenovirus

construct and its efficacy was tested both in vitro and in vivo using PC3 xenograft murine model (Lin et al, 1999). AdC-CAM1 construct manifested a strong antitumoral activity on PC3 tumor cells grown in nude mice. Therefore, selective use of cell adhesion molecules might be beneficial for the treatment of prostate carcinoma. Moreover, combining C-CAM1-based therapy with TNP-470, a potent angiogenesis inhibitor, induced greater growth suppression on DU145 tumor xenografts than by either Ad-C-CAM1 or TNP-470 application alone (Pu et al, 2002).

Vascularization of a solid tumor is required for cancer growth. Recently, preventing vascularization through inhibition of angiogenesis was a popular target for cancer gene therapy. For example, a 16-kDa prolactin protein (PRL) has previously been shown to possess an antiangiogenic activity (Galfione et al, 2003). Not surprisingly, adenovirus delivery of PRL protein manifested a significant antitumoral activity in vivo (Kim et al, 2003). In addition, vascular endothelial growth factor (VEGF) receptor signaling is another relevant pathway, which modulates the vascularization of newly growing tumors. Interfering with such a signaling pathway might be valuable in controlling the tumor growth. In fact, when fused to an Fc domain and cloned into the recombinant adenovirus construct, the ligand-binding ectodomain of VEGF receptor 2 (Flk1) manifested a considerable reduction in tumor growth induced by a drastic decline in the microvessel density in SCID mice carrying human LNCaP xenografts (Becker et al, 2002).

Growth factors are needed for survival of cancer cells and molecular chaperones are required for functional production of these molecules. A new member of the heat shock protein family functioning as a molecular chaperone in the endoplasmic reticulum was recently discovered and named as 150-kDa oxygen-regulated protein (ORP150). Since prostate cancer cells exhibited an upregulation of ORP150 protein and VEGF, adenovirus delivery of an antisense ORP150 cDNA approach was used to reduce angiogenicity and tumorigenicity through inhibition of VEGF secretion. This approach indeed suppressed the growth of DU145 prostate carcinoma cell line in a xenograft model (Miyagi et al, 2002).

IX. Replication competent adenovirus vectors

Replication competent adenoviral vectors provide powerful means to kill cancer cells through cell lysis. Since they only replicate in tumor cells, the therapeutic range is limited to cancer cells. Two replication-competent adenoviruses, CV706 and CV787, were generated in order to selectively destroy PSA producing prostate cancer cells. It has been demonstrated earlier that prostate-specific antigen (PSA)-selective replication-competent adenovirus variant CV706 specifically eliminated tumors in human prostate cancer xenografts in preclinical models (Rodriguez et al, 1997). Since adenovirus E1A is known to be a potent inducer of chemosensitivity and radiosensitivity through p53-dependent and independent mechanisms, the potential radiosensitizing effects of CV706 on prostate cancer cells were evaluated (Chen et al, 2001). The CV706 construct demonstrated a synergistic antitumoral effect both on irradiated human prostate cancer cells and tumor xenografts. Moreover, in order to investigate the safety and the functionality of intraprostatic delivery of CV706 for the treatment of patients with locally recurrent prostate cancer following radiation therapy, a Phase I dose-escalation study was conducted (DeWeese et al, 2001). Results from this study suggested that even at high doses, intraprostatic delivery of the CV706 was relatively safe for patients and CV706 construct demonstrated high therapeutic activity as reflected by the reduction in serum PSA. This was the first clinical trial of a prostate-specific, replication-restricted adenovirus for the treatment of prostate cancer. Another prostate-specific replication-competent adenovirus carrying not one, but two, cell type specific promoters (CV787) was constructed. This construct contained E1B gene driven by the human prostate-specific enhancer/promoter and the adenovirus type 5 (Ad5) the E1A gene under the control of prostate-specific rat probasin promoter. The Ad5 E3 region was also conserved in the vector to improve the efficacy. A single tail vein injection of CV787 eliminated LNCaP xenografts within 4 weeks in nude mice (Yu et al, 1999). When the prostate cancer-specific adenovirus CV787 was combined with chemotherapeutic agents like taxanes (paclitaxel and docetaxel), a synergistic antitumoral effect was observed in mice carrying human prostate cancer xenografts (Yu et al, 2001b).

Heat-inducible gene expression is another approach used in the context of suicide gene therapy. A recombinant adenovirus containing the CD-TK fusion gene controlled by the human inducible heat shock protein 70 promoter (Ad.HS-CDTK) was generated for this purpose. Heat application at 41^{o}C for 1 hour induced therapeutic gene expression from this vector. Despite the fact that the Ad.HS-CDTK construct induced CD-TK expression in human prostate cancer cells, a therapeutic benefit was not observed due to lower transduction efficiency of tumors in vivo. Instead, a replication-competent, E1B-attenuated adenoviral vector containing the hsp70 promoter-driven CD-TK gene (Ad.E1A^{+}HS-CDTK) was generated to increase CD-TK gene expression to achieve a therapeutic effect (Lee et al, 2001). Contrary to replication incompetent Ad.HS-CDTK, replication competent Ad.E1A^{+}HS-CDTK construct yielded severe cytotoxicity and greater levels of therapeutic index in the presence of prodrugs. This approach revealed the beneficial effects of using replication competent virus complemented with a heat inducible suicide gene therapy approach for prostate carcinoma.

X. Adenovirus vectors with cell type specific and inducible promoters

Even though adenovirus-mediated HSVTK suicide gene therapy approach manifested a satisfactory toxicity profile in Phase I clinical trials, the toxicity studies using adenovirus vectors were very restricted in numbers.

However, it was known that the promoter of choice might influence the level of toxicity. In order to study the promoter effect on adenovirus mediated toxicity the mouse caveolin 1 promoter was cloned into the adenovirus HSV-tk vector (Adcav-1tk) because this promoter was highly active in metastatic and androgen-resistant prostate cancer cells (Pramudji et al, 2001). The efficacy of this vector for suicide gene therapy was compared to those of AdHSV-tk vectors carrying either cytomegalovirus (AdCMV-tk) or rous sarcoma virus (AdRSV-tk) promoters in mice transplanted with mouse prostate cancer cells. Following GCV administration, all the HSV-tk expressing vectors regressed the tumor growth in situ. Interestingly, the efficacy of Adcav-1tk vector was much greater in terms of inducing necrosis and microvessel density. In order to evaluate the toxicity profile of adenovirus vectors carrying CMV, RSV or mouse caveolin promoter-driven HSV-tk transgenes, these vectors were also injected systemically into mice (Ebara et al, 2002). Adenovirus vectors with CMV and RSV promoters, but not caveolin promoter, exhibited significant levels of liver damage. These results suggested that the promoter selection greatly influences the toxicity profile of adenovirus-mediated suicide gene therapy approach. In order to increase the number of promoters available for prostate specific gene expression, transgenic mice were generated expressing a reporter gene (SV40 Tag) directed by prostate secretory protein of 94 amino acids (PSP94) (Gabril et al, 2002). PSP94 gene promoter/enhancer region directed SV40 Tag expression exclusively in prostate leading to prostatic intraepithelial neoplasia and eventually to high-grade prostate carcinoma. These studies suggested that this PSP94 gene promoter/enhancer strategy could be employed for the treatment of prostate carcinoma.

One conventional way to limit the toxicity of virus mediated suicide gene therapy is to use cell type specific promoters as suggested above. Although adenovirus vectors with the native PSA enhancer and promoter (PSAP) provided prostate-specific expression, lower transcriptional activity observed in prostate challenged its use in prostate-targeted gene therapy. To improve the activity and specificity of the prostate-specific PSA enhancer for gene therapy, various studies were carried out by exploring the properties of the natural PSA control regions. Chimeric PSA enhancer constructs were generated with tandem copies of the proximal ARE elements and then inserted into adenovirus constructs (Ad-PSE-BC-luc) (Wu et al, 2001). This construct was highly inducible with androgens as shown by systemic administration into SCID mice carrying LAPC-9 human prostate cancer xenografts while retaining prostate specific gene expression. Furthermore, the CreLoxP system was also utilized to enhance the activity of PSAP. CD suicide gene therapy approach using adenoviral vectors with CRELoxP augmented PSAP activity effectively inhibited subcutaneous LNCaP tumor growth in nude mice (Yoshimura et al, 2002). In addition, hormone refractory prostate cancer cells retain the expression of prostate-specific membrane antigen (PSMA) and prostate-specific antigen (PSA). An adenovirus construct with an artificial chimeric enhancer (PSES) composed of two modified regulatory elements of PSA and PSMA genes (Ad-PSES-luc) was generated and tested for its promoter activity for the treatment of prostate cancer (Lee et al, 2002a). Systemic injection of Ad-PSES-luc construct into mice produced very low levels of reporter gene expression in major organs. However, when injected directly into prostate, only the prostate but not other tissues produced high levels of reporter gene expression. These results encouraged the use of PSES for the treatment of androgen-independent prostate carcinoma. Even though prostate-specific antigen (PSA/hK3) provided prostate specific gene expression, its expression displayed an inverse correlation with prostate cancer grade and stage, giving reason to doubt its effectiveness for advanced stage of prostate carcinoma. A new approach was developed in order to generate gene therapy vectors targeting higher grades especially of prostate carcinoma. The human glandular kallikrein 2 (hK2) is upregulated in an advanced form of prostate cancer with a higher grade. Therefore the hK2 promoter was cloned into adenovirus construct in combination with EGFP reporter gene (ADV.hK2-E3/P-EGFP) in order to obtain preferential expression of EGFP in prostate cancer (Xie et al, 2001a). Indeed ADV.hK2-E3/P-EGFP injection led to a robust but tumor-restricted EGFP expression in subcutaneously generated LNCaP tumors. These results showed that adenovirus constructs with the hk2 multienhancer/promoter driven therapeutic genes might be a powerful tool for gene therapy of advanced prostate cancer.

Previous studies have shown that the bone matrix protein osteocalcin is predominantly expressed in prostate cancer epithelial cells, fibromuscular stromal cells and osteoblasts. A conditional replication competent adenovirus vector carrying the osteocalcin promoter driven early E1A gene (AdOCE1A) was generated to co-target both prostate cancer cells and their surrounding stromal cells (Matsubara et al, 2001). Both PSA-producing (LNCaP) and non-producing (DU145 and PC3) human prostate cancer cell lines as well as human stromal cells and osteoblasts were effectively killed by this recombinant virus in vitro. In addition a single systemic intravenous injection of the AdOCE1A construct significantly destroyed prostate tumor cells transplanted in SCID mice. This co-targeting strategy appeared to have a broader effect compared to other recombinant constructs tested on the preclinical models of human prostate cancer. These promising results initiated first gene therapy trial (phase I) in which adenoviruses carrying the osteocalcin promoter driven HSV-tk gene (AdOCHSVTK) were directly injected into prostate cancer lymph node and bone metastasis (Kubo et al, 2003). The results of this trial suggested that adenoviruses did not display any adverse effects and the treatment was well tolerated in all patients. In addition, 63 % of the patients had local cell death in treated lesions. Further studies are suggested in order to assess the efficacy of this approach for androgen-independent prostate carcinoma. A new treatment modality to enhance adenoviral replication by vitamin D_3 in androgen-independent human prostate cancer cells and tumors was tested using a novel replication-competent adenoviral vector, Ad-hOC-E1, carrying the human

osteocalcin (hOC) promoter to drive both the early viral E1A and E1B genes (Hsieh et al, 2002). While the replication properties of Ad-hOC-E1 vector were restricted to OC-expressing cells, vitamin D_3 exposure further enhanced viral replication by 10 fold. The growth of both androgen-dependent and androgen-independent prostate cancer cells was suppressed by Ad-hOC-E1 infection, irrespective of the cells' androgen responsiveness and PSA status. This is in contrast to Ad-sPSA-E1 vector, which only replicated in PSA-expressing cells with androgen receptor (AR). Ad-hOC-E1 injection inhibited the growth of DU145 (an AR and PSA-negative cell line) tumor xenografts in mice. Consequently, vitamin D_3-enhanced Ad-hOC-E1 viral replication represented an alternative for the treatment of localized or osseous metastatic prostate cancer. Prostate specific antigen promoter (PSAP) and rat probasin (rPB) promoter are currently employed to drive the therapeutic transgene expression in prostate cancer cells. However, since these promoters require the binding of androgen to androgen receptor for activation, they were only functional in androgen-dependent prostate carcinoma cells. Because androgen refractory prostate carcinoma cells lose the expression of androgen receptor along the way, constructs with PSAP or rPB promoters are not useful for treating patients with androgen-independent prostate carcinoma. In order to circurment this problem, prostate specific promoters were modified so that they were activated in response to the retinoids-retinoid receptor complex in place of the androgen-AR complex. As a result, retinoid treated androgen-independent prostate cancer cells were sensitized to HSVTK-ganciclovir gene therapy using promoters responding to retinoids (Furuhata et al, 2003).

Apart from promoters providing tissue specific gene expression, expression inducible promoters were cloned into adenovirus constructs to control the onset and the duration of gene expression. Tetracycline-inducible adenovirus vectors expressing the cytokine interleukin-12 were successfully tested in an immunotherapy model for prostate cancer (Nakagawa et al, 2001). Thus, recombinant adenovirus vectors with tetracycline-inducible gene expression opened up new avenues while improving the safety of viral vector administration for cancer gene therapy. Limitation of cytotoxic gene expression only to tumor cells is very much desired in adenovirus-mediated gene therapy approach for cancer. Unfortunately, the expression levels of many tumor and tissue-specific promoters are much lower than the constitutively active promoters. A complex adenoviral vector was generated by fusing the tetracycline transactivator gene to a prostate-specific ARR2PB promoter while placing a mouse FASL-GFP fusion gene under the control of the tetracycline responsive promoter. This allowed the joining of cell-type specificity with high-level regulation of transgene expression (Rubinchik et al, 2001). The doxycycline regulated, ARR2PB driven FASL-GFP vector generated higher levels of prostate-specific FASL-GFP expression than FASL-GFP expression directed with ARR2PB alone, leading to apoptosis in LNCaP cells. Systemic delivery of both the prostate-specific and the prostate-specific/tet-regulated vectors was well tolerated in animals at doses that were lethal for adenovirus vectors with CMV-driven FASL-GFP expression. This approach improved the safety and efficacy of adenovirus-mediated cytotoxic gene delivery for the treatment of prostate carcinoma.

The prostate-specific adenovirus gene expression technology can also be used for the identification of metastatic lesions of prostate cancer through the use of non-invasive imaging. A prostate-specific adenovirus vector expressing a luciferase reporter gene (AdPSE-BC-luc) and a charge-coupled device-imaging system were employed for this purpose (Adams et al, 2002). A robust expression from AdPSE-BC-luc construct was found in the prostate, especially in the androgen-independent tumors. Furthermore, metastatic lesions in the lung and spine with prostatic origin were identified successfully through repetitive imaging over a three-week period after AdPSE-BC-luc injection into tumor-bearing mice. These results demonstrate that adenovirus gene delivery specific to the prostate can be coupled to a non-invasive imaging modality for therapeutic and diagnostic strategies for prostate cancer.

XII. Adenovirus vectors for vaccination and adjuvant gene therapy

CAR receptors and MHC class I heavy chains are important mediators of adenovirus entry into tumor cells. Contrary to the cell lines derived from other malignancies, down regulation of CAR or MHC class I expression is relatively rare in both human and murine prostate carcinoma cells. This brought the possibility of developing vaccine strategies for prostate cancer based on the modification of prostate cancer cells using recombinant adenovirus vectors (Pandha et al, 2003). The expression of prostate-specific antigen (PSA) is highly restricted to prostatic epithelial cells. In fact, 95 % of patients with prostate carcinoma express PSA, making this antigen a good candidate for targeted immunotherapy. A recombinant PSA adenovirus type 5 (Ad5-PSA) was generated in order to activate PSA-specific T-cell response with the potential of eliminating prostate cancer cells (Elzey et al, 2001). Ad5-PSA immunized mice displayed a PSA-specific cellular immunity involving $CD8^+$ T lymphocytes. This approach deterred subcutaneous tumor formation with RM11 prostate cancer cells expressing PSA (RM11psa). However, this did not affect the growth of existing RM11psa tumors. On the contrary, Ad5-PSA administration followed by intratumoral injection of recombinant canarypox viruses (ALVAC) encoding interleukin-12 (IL-12), IL-2, and tumor necrosis factor-α effectively eliminated established RM11psa tumors.

Surgery is one of the conventional treatment modalities used against solid tumors. Due to the fact that minor residual tumors following surgical operation may result in local recurrence, surgery is neither efficient nor plausible for the treatment of metastatic disease. Although AdHSV-tk gene therapy followed by ganciclovir administration has been evaluated extensively as a potential treatment modality for numerous tumors, it has not yet been proven to achieve a complete cure on its own.

Prostate-derived tumor models were used to evaluate the effects of AdHSV-tk gene therapy as an adjuvant to surgery (Sukin et al, 2001). Lung nodules of prostate cancer cells were generated by intravenous injection of tumor cells in order to evaluate systemic effects. Following resection of subcutaneous tumors, AdHSV-tk was delivered to the resection site. Toxicity, local tumor recurrence, survival, and lung nodule formation were evaluated in animals; increased survival and decreased recurrence accompanied by no systemic toxicity were observed. Adjuvant AdHSV-tk gene therapy resulted in a significant reduction in lung nodules as well. This study suggested that AdHSV-tk gene therapy might be beneficial as an adjuvant for patients undergoing surgical treatment of cancer.

XIII. Current progress to overcome rate-limiting steps in adenovirus-mediated gene therapy for prostate carcinoma

The success of adenovirus mediated gene therapy for prostate carcinoma is effected by several factors including the level of expression of the receptor which facilitates the entry of the viral vectors into the cells, penetration of transgenes to surrounding tissues, and finally the expression of the delivered gene. Enhancing these factors has been the focus of many laboratories working on adenovirus-mediated gene therapy for prostate carcinoma. Although a limited number of studies have been completed regarding these issues, effectiveness of prostate cancer gene therapy will certainly benefit from the progress in this field.

A. Receptor abundance

The presence of the coxsackie adenovirus cell surface receptor, CAR, is required for an effective adenovirus infection of target cells. CAR expression patterns of normal prostate and prostate carcinoma were compared using immunohistochemical approaches in order to assess the feasibility of adenovirus mediated gene therapy for prostate cancer (Rauen et al, 2002). While a robust membrane staining for CAR was detected in the metastatic prostate specimens with higher Gleason scores, just lumenal and lateral cell membrane staining were detected in the benign prostate epithelia. Therefore, adenovirus mediated gene delivery should be more effective for aggressive prostate tumors than it is for benign cases.

B. Penetration of hybrid therapeutic transgenes to the surrounding tissue

Despite the fact that adenovirus could transduce cells very efficiently in vitro, adenovirus mediated gene delivery is restricted by the inefficient transduction of surrounding cells for a given tumor. In order to overcome this obstacle, an important intercellular transport protein named VP22, was first fused to the therapeutic transgene of interest (p53 gene) and then cloned into adenovirus vector (Roy et al, 2002). Infection of p53 negative human prostate cancer cells (LNCaP) by this approach generated very efficient gene delivery of p53, inducing apoptosis not only in the infected cells but also in the surrounding uninfected cells.

C. Enhancement of transgene expression through transcriptional regulation

Although the use of prostate specific promoters is necessary to limit the transgene toxicity, the low level of transgene expression directed by these promoters represents a barrier to gene therapy. The observation, which led to the idea that chemotherapeutics enhanced the transgene expression from viral promoters, represented a new approach to overcome this barrier. Two recombinant adenovirus constructs were used to deliver p21WAF-1/CIP1 and p53 protein c-DNA under the control of cytomegalovirus promoter to the metastatic androgen independent prostate cancer cells treated with chemotherapeutic agents docetaxel or paclitaxel (Li et al, 2002b). Both chemotherapeutics appeared to enhance adenovirus mediated transgene expression in androgen independent prostate cancer cell lines. This increase in transgene expression was attributed to the enhancement of CMV promoter activity rather than the increased viral uptake. Therefore, the observed synergy of gene therapy with these chemotherapeutics may become useful when the transgene expression is a limiting factor for the treatment of the metastatic androgen independent prostate cancer. The possible use of other chemotherapeutic agents and their effect on prostate specific promoters should also be explored.

XIV. Summary of clinical trials

There are 636 clinical protocols involving 3496 patients employed in gene therapy worldwide as reported to the Journal of Gene Medicine website by the year 2002. 403 clinical studies (63.4 %) with regard to gene therapy for cancer were tested on 2392 (68.5 %) patients. Adenovirus was the vector of choice in 171 of these protocols (27 %), and 644 patients (18.4 %) received the adenovirus vector for gene therapy. 22 out of 171 clinical protocols were engaged in adenovirus mediated gene therapies targeting the prostate only as summarized in **Table 1**. 13 of these were reported to be in Phase I, 3 trials in Phase II and the rest (5) were in Phase I/II. There is no Phase III clinical study reported using adenovirus vectors targeting prostate yet. Some of the adenovirus mediated gene therapy approaches were complemented either with radiotherapy or radical prostatectomy. The percentage of the choice of gene therapy modalities targeting prostate is provided in **Figure 3**. The use of selectively replicating adenovirus constructs leads other approaches followed by suicide gene therapy. This is partly because not long ago astonishing results were obtained with selectively replicating adenovirus constructs in the preclinical animal models. It is also interesting to note that two of these clinical trials utilize suicide gene therapy in combination with the selectively replicating adenovirus approach

(**Figure 3**). No clinical studies have been carried out using the death ligand-mediated gene therapy approach and adenovirus vectors up to date. However we should not be surprised if such trials are being initiated and we encounter some of these in the near future. Although preliminary results are very encouraging from these clinical investigations, clear conclusions can be drawn only upon completion of these studies.

Considering all these preclinical and clinical studies, we concluded that great progress in adenovirus mediated gene therapy for prostate carcinoma has been made within the last 3 years. While the molecular mechanisms responsible for prostate carcinoma are not fully understood, the effectiveness of gene therapy is still quite amazing. As more data become available on the understanding of prostate carcinoma, we anticipate that more effective treatment modalities will be developed using adenovirus to target prostate cancer.

Table 1. A summary of ongoing clinical trials of adenovirus mediated gene therapy targeting prostate as of 2002. The data was collected from the Journal of Gene Medicine web site (www.wiley.co.uk/genmed/clinical) and published with the permission from ã John Wiley and Sons 2002.

Country	**Investigator**	**Mode of Therapy**	**Phase**
Canada	A. K. Stewart	Immunotherapy (IL-2)	I
Canada	J. Dancey	Immunotherapy (IL-2)	I
USA	Peter T. Scardino	Suicide gene therapy (HSV-tk) + radiotherapy	I
USA	Simon J. Hall	Neo-adjuvant suicide gene therapy (HSV-tk) + radical prostatectomy	I
USA	Arie Belldegrun	Tumor suppressor gene therapy (p53)	I
USA	Christopher J. Logothetis	Tumor suppressor gene therapy (p53)	I/II
USA	Dov Kadmon	Neo-adjuvant suicide gene therapy (HSV-tk) + radical prostatectomy	I
USA	Jonathan W. Simons	Selectively replicating adenovirus (CN706)	I
USA	Thomas A. Gardner	Suicide gene therapy (HSV-tk)	I
USA	Jae Ho Kim	Suicide gene therapy (CD/Tk) with selectively replicating adenovirus + radiotherapy	I
USA	E. Brian Butler	Suicide Gene Therapy (HSV-tk) + radiotherapy	I/II
USA	Jeffrey R. Gingrich	Neo-adjuvant CDK inhibitor (p16) + radical prostatectomy	I
USA	Martha K. Terris	Selectively replicating adenovirus (CV787) + Radiotherapy	I/II
USA	George Wilding	Selectively replicating adenovirus (CV787)	I/II
USA	Alan Pollack	Tumor suppressor gene therapy (p53) + radiotherapy	II
USA	Thomas A. Gardner	Selectively replicating adenovirus with osteocalcin promoter (Ad-OC-E1A)	I
USA	David M. Lubaroff	Immunotherapy (PSA)	I
USA	Brian J. Miles	Immunotherapy (IL-12) + radiotherapy	I
USA	Theodore L. DeWeese	Selectively replicating adenovirus (CV706)	II
USA	Eric J. Small	Selectively replicating adenovirus (CV787) + chemotherapy	II
USA	Svend O. Freytag	Neo-adjuvant suicide gene therapy (CD/Tk) with selectively replicating adenovirus + Radiotherapy	I
USA	John M. Corman	Selectively replicating adenovirus (CG7060) + radiotherapy	I/II

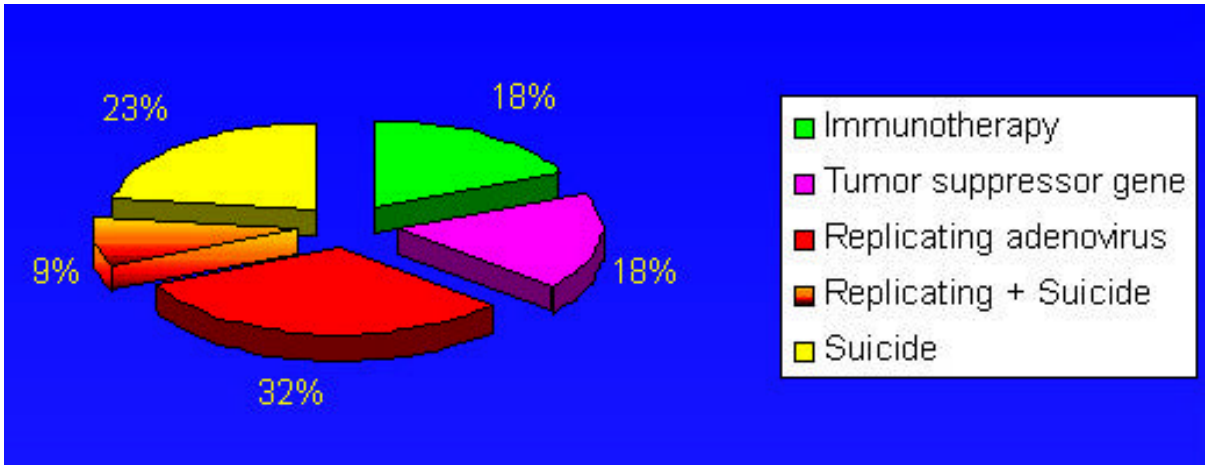

Figure 3. Adenovirus mediated clinical gene therapy modalities for prostate. The types of clinical gene therapy modalities for prostate are represented as percentages in a pie graph in order to better appreciate the contribution of each treatment modality.

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Dr. Salih Sanlioglu