Expert Opinion

Gene delivery and gene therapy of prostate cancer

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Surgery, radiation or hormonal therapy are not adequate to control prostate cancer. Clearly, other novel treatment approaches, such as gene therapy, for advanced/recurrent disease are desperately needed to achieve long-term local control and particularly to develop effective systemic therapy for metastatic prostate cancer. In the last decade, significant progress in gene therapy for the treatment of localised prostate cancer has been demonstrated. A broad range of different gene therapy approaches, including cytolytic, immunological and corrective gene therapy, have been successfully applied for prostate cancer treatment in animal models, with translation into early clinical trials. In addition, a wide variety of viral and nonbiological gene delivery systems are available for basic and clinical research. Gene therapy approaches that have been developed for the treatment of prostate cancer are summarised.

Keywords: gene delivery methods, gene therapy, prostate cancer

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1. Introduction

Prostate cancer is the most commonly diagnosed type of male malignancy, which is the second leading cause of cancer death in men, and is exceeded only by lung cancer. Conventional prostate cancer therapies used in routine clinical practice, including surgical (for local disease), radiation or androgen-withdrawal hormonal therapy as monotherapy or in combination, does not appear to be adequate to control prostate cancer, which ultimately leads to distant metastasis and morbidity [1]. The development of resistance to these treatment regimens often occurs in advanced or recurrent cancer. A major disadvantage of conventional prostate cancer therapies is the limited effect on metastasis development. Because androgen-independent prostate cancer cells eventually lead to death, successful strategies to modify the biological behaviour of these cells may have the most significant clinical impact [2]. Clearly, other novel treatment approaches, such as gene therapy, for advanced/recurrent and metastatic disease are desperately needed to achieve long-term local control and particularly to develop effective systemic therapy for metastatic prostate cancer [3].

A growing body of evidence suggests that prostate cancer development is a multistep process characterised by a combination of genetic and phenotypic alterations of prostate cells as well as surrounding normal cells that result in tumour expansion, invasion and metastasis. To our knowledge the molecular pathogenesis of prostate cancer initiation and development is not completely defined so far; several factors (such as alterations in signal transduction pathways, stimulation of angiogenesis, inhibition of apoptosis) that correlate with tumour progression have been discovered [4-7].

Manipulation of genes involved in tumour progression represents an important approach for therapeutic intervention in prostate cancer. Cancer gene therapy is the selective transfer of recombinant DNA, using viral and nonviral gene delivery vectors, into malignant cells as well as tumour-associated cells for therapeutic purposes. Gene therapy has emerged as a potential approach for both localised and

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metastatic prostate cancer. Several biological features of the prostate make it an attractive target for the development of gene therapy strategies in combination with radiotherapy or chemotherapy for the treatment of prostate cancer. The accessibility of the prostate gland allows local administration of a desirable gene therapy vector for treatment of localised disease, which should prevent major obstacles associated with systemic gene delivery methods, including hepatic clearance, broad tropism and immune system response. It is important that the prostate gland is an accessory organ and, as such, cytoreductive approaches that eliminate prostate cancer cells are tolerable. Also, the prostate represents a tissue that is suitable for gene therapy because several unique tissue-specific antigens including human osteocalcin (hOC), prostate-specific antigen (PSA), prostate-specific membrane antigen, human kallikrein 2 and probasin are highly expressed in prostate cancer [8]. So far, several prostate tissue-specific, tumour-specific and synthetic promoters have been successfully applied for transcriptional targeting of prostate cancer. This circumstance provides a degree of greater latitude in the gene therapy specificity that may not be appropriate for other tumour types [9]. In general, the current available strategies for gene therapy for prostate cancer can be divided into two large groups: cytoreductive (including cytolytic gene therapy and immunomodulation) and corrective (including restoration of tumour suppressor genes or repression of oncogenes) (Table 1). Several approaches including immunomodulatory [10-13] or gene-directed enzyme/prodrug gene therapy [14,15] have shown therapeutic effect in clinical trials without substantial toxic effects when used alone or in combination with radiotherapy. In this review, gene therapy and delivery approaches that were developed in the last decade for the treatment of prostate cancer will be summarised.

2. Gene therapy cytoreductive approaches

2.1 Gene-directed enzyme/prodrug therapy

Gene-directed enzyme/prodrug therapy, or suicide gene therapy, involves the delivery of a specific enzyme that can produce cell death through the conversion of an inactive nontoxic prodrug into a cytotoxic drug metabolite. Specifically targeted expression of the prodrug-activating enzyme avoids systemic toxicity, and results in high drug concentration in the tumour mass and an improved therapeutic index compared with systemic drug administration. The key element of a gene-directed enzyme/prodrug therapy system is a gene that encodes an enzyme, which converts a prodrug to an active cytotoxic drug. Importantly, prodrug-activating enzymes are normally absent or poorly expressed in mammalian cells. This means that tumour-targeting of gene therapy, using specific delivery vehicles, restricts enzyme expression to the transduced tumour cells and adjacent surrounding tumour cells through diffusion of the drug metabolite to generate a bystander effect.

One of the most widely used suicide gene/prodrug systems for prostate cancer uses the herpes simplex virus-thymidine kinase (HSV-TK) in combination with antiherpetic drugs such as ganciclovir (GCV) or its analogues (acyclovir and valacyclovir) [16]. The HSV-TK/GCV system is characterised by the effective phosphorylation of GCV into a toxic compound that is incorporated into DNA during its replication. This incorporation into guanine sites in newly synthesised DNA chains causes termination of synthesis and the selective killing of dividing cells by activation of apoptosis pathways. A number of adenoviral (Ad) vectors encoding the *HSV-tk* gene were injected intratumourally and produced tumour growth inhibition in subcutaneous prostate cancer xenografts in combination with GCV administration [16-19]. In addition, the HSV-TK/GCV system sensitised prostate cancer cells to ionising radiation [20] or castration-induced androgen withdrawal therapy [21].

Another well-studied gene-directed enzyme/prodrug system is cytosine deaminase/5-fluorocytosine (CD/5-FC), which has been investigated intensely during the last decade. Several studies have evaluated the effects of the CD/5-FC system in prostate cancer [22-26]. CD is a bacterial or yeast enzyme that can convert the antifungal agent 5-FC into the chemotherapy agent 5-fluorouracil (5-FU). Importantly, 5-FU is able to diffuse across the cell membrane into adjacent cells without passing through the gap junctions, which results in a more powerful bystander effect [27]. However, certain cancers demonstrate relative resistance to 5-FU because of the poor efficiency of conversion of 5-FU into its toxic metabolites. *Escherichia coli* uracil phosphoribosyltransferase (UPRT) is a pyrimidine salvage enzyme that catalyses the synthesis of uridine 5′-monophosphate (UMP). As 5-FU is directly converted into 5-FUMP by UPRT in microorganisms, the transfer of UPRT into tumour cells reportedly induces a more efficient conversion of 5-FU into 5-FUMP, which results in enhanced cytotoxicity of 5-FU. Furthermore, a combination of UPRT with the CD/5-FC system has been reported to increase 5-FC killing of cancer cells including prostate carcinoma [25]. In an extensive series of preclinical studies the replication-competent Ad5-CD/TKrep encoding *CD:HSV-TK* fusion gene demonstrated potent tumour cell radiosensitisation activity [15].

Currently, several alternative suicide gene/prodrug systems using various prostate cancer-specific promoters and gene delivery systems are under intense investigation; for example, the prodrug CB-1954 (5-[aziridin-1-yl]-2,4-dinitrobenzamide) is activated by *E. coli* nitroreductase (NTR) to a potent DNA-crosslinking agent. A system based on purine nucleoside phosphorylase and 9-([f]-M-2-deoxyerythropento-furanosyl) 6-methylpurine prodrug has also been used in prostate cancer [28]. A series of clinical trials of CTL-102, a replication-defective adenovirus expressing *NTR*, was initiated in patients with primary or secondary liver cancer, head and neck cancer, or prostate cancer [29]. In addition, Ad-mediated *NTR* expression under the control of a PSA promoter followed by CB-1954 treatment produced prostate cancer cell death *in vitro* [30] and in subcutaneous xenograft models [31]. Another *E. coli* enzyme, purine nucleoside phosphorylase

Table 1. The current strategies for prostate cancer gene therapy .

Vector	Transgene	Promoter	Ref.
Suicide gene therapy			
Ad	HSV-TK	RSV	[16]
Ad	CD:HSV-TK	HSP70	[17]
Ad	CD	PSMA	[22]
Ad	UPRT	CAG	[25]
Ad	NTR	CMV	[31]
Ad	PNP	PSA	[32]
Induction of apoptosis			
Ad	Bax	GT	[34]
Ad	Bad	ARR_2PB	[37]
Ad	TRAIL	CMV	[40]
Ad	FasL	CMV	[45]
Ad	mda-7/IL-24	CMV	[47]
Ad	RTVP-1	CMV	[52]
Oncolytic virotherapy			
HSV-1			[53]
NDV			[55]
VSV			[56]
Ad	E1A	PSA/PSE	[57]
Ad	E1A	hOC	[59]
	E1B	hOC	[60]
Ad	E1A	Probasin	[61]
	E1B	PSA/PSE	[62]
Ad	E1A	PSA/PSE	
	E1B	hK2	
Ad	E1A	hTERT	
Cytotoxic gene therapy			
Plasmid	DTA	PSA	[63]
Ad	rNIS	CMV	[66]
Immunomodulatory gene therapy			
Ad	mIL-12	CMV	[72]
Ad	mIL-12 + B7-1	CMV	[73]
Retrovirus	IFN-β	MoMuLV(p/e)	[74]
Ad	PSA	CMV	[76]
Plasmid	IL-2	CMV	[11]
Ad	mIL-3	CMV	[78]
Restoration of tumour suppressor gene			
Ad	p21	CMV	[81]
Ad	p53	CMV	[83]
Ad	p16	CMV	[90]
Ad	pHyde	RSV	[96]
Ad	C-CAM1	CMV	[97]
Ad	PML	CMV	[99]
Suppression of oncogene			
Antisense oligonucleotides			
	c-myc		[100]
	Bcl-2		[102]
	C-raf		[105]
	clusterin		[106]
	cIAP-1		[107]
	MDM2		[108]
	PKCα		[109]
	Insulin-like growth factor binding protein-5		[110]
	DNA polymerase-α/ topoisomerase II α	PSA	[111]
Ribozymes			
	AR		[113]
	IGF-I		[114]
	RAD51		[116]
	Human metallothionein class II		[117]
	FGF-BP		[118]
	PKCα		[119]
	Survivin		[120]
	c-Met		[121]
Ad	sKDR	VEGF	[122]
Ad	sFGFR1	CMV	[123]
Ad	FGFR2IIIβ	CMV	[124]
Ad	sFv against HER2/neu	CMV	[125]

ARR_2PB: Dihydrotestosterone-inducible probasin-derived promoter; Bcl: B-cell leukaemia; CAG: Composed cytomegalovirus immediate-early enhancer and a chicken f-actin promoter; C-CAM1: Cell adhesion molecule 1; CD: Cytosine deaminase; cIAP-1: Inhibitor of apoptosis protein-1; CMV: Cytomegalovirus; DTA: Diphtheria toxin A chain; FasL: Fas ligand; FGF-BP: Fibroblast growth factor-binding protein; GT: Synthetic GAL4-responsive promoter consisting of five GAL4-binding sites and a TATA box; HER: Human epidermal growth factor receptor; hOC: Human osteocalcin; hK2: Human glandular kallikrein promoter; HSP70: Human inducible heat-shock protein 70 promoter; HSV: Herpes simplex virus; hTERT: Human telomerase reverse transcriptase; IFN: Interferon; IGF: Insulin-like growth factor; MDM2: Mouse double minute 2; mIL: murine interleukin; MoMuLV(p/e): Moloney murine leukaemia viral promoter/enhancer; NDV: Newcastle disease virus; NTR: Nitroreductase; PKCα: Protein kinase Cα; PML: Promyelocytic leukaemia gene; PNP: Purine nucleoside phosphorylase; PSA: Prostate-specific antigen; PSE: Prostate-specific enhancer; PSMA: Prostate-specific membrane antigen; rNIS: Rat sodium iodide symporter; RSV: Rous sarcoma virus; RTVP: Related to testes-specific, vespid and pathogenesis protein; sFGFR: Soluble fibroblast growth factor receptor; sKDR: Soluble vascular endothelial growth factor receptor 2; TK: Thymidine kinase; TRAIL: Tumour necrosis factor-related apoptosis-inducing ligand; UPRT: Uracil phosphoribosyltransferase; VSV: Vesicular stomatitis virus.

(PNP), converts the prodrug 9-(β-D-2-deoxy-erythropento-furanosyl)6-methylpurine to the toxic compound 6-methyl-purine, which is capable of killing quiescent and proliferating cells when incorporated in mRNA or DNA during synthesis.

The *PNP* gene was delivered by a replication-deficient Ad vector under the control of a PSA promoter fragment, which induced cytotoxicity *in vitro* and significantly suppressed the growth of human prostate tumours in nude mice as well as increased their survival compared with control animals [32]. Recently, ovine Ad vectors encoding the *PNP* gene under transcriptional control of the Rous sarcoma virus (RSV) or PSME/probasin hybrid promoter were formulated with cationic lipid for enhanced infectivity, and were injected intratumourally and produced tumour growth inhibition in subcutaneous androgen-sensitive and -independent human prostate cancer xenografts in combination with fludarabine phosphate; an approved chemotherapy agent for leukaemia treatment [33].

2.2 Induction of apoptosis

The most common form of programmed cell death is apoptosis, which regulates tissue homeostasis. Clonal expansion and tumour growth are the result of the deregulation of the balance between cell proliferation and apoptosis. Suppressed apoptosis is an important distinctive feature that contributes to the malignant phenotype of prostate cancer. In addition, the ability of a cancer cell to respond to a chemotherapeutic agent is believed to be due, in part, to its apoptotic capacity. Thus, specific induction of apoptotic potential is a rational approach for cancer gene therapy. The cellular apoptotic machinery is mediated by a family of cysteine proteases known as caspases. Two of the major pathways for caspase activation in mammalian cells have been discovered. The intrinsic apoptotic pathway can be induced by the release of cytochrome c from mitochondria through elevations in the levels of pore-forming pro-apoptotic Bcl-2 family proteins such as Bax. Translocation of Bax homodimer to the mitochondria can trigger the release of cytochrome c and activate the caspase pathway causing apoptotic death of mammalian cells. In the cytosol, cytochrome c binds and activates Apaf-1, allowing it to bind and activate procaspase-9. Active caspase-9 and -8 have been shown to directly cleave and activate the effector protease, caspase-3. Several Ad vectors encoding the *bax* gene under transcriptional control of tumour-specific promoters were tested *in vitro* and in prostate cancer xenograft models. These studies demonstrated that *bax* overexpression produced prostate tumour cell death by activating the mitochondrial pathway [34-36]. In addition, gene therapy using *bax* in combination with the pro-apoptotic gene *bad* was more effective in experimental models of androgen-dependent or -independent prostate cancer in comparison with *bax* or *bad* alone [37]. Ad-mediated overexpression of caspase-7 induced apoptosis in parental LNCaP prostate cancer cells and in Bcl-2-transfected cells, which express this antiapoptotic gene [38]. Replication-deficient Ad vectors expressing caspase-1 and -3 under the transcriptional control of the cytomegalovirus (CMV) promoter were also evaluated for experimental prostate cancer therapy [39].

The extrinsic pathway can be induced by the engagement of death receptors on the cell surface by members of the TNF superfamily of cytokines, which includes the TNF-related apoptosis-inducing ligand (TRAIL/Apo2L) and Fas ligand (FasL; CD95L/APO-1L). These proteins recruit adapter proteins to their cytosolic death domains, which then bind the death effector domain containing procaspases, particularly procaspase-8. TRAIL is of particular interest in the development of cancer therapeutics as it preferentially induces apoptosis of tumour cells with little or no effect on normal cells. TRAIL can trigger caspase activation and result in rapid apoptosis through the binding of specific proapoptotic receptors. It has been demonstrated that TRAIL gene delivery using Ad vectors produces prostate cancer cell killing through the induction of apoptosis pathways *in vitro* and inhibits tumour growth after intratumoural injection in human prostate cancer xenografts [40,41]. Furthermore, doxorubicin pretreatment sensitised TRAIL-resistant cells to TRAIL-induced apoptosis [42], and the administration of an Ad vector encoding TRAIL at the site of tumour growth in combination with radiation treatment produced significant suppression of the growth of DU145 androgen-independent human prostate tumour xenografts in athymic nude mice [43].

In addition, *FasL* gene delivery, using Epstein–Barr virus-based plasmid vectors conjugated with cationic polymers [44] or recombinant Ad vectors [45,46], has been shown to induce apoptosis in a number of prostate cancer cells that were resistant to apoptosis induction by the anti-Fas antibody and membrane-bound FasL. A melanoma differentiation-associated gene-7, *mda-7/IL-24*, has demonstrated profound and selective activity against prostate carcinoma *in vitro* and *in vivo*, with minimal toxicity to normal cells. Ad-mediated expression of *mda-7/IL-24* resulted in growth inhibition in human prostate cancer cell lines via the induction of apoptosis. *mda-7/IL-24* selectively induces G_2/M arrest and apoptosis in cancer cells by promoting mitochondrial dysfunction and reactive oxygen species production, as well as an alteration of the ratio of pro- to antiapoptotic Bcl-2 protein family members [47-49]. In addition to its direct effect on cancer cells resulting in apoptosis, the secreted form of the MDA-7/IL-24 protein acts as a cytokine and has shown antiangiogenic activity [50]. Recently, a mouse related to testes-specific, vespid and pathogenesis protein-1 (*mRTVP-1*) gene was identified as a direct target of p53, with proapoptotic activities in various cancer cell lines including prostate cancer [51]. AdmRTVP-1-mediated gene overexpression increased apoptosis and demonstrated therapeutic efficacy in an orthotopic metastatic mouse model of prostate cancer [52].

2.3 Oncolytic virotherapy

Conditional oncolytic virotherapy has employed replication-competent viral vectors that specifically kill tumour cells and represents a promising tool for prostate cancer treatment. A major advantage of replicative oncolytic viral vectors is that their number should increase via viral replication within

infected tumour cells, and the resulting viral progeny can infect neighbouring cancer cells within the tumour mass. G207 is an attenuated/replication-competent HSV-1 mutant that lacks both copies of *ICP34.5* (*RL1*) gene and contains an insertion of *lacZ* in the *ICP6* (*UL39*) gene. These gene modifications offer a new modality in cancer therapy through the ability of G207 to replicate selectively within and kill malignant cells with minimal toxicity to normal tissues. G207 induced a significant inhibition of the tumour growth alone and in combination with radiation in human prostate tumour xenograft models [53]. Incorporation of a gene encoding a cell membrane fusion glycoprotein of the gibbon ape leukaemia virus into the HSV genome significantly increased the antitumour potency in mouse models of primary and metastatic human prostate cancer [54]. An avian paramyxovirus Newcastle disease virus strain 73-T caused significant inhibition of the growth of PC-3 human prostate xenografts in athymic mice [55]. The comparative study of a recombinant vesicular stomatitis virus (VSV), containing a wild-type M protein and an isogenic M protein mutant virus, showed that LNCaP cells were sensitive to killing by both wild-type and mutant viruses, whereas PC-3 cells were highly resistant to VSV-induced cell killing. Both viruses were equally effective at reducing LNCaP tumour volume *in vivo* following intratumorally or intravenous inoculation in nude mice, whereas PC-3 tumours were resistant to VSV treatment [56].

CN706 is an oncolytic conditionally replicating Ad vector, in which the essential *E1A* viral replication gene is under the control of an exogenous minimal enhancer/promoter construct derived from the 5′ flank of the human *PSA* gene promoter that is preferentially active in prostate cancer cells [57]. CN706 has been shown to kill human prostate cancer xenografts in preclinical models [58]. A replication-competent Ad vector, Ad-hOC-E1, which contains a single bidirectional hOC promoter to drive both the early viral *E1A* and *E1B* genes, was constructed. This vector selectively replicated in OC-expressing prostate cancer cells and viral replication was enhanced at least 10-fold with vitamin D3 exposure. Unlike Ad-sPSA-E1, an Ad vector with viral replication controlled by a strong super prostate-specific antigen (sPSA) promoter, which only replicates in PSA-expressing cells with androgen receptor (AR), Ad-hOC-E1 retarded the growth of both androgen-dependent and -independent prostate cancer cells irrespective of their basal level of AR and PSA expression. A single intravenous administration of Ad-hOC-E1 virus inhibited the growth of previously established s.c. DU145 (AR- and PSA-negative) tumours. Viral replication was enhanced by intraperitoneal administration of vitamin D3 [59]. CV787, a prostate-selective replication-competent Ad with improved efficacy, contains the prostate-specific rat probasin promoter, driving the *E1A* gene, and the human prostate-specific enhancer/promoter, driving the *E1B* gene. In nu/nu mice carrying LNCaP xenografts, a single tail vein injection of CV787 eliminated tumours within 4 weeks [60]. In an attempt to improve both the efficacy and safety of oncolytic virotherapy, a replication-competent Ad vector encoding a *CD:HSV-TK* fusion gene was constructed. Phase I studies demonstrated the safety of intraprostatic administration of this Ad in combination with conventional-dose three-dimensional conformal radiation therapy in patients with intermediate- to high-risk prostate cancer [15,61]. Studies using an Ad vector encoding the *E1A* gene under transcriptional control of the human telomerase reverse transcriptase promoter have shown that viral genome replication and productive infection is primarily restricted to telomerase-positive tumour cells. Administration of the virus into nude mice bearing human prostate xenografts produced significant tumour reduction [62].

In addition, several strategies of cytoreductive gene therapy were employed for the treatment of prostate cancer; for example, diphtheria toxin is a potent cellular toxin that poisons protein synthesis by catalysing ADP-ribosylation of elongation factor 2 and produced LNCaP cell death by an apoptosis-mediated pathway [63]. The use of a HIV-1-based lentiviral vector [64] or an Ad vector [65] for prostate-specific transcriptional targeting of the active domain of diphtheria toxin in cancer cells has been reported for gene therapy of prostate cancer. In other studies, a therapeutic effect of ^{131}I has been demonstrated in prostate cancer cells transfected with the human sodium iodide symporter gene [66].

2.4 Immunomodulatory gene therapy

Immunomodulation represents an approach to cancer therapy, in which induction of an antitumour immune response leads to prostate tumour cell killing. Prostate cancer cells express several unique tissue-specific proteins that can potentially function as target antigens for genetic immunotherapy. Although a large number of tumour-associated antigens that can be recognised by the immune system are expressed on the prostate cancer cell membrane, tumour cells demonstrate relatively low immunogenicity. At the present time, mechanisms that permit prostate cancer cells to avoid the immunological response are not completely understood; several pathways controlling the tumour immune system recognition and inhibition of tumour antigen presentation have been identified; for example, in contrast to the normal human leukocyte antigen (HLA) class I expression of normal prostate tissue, complete loss of HLA class I expression occurred in 34 and 80% of primary prostate cancers and lymph node metastases, respectively. In addition, the downregulation of HLA class I expression was observed in 85% of primary prostate cancers and in 100% of metastases [67]. Tumour cells can also evade immune attack by downregulation of the CD95 (APO-1/Fas) receptor and killing of lymphocytes through the expression of CD95L [68]. In contrast, malignant cells appear to be relatively resistant to Fas ligand [69]. Metastatic prostate cancer cells can escape T-cell recognition by specific underexpression of the *TAP-2* gene product or deficient class I major histocompatibility complex (MHC) heavy-chain function [70].

A number of approaches have been developed to generate or enhance the antitumour immune response, including delivery

of genes encoding tumour-specific or tumour-associated antigens as well as certain cytokines with immunomodulatory activity. In animal experiments, hormone-refractory prostate tumours were treated with irradiated prostate cancer cells genetically engineered to secrete human granulocyte-macrophage colony-stimulating factor (GM-CSF), and showed longer disease-free survival compared with either untreated or control rats receiving the prostate cancer cell vaccine mixed with soluble human GM-CSF [71]. IL-12 demonstrates a wide range of immunoregulatory activity including the generation of specific antitumoural immunity through stimulation of antigen presentation, enhancement of cytolytic natural killer (NK) cell activity and differentiation of $CD4^+$ and $CD8^+$ T cells. A recombinant Ad vector encoding the murine *IL-12* gene demonstrated significant growth suppression and increased mean survival time in orthotopic murine prostate carcinomas generated from a poorly immunogenic cell line [72]. Ad gene therapy using a combination of the *IL-12* gene with the *B7-1* (*CD80*) gene, which is poorly expressed on most tumour cells and whose interaction with a specific receptor on T cells (CD28) generates long-lasting antitumour responses, showed a dose-dependent reduction of tumour weight, effectively suppressing the development and growth of orthotopic tumours in comparison with Ad-mediated *IL-12* expression alone [73]. IFN-β, a type I IFN together with IFN-α, can directly inhibit the growth of tumour cells by inducing differentiation, S phase accumulation and apoptosis. Whereas IFN-β and -α function through the same receptor, prostate cancer cells are more sensitive to IFN-β. In addition, IFN-β demonstrates antiangiogenic and antimetastatic activity. Human prostate carcinoma cells were infected with a retroviral vector encoding the murine *IFN-β* cDNA. The inhibition of angiogenesis and activation of host effector cells resulted in the suppression of tumorigenicity and metastasis [74]. Intratumoural delivery of the *IFN-β* gene employing an Ad vector suppressed orthotopic tumour growth and the development of metastasis by 80%, and eradicated the tumours in 20% of mice [75]. PSA-specific immune response was induced *in vivo* by the immunisation of mice with the plasmid encoding the *PSA* gene under the control of a CMV promoter. PSA immunisation induced a strong and persistent antibody response and MHC Class I $CD8^+$ T-cell-restricted cytotoxic T lymphocyte response against tumour cell targets expressing PSA [76]. In another study, mice were immunised with recombinant Ad5-PSA encoding *PSA* gene. Induction of PSA-specific cellular and humoral immunity resulted in the protection against a subcutaneous challenge with prostate cancer cells expressing PSA. Although Ad5-PSA immunisation prior to challenge was protective, Ad5-PSA immunisation alone was not able to control the growth of existing tumours. In contrast, established tumours were efficiently eliminated if Ad5-PSA priming was followed 7 days later by intratumoural injection of recombinant canarypox viruses encoding IL-12, IL-2 and TNF-α [77].

As mentioned previously, the low immunogenicity of prostate cancer may be due to a defect in cell-surface expression of MHC class I. Thus, to overcome the immune effector cell anergy, gene delivery methods employing exogenous cytokines (e.g., IL-2, which is not dependent on MHC class I antigen processing) were developed. In a Phase I clinical trial using gene-based immunotherapy, the plasmid DNA vector pVCL-1102 containing the human *IL-2* gene was administered intraprostatically into the tumour lesion. Evidence of systemic immune activation was observed after *IL-2* gene therapy, based on an increase in the intensity of T-cell infiltration in the injected tumour sites and increased proliferation rates of peripheral blood lymphocytes in patient serum collected after treatment [11]. *In situ IL-2* cytokine gene transfer using an *E1*- and *E3*-deleted Ad vector containing the human *IL-2* gene, under control of the CMV promoter, produced minimal toxicity and demonstrated PSA decreases ranging 17 – 69% from the initial values in the Phase I study [13]. IL-3 has pleiotropic effects on haematopoietic cells, which include the activation of $CD4^+$ and $CD8^+$ T lymphocytes and antigen presentation by dendritic cells. Intratumoural injection of a replication-defective Ad-mIL-3 with a murine *IL-3α* gene showed no tumour growth delay without radiation treatment; however, the combination of Ad-mIL-3 injection with ionising radiation produced tumour growth delay that was significantly greater than that of radiation alone [78].

In summary, cytoreductive gene therapy is a powerful approach for prostate cancer treatment. So far, specifically targeted suicide gene therapy has been tested in a number of clinical trials in patients with prostate cancer [14,15]. Importantly, it was shown that the bystander effect produced by HSV-TK/GCV and CD/5FC systems resulted in an improved therapeutic index. In addition, transductional/transcriptional targeted oncolytic virotherapy is a promising strategy for prostate cancer treatment due to their ability to replicate within infected tumour cells and infect neighbouring cancer cells within the tumour mass. Data from experimental and clinical studies provide strong evidence that apoptosis-inducing gene therapy in combination with ionising radiation and chemotherapy drugs may override resistance to conventional prostate cancer treatment regimens [42,43]. Currently, a number of immunomodulatory gene therapy strategies are being successfully used in clinical trials [11,13].

3. Gene therapy approaches inducing modification of signalling pathways

3.1 Restoration of tumour suppressor signalling pathways

A different strategy to prostate cancer gene therapy is the specific regulation of gene expression involved in the cell cycle, such as the expression of tumour suppressors (including cell cycle control genes *p53*, *p16* and *p21*) or oncogenes (*c-myc*, *bcl-2*), to avoid the neoplastic transformation of prostate cells and to alter the malignant phenotype. A large number of studies have shown that alterations in the $p14^{ARF}$/MDM2/p53 signalling pathways are common in most human prostate

cancers, resulting in resistance to apoptosis that produces a more aggressive phenotype. Mutations in the *p53* gene are observed in 20 – 75% of prostate tumours, and levels of *p53* alteration were frequently correlated with cancer progression from untreated primary to hormone-refractory and metastatic disease [79,80]. Ad-mediated *p53* transgene delivery was associated with enhanced apoptosis and suppression of growth of prostate cancer cells *in vitro* [81-83]. In addition, the efficacy of *p53* corrective gene therapy alone or in combination with ionising radiation and chemotherapy drugs has been shown in multiple prostate cancer animal models [83,84]. Another approach to enhance *p53* gene therapy used an Ad vector encoding VP22-p53 fusion protein on p53-negative prostate cancer cells was investigated. VP22-p53 showed efficient translocation into tumour cells, inhibition of tumour cell proliferation and induction of apoptosis [85].

Deregulation of the p16/cyclin D/pRB/E2F-1 signalling pathway, which controls the expression of genes during the early phase of cell cycle progression (in particular the G_1/S phase transition), is a potential target for most cancers. Alterations in the *p16*^{INK4a} tumour suppressor gene occur frequently in prostate tumour cells, and have been linked to malignant behaviour of prostate carcinoma and associated with a poor prognosis [86-89]. Several independent groups have shown that using Ad vectors encoding the *p16* gene under the control of CMV or RSV long-terminal repeat promoters produced prostate cancer cell killing *in vitro* and demonstrated significant tumour regressions in human prostate cancer xenografts in nude mice and prolonged animal survival [90-92].

p21$^{(WAF-1/CIP1)}$ (p21), a cyclin-dependent kinase (CDK) inhibitor, was identified as a transcriptional target of the *p53* tumour suppressor gene. p21 expression induces G_1 arrest through inhibition of CDK, which directly prevents DNA synthesis. Loss of these negative regulators of the cell cycle contributes to increased cell proliferation, which may in turn lead to tumorigenesis [93]. The *p21* gene modulates p53-induced apoptosis, as well as prostate cancer cell survival, after exposure to DNA-damaging agents and growth factor deprivation, and its expression is associated with the progression to androgen-independent prostate cancer [94,95]. An *in vitro* study demonstrated significantly higher growth suppression after Ad5CMV-p21-mediated *p21* gene delivery compared with Ad5CMV-p53 infection of prostate cancer cells, and was associated with downregulation of the activity of the CDK-2 by ~ 65%. In addition, *p21* gene therapy produced significant prostate cancer growth inhibition and extended animal survival [81].

Various potential targets for corrective gene delivery that suppress prostate cancer growth, including pHyde, C-CAM1 and promyelocytic leukaemia (*PML*) gene, have been evaluated. A recent study demonstrated that Ad-mediated *pHyde* gene expression induced growth inhibition both *in vitro* and *in vivo* by p53-dependent apoptosis in prostate cancer cells [96]. It was demonstrated that an androgen-regulated immunoglobulin-like cell adhesion molecule, C-CAM1, was diminished in both prostate intraepithelial neoplasia and cancer lesions, indicating that loss of C-CAM1 expression may be involved in the early events of prostate carcinogenesis. *C-CAM1* gene delivery using the Ad-C-CAM1 vector was able to repress the growth of PC3-induced tumours in nude mice for at least 3 weeks [97]. In contrast, 14 of the 18 tumours receiving three fractionated Ad-C-CAM1 injections regressed completely, whereas the other 4 tumours shrank to significantly smaller sizes [98]. *PML*, a growth and transformation suppressor, plays an important role in essential cellular functions such as cell proliferation, differentiation, survival and tumour suppression. Overexpression of PML mediated by a replication-deficient Ad vector (Ad-PML) reduced prostate cancer cell growth, and direct injection of Ad-PML into DU145 tumours was able to repress tumour growth in nude mice by 64% [99].

3.2 Suppression of oncogene signalling pathways

The identification of specific changes in the gene expression profile during tumorigenesis resulted in the discovery of a large number of overexpressed genes that are attractive targets for prostate cancer therapy. This direct activation of oncogene signalling pathways in cancer cells produces uncontrolled proliferation often in combination with resistance to apoptosis. A range of strategies to specific suppression of oncogene signalling pathways showed promise for corrective gene therapy of prostate carcinoma that include using antisense oligonucleotides, catalytic RNA (ribozymes) or genes that interrupt growth factor stimulation of prostate cancer development.

Antisense oligonucleotide is a short chemically modified deoxyribonucleic acid, which is able to bind with a complementary mRNA transcript and prevent translation of the target protein. The overexpression of c-*myc* proto-oncogene associated with uncontrolled cell proliferation is a frequent genetic event in androgen-refractory prostatic neoplasia. Time- and dose-dependent decreases in DNA synthesis and viability were noted for prostate cancer cells after c-myc-antisense-oligonucleotide treatment *in vitro* [100]. The mechanism for the antitumour effect of an antisense c-myc replication-incompetent retrovirus that contained the mouse mammary tumour virus (MMTV) promoter seems to be suppression of c-myc mRNA and protein expression. The *in vivo* transduction of DU145 prostate cancer cells with MMTV-antisense c-myc retrovirus reduced tumour growth, resulting in the downregulation of Bcl-2 expression, tumour cell differentiation, decreased invasion and a marked stromal response [101]. Overexpression of the antiapoptotic Bcl-2 and *bcl-xl* genes has been observed in prostate cancer cells and was linked with resistance to ionising radiation and chemotherapy drug treatment. Thus, treatment of LNCaP cells with antisense Bcl-2 oligodeoxynucleotides combined with androgen ablation resulted in the interruption of development to androgen independence and increased sensitivity to cytotoxic chemotherapy [102,103]. Adjuvant administration of antisense Bcl-xl and Bcl-2 oligodeoxynucleotides in combination with paclitaxel therapy

significantly delayed time to tumour recurrence [104]. A number of oncogenes, including *C-raf* [105], clusterin/testosterone-repressed prostate message-2 [106], BIR domain of cIAP-1 [107], *MDM2* [108], human protein kinase C-α [109], insulin-like growth factor binding protein-5 [110], PSA promoter-driven DNA polymerase-α and topoisomerase II a [111], and *AR* [112], were also evaluated as targets for antisense oligonucleotide treatment of prostate carcinoma, and demonstrated therapeutic potential alone and in combination with chemotherapy *in vitro* as well as *in vivo*.

Ribozymes are catalytic RNA molecules with site-specific activity to cleave complementary mRNA of essential cellular genes that are expressed predominantly/exclusively in prostate cancer cells. Sequence-specific hammerhead ribozymes have been successfully used to selectively degrade mRNAs, encoding a number of oncoproteins, such as AR [113], human insulin-like growth factor-I [114], Bcl-2 [115], RAD51 [116], human metallothionein class II [117], fibroblast growth factor-binding protein [118], protein kinase Cα [119], survivin [120], and hepatocyte growth factor scatter factor tyrosine kinase receptor c-Met [121], in prostate cancer cells *in vitro* and *in vivo*. Expression of hammerhead ribozymes in prostate cancer cells induced apoptosis, produced cytotoxicity and increased susceptibility to ionising radiation [116] and platinum chemotherapy drug treatment [119].

Data from experimental and clinical studies of prostate cancer indicate that tumour–microenvironment interaction has a fundamental role in tumorigenesis. Growth, progression, invasion and metastasis of prostate cancers are accompanied by remarkable changes in the gene expression profile of surrounding normal cells. Cross-talk between malignant cells and other tumour-associated cells represents a new target for anticancer therapy, which has been explored through different forms of intervention. Strategies to inhibit tumour–microenvironment interaction include: inhibition of expression of growth factor and of their receptors; inhibition of growth factor signalling pathways; abrogation of the binding between ligands and their receptors; and through the prevention of intracellular transduction of the growth factor signal. The accumulation of cancerous cells within a growing prostate tumour can deprive them of adequate vascular support. Overexpression of vascular endothelial growth factor (VEGF) and its cognate receptors has been linked to a more aggressive phenotype of human prostate carcinomas. The importance of signal transduction through the VEGF receptor 2 (KDR) is illustrated by the use of soluble KDR, which binds to VEGF and sequesters this ligand before its binding to a cellular receptor. AdVEGF-sKDR-mediated sKDR overexpression under control of the VEGF promoter significantly reduced human vascular endothelial and prostate cancer cell proliferation and sensitised cancer cells to ionising radiation. *In vivo* tumour therapy studies demonstrated significant inhibition of DU145 tumour growth in mice that received combined AdVEGF-sKDR infection and ionising radiation versus AdVEGF-sKDR alone or radiation therapy alone [122]. In addition, overexpression of soluble fibroblast growth factor receptor 1 or restoration of FGFR2IIIβ expression significantly suppresses proliferation and invasion of prostate cells [123,124]. Infection with Ad, encoding the intracellular single-chain antibody (sFv) against the human epidermal growth factor receptor-2 (EGFR-2, HER2/neu), resulted in cytotoxicity to the HER-2/neu-positive prostate carcinoma cell lines [125].

In conclusion, a growing body of evidence suggests that a combination of genetic alterations in signal transduction pathways of prostate cancer cells results in tumorigenesis. Thus, the replacement of deficient tumour suppressor genes or inhibition of oncogene expression is an attractive approach for prostate cancer gene therapy. However, heterogeneity of the cancer cell populations within individual tumours, as a result of multiplicity of gene mutations, decreases the efficacy of modification of signalling pathways as an approach for gene therapy. In addition, current gene therapy protocols cannot achieve stable expression of therapeutic genes in all cancer cells. Another promising target for anticancer therapy is selective inhibition of the interaction between malignant cells and other tumour-associated cells that can enhance gene therapy in combination with conventional prostate cancer therapies.

4. Gene delivery systems

Gene therapy approaches are based on the employment of genetically engineered vectors that promote the transfer of the transgene expression unit into the cancer cells and allow transient or stable expression of the therapeutic gene. The search for methods to introduce genes into cancer cells led to many advances in viral and nonviral vector development, which are summarised in Table 2. Vectors derived from adenoviruses, adeno-associated viruses, herpesviruses, poxviruses and retroviruses are the most commonly used gene carriers in cancer gene therapy. Nonviral gene transfer systems include DNA transfection using chemical methods such as in use of a variety of liposomes, cationic polymers or peptide delivery systems, as well as the disruption of the cell membrane by physical methods (e.g., electroporation or ultrasonography). Many of these gene delivery systems have been tested for prostate cancer therapy. Each group of gene vectors demonstrates a number of advantages as well as significant limitations. Most commonly used in cancer gene therapy, recombinant viral vectors are biological systems derived from naturally evolved pathogens, which as part of the infection process transfer their genomes into the target tumour cells. The common restrictions associated with viral vectors are related to their safety, immunogenicity, pre-existing immune response and limited capacity for transgene insert.

4.1 Adenoviruses

At present, human and nonhuman adenoviruses have been modified extensively and are commonly used vectors in clinical trials of gene therapy for prostate cancer. Most clinical trials on gene therapy for patients with prostate cancer have

Table 2. Current vectors and delivery systems for cancer gene therapy.

Viral	Nonviral
Adenovirus	Liposome-based
Adeno-associated virus	DNA-peptide complex
Herpes simplex virus	Naked DNA/RNA
Vaccinia virus	Antisense oligonucleotide
Retrovirus	Ribozyme

administered vectors directly into the tumour, metastatic lesions of bone or lymph nodes. A strong argument for the employment of Ad vectors in the treatment of prostate cancer is that Ads can infect quiescent as well as dividing cells. In fact, prostate carcinoma demonstrates relatively slow growth in comparison with other cancers, which is responsible in part for the resistance of prostate cancer cells to conventional treatments such as chemotherapy or radiotherapy. The Ad vectors can accommodate up to a 30-kb DNA insert, and demonstrate a high transduction efficiency and relatively simple production and manipulation. In contrast with parvoviruses and retroviruses, Ads do not integrate into the cell genome and, therefore, pose no risk of insertional mutagenesis. A substantial problem associated with Ad vectors is high immunogenicity in combination with proinflammatory effects that limit transgene expression and impede systemic re-administration [126]. However, an appropriate immune response induced by Ad vectors may be beneficial in some cancer gene therapy methods related to regulation of the host immune system.

4.2 Adeno-associated viruses

AAV vectors are replication-defective parvoviruses and require a helper Ad for successful replication. Site-specific or random AAV vector integration into the host cell genome, in the absence of a helper virus, results in long-term transgene expression. In addition, AAVs demonstrate high transduction efficiency in a broad range of quiescent and dividing target cells. In contrast to Ad, AAV vectors do not produce a significant immune response [127]. A major limitation of using AAV is a relatively small transgene size (4 kb), a necessity of helper Ad for successful transduction, and integration into the genome of target tumour cells as well as host cells, which could lead to mutagenesis.

4.3 Herpes simplex viruses

The herpesviruses (including HSV-1) are receiving increasing attention because of their ability to replicate in the nucleus as latent episomes and kill dividing and nondividing tumour cells. Attenuated and replication-competent HSV-1 mutants offer the capability to insert up to almost a 40-kb transgenic sequence. However, low and transient transfection efficiency as well as elicited host immune response and severe symptoms associated with wild-type viruses are serious obstacles for HSV-based gene therapy [128].

4.4 Poxviruses

Similar to HSV-1, the vaccinia virus has a large genome (~ 186 kb) and can accommodate up to 25 kb of foreign DNA. Recombinant vaccinia viral vectors have been used mainly for the delivery of cancer-specific antigens (e.g., *PSA*) and immunoregulatory genes for vaccine-based gene therapy, which is based on the long history of use of these viruses for vaccination. However, the strong immune response as a result of pre-existing antivaccinia immunity following childhood smallpox immunisation limits recombinant vaccinia viral vector gene therapy [129].

4.5 Retroviruses

Retroviruses continue to be employed as gene delivery vehicles, although recent adverse events following retroviral gene therapy have raised concerns about potential insertional mutagenesis [130]. Advantages of retroviral vectors for gene therapy include the potential long-term transgene expression due to integration into the target tumour cells or host genome and low immunogenicity. However, most retroviruses demonstrate low levels of transduction efficiency and only infect dividing cells during mitosis [131].

4.6 Nonviral vectors

In an attempt to improve both the efficacy and safety of viral-mediated gene therapy, many vectors have been modified. However, several obstacles, including limitation of the size of the inserted genetic material, difficulty of propagation and purification, and toxic adverse events (in particular immunogenic and oncogenic activity) have encouraged researchers to focus on nonbiological gene transfer systems as an alternative to viral vectors. Progress has been made using a variety of liposomes, cationic polymers and peptides. In this approach, recombinant DNA is associated with positively charged lipid bilayers, cationic polymers or peptides and can enter cells either by endocytosis or by fusion with the cell membrane. DNA–protein complexes have been developed employing natural or synthetic peptides that allow the use of these gene delivery vehicles in cell-targeting strategies. In contrast with recombinant viruses, these nonviral gene transfer systems are characterised by low cost and simple production, large gene insert size without toxic effects, and lack of an immune response after re-administration. However, nonbiological gene transfer systems produce short-term expression and their efficiency and specificity of gene delivery is less compared with viral vectors [132].

To summarise, Ads as well as retroviruses and liposomes are the most commonly used gene therapy vectors in prostate cancer treatment with varying results. Unfortunately, in spite of the growing number of gene transfer systems tested in the last decade, there is little evidence of an effective, safe and inexpensive vector for prostate cancer gene therapy. An appropriate method of systemic gene delivery is also important for efficacy and safety of prostate cancer gene therapy. At present, most gene therapy studies use only local intratumoural gene

delivery such that gene expression is restricted to the area surrounding the injection site. This method is characterised by relatively low toxic effects as well as low therapeutic efficacy due to limited delivery of the cytotoxic gene to each cancer cell. For some approaches of gene therapy, such as restoration of tumour suppressor signalling pathways or induction of apoptosis, gene delivery to each cancer cell is mandatory. On the other hand, to attempt increased efficacy, production of a bystander effect following gene-directed enzyme/prodrug therapy was successfully tested. Another example of local gene delivery with the potential for antimetastasis activity is immunoregulatory gene therapy, which may result in systemic immune system activation. In general, many experimental models demonstrate limited relevance to the situation in patients with metastatic disease, wherein systematic gene delivery approaches are required. Thus, the development of safe and targeted gene therapy vectors, which can be delivered systemically, is one of the major challenges facing prostate cancer gene therapy. To increase efficacy and limit potential systemic toxicity, which would occur after intravenous administration of gene delivery vehicles, a number of modifications of gene therapy vectors will be required; for example, the evolution of several generations of Ad vector systems, from replication-deficient Ads to conditionally replicating vectors combined with transcriptional or/and transductional targeting of therapeutic gene expression is an approach to increase the efficacy and reduce the toxicity of Ad-mediated prostate cancer gene therapy. However, development of new vectors that can be delivered to metastatic tumour sites will continue to be critical for the successful treatment of metastatic prostate cancer.

5. Conclusion

Gene therapy offers new opportunities for the treatment of prostate cancer through the use of selective and relatively effective approaches. In recent years, a broad range of different gene therapy approaches, including cytolytic, immunological and corrective gene therapy, have been successfully applied for prostate cancer treatment in experimental systems. Some of these strategies have already progressed to clinical evaluation in patients. A wide variety of gene carriers, including viral and synthetic vectors, have been explored as therapeutic gene transfer systems. Thus, in combination with conventional prostate cancer treatments, including radiation and hormone treatment, gene therapy may provide additional beneficial options with the potential to affect advanced/recurrent and metastatic disease.

6. Expert opinion

Although in recent years significant progress in gene therapy for the treatment of experimental and localised prostate cancer has been achieved in animal models, development of recurrent and metastatic disease remains a major problem for successful control of prostate cancer in humans. Obviously, novel approaches and delivery systems for gene therapy of metastatic disease are needed. A growing body of evidence suggests that interaction between prostate cancer cells and surrounding normal cells controls local tumour development, invasion and metastasis. Thus, intervention against the interaction between tumour cells and their microenvironment is a promising area for the development of novel therapeutic anticancer modalities. Important advances in gene therapy over the last decade are a result of improved understanding of the molecular mechanisms that mediate cancer progression and resistance to therapeutic treatment. Therefore, targeting of genes involved in metastasis may be useful for prostate cancer gene therapy to prevent the development of metastatic disease; for example, the *mda-7/IL-24* gene, which was isolated and characterised almost a decade ago, shows potential as a therapeutic transgene because of its profound and selective anticancer and antiangiogenesis activities, with negligible toxicity to normal cells. Another novel proapoptotic gene, *mRTVP-1*, produced significant antiangiogenesis, antimetastasis and immunomodulatory activity in an orthotopic model of metastatic prostate cancer. Modifications of gene delivery systems, such as using tumour-specific promoters for selective expression of therapeutic genes or the development of new oncolytic virotherapy vectors that are designed to selectively replicate in tumour cells or tumour-associated cells, should also significantly increase the efficacy and safety of prostate cancer gene therapy. Promising data have been demonstrated in studies where prostate cancer gene therapy was employed in combination with chemotherapy or radiotherapy. For example, Phase I studies demonstrated that combination of replication-competent Ad5-CD/TKrep-mediated double-suicide prostate cancer gene therapy with 5-FC and GCV prodrug therapy and conformal radiation therapy can be safely applied to humans and produce a significant decline in serum PSA [15]. Other approaches to enhance the effectiveness of radiation therapy have been described [133-135]. Hence, it seems that combination gene therapy approaches with conventional treatments will increase the efficacy of prostate cancer therapy.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. SCHELLHAMMER PF, LYNCH DF: Are monotherapy options reasonable for T3 prostate cancer? *Semin. Urol. Oncol.* (1997) 15(4):207-214.
2. ISAACS WB: Molecular genetics of prostate cancer. *Cancer Surv.* (1995) 25:357-379.
3. STEINER MS, GINGRICH JR, CHAUHAN RD: Prostate cancer gene therapy. *Surg. Oncol. Clin. N. Am.* (2002) 11(3):607-620.
4. GIOELI D: Signal transduction in prostate cancer progression. *Clin. Sci.* (2005) 108(4):293-308.

• This paper identifies growth factor pathways as effective targets for prostate cancer therapy.

5. CHUNG LW, BASEMAN A, ASSIKIS V, ZHAU HE: Molecular insights into prostate cancer progression: the missing link of tumor microenvironment. *J. Urol.* (2005) 173(1):10-20.

•• The role of cancer-associated surrounding stroma in prostate cancer progression and metastasis are comprehensively reviewed in this paper.

6. CHIN JL, REITER RE: Molecular markers and prostate cancer prognosis. *Clin. Prostate Cancer* (2004) 3(3):157-164.
7. KARAYI MK, MARKHAM AF: Molecular biology of prostate cancer. *Prostate Cancer Prostatic Dis.* (2004) 7(1):6-20.

• This is a good review of the molecular basis for prostate cancer therapy.

8. AST G: Drug-targeting strategies for prostate cancer. *Curr. Pharm. Des.* (2003) 9(6):455-466.
9. MABJEESH NJ, ZHONG H, SIMONS JW: Gene therapy of prostate cancer: current and future directions. *Endocr. Relat. Cancer* (2002) 9(2):115-139.
10. SIMONS JW, MIKHAK B, CHANG JF *et al.*: Induction of immunity to prostate cancer antigens: results of a clinical trial of vaccination with irradiated autologous prostate tumor cells engineered to secrete granulocyte-macrophage colony-stimulating factor using *ex vivo* gene transfer. *Cancer Res.* (1999) 59(20):5160-5168.
11. BELLDEGRUN A, TSO CL, ZISMAN A *et al.*: Interleukin 2 gene therapy for prostate cancer: Phase I clinical trial and basic biology. *Hum. Gene Ther.* (2001) 12(8):883-892.
12. GULLEY J, CHEN AP, DAHUT W *et al.*: Phase I study of a vaccine using recombinant vaccinia virus expressing PSA (rV-PSA) in patients with metastatic androgen-independent prostate cancer. *Prostate* (2002) 53(2):109-117.
13. TRUDEL S, TRACHTENBERG J, TOI A *et al.*: A Phase I trial of adenovector-mediated delivery of interleukin-2 (AdIL-2) in high-risk localized prostate cancer. *Cancer Gene Ther.* (2003) 10(10):755-763.
14. TEH BS, AGUILAR-CORDOVA E, KERNEN K *et al.*: Phase I/II trial evaluating combined radiotherapy and *in situ* gene therapy with or without hormonal therapy in the treatment of prostate cancer-a preliminary report. *Int. J. Radiat. Oncol. Biol. Phys.* (2001) 51(3):605-613.
15. FREYTAG SO, STRICKER H, PEGG J *et al.*: Phase I study of replication-competent adenovirus-mediated double-suicide gene therapy in combination with conventional-dose three-dimensional conformal radiation therapy for the treatment of newly diagnosed, intermediate-to high-risk prostate cancer. *Cancer Res.* (2003) 63(21):7497-7506.

• This paper describes the clinical results with a combination of suicide gene therapy and radiation therapy of prostate cancer.

16. EASTHAM JA, CHEN SH, SEHGAL I *et al.*: Prostate cancer gene therapy: herpes simplex virus thymidine kinase gene transduction followed by ganciclovir in mouse and human prostate cancer models. *Hum. Gene Ther.* (1996) 7(4):515-523.
17. BLACKBURN RV, GALOFORO SS, CORRY PM, LEE YJ: Adenoviral-mediated transfer of a heat-inducible double suicide gene into prostate carcinoma cells. *Cancer Res.* (1998) 58(7):1358-1362.
18. THOMPSON TC: *in situ* gene therapy for prostate cancer. *Oncol. Res.* (1999) 11(1):1-8.
19. CHEON J, KIM HK, MOON DG, YOON DK, CHO JH, KOH SK: Adenovirus-mediated suicide-gene therapy using the herpes simplex virus thymidine kinase gene in cell and animal models of human prostate cancer: changes in tumour cell proliferative activity. *BJU Int.* (2000) 85(6):759-766.
20. ATKINSON G, HALL SJ: Prodrug activation gene therapy and external beam irradiation in the treatment of prostate cancer. *Urology* (1999) 54(6):1098-1104.
21. HALL SJ, MUTCHNIK SE, YANG G *et al.*: Cooperative therapeutic effects of androgen ablation and adenovirus-mediated herpes simplex virus thymidine kinase gene and ganciclovir therapy in experimental prostate cancer. *Cancer Gene Ther.* (1999) 6(1):54-63.
22. O'KEEFE DS, UCHIDA A, BACICH DJ *et al.*: Prostate-specific suicide gene therapy using the prostate-specific membrane antigen promoter and enhancer. *Prostate* (2000) 45(2):149-157.
23. YOSHIMURA I, IKEGAMI S, SUZUKI S, TADAKUMA T, HAYAKAWA M: Adenovirus mediated prostate specific enzyme prodrug gene therapy using prostate specific antigen promoter enhanced by the Cre-loxP system. *J. Urol.* (2002) 168(6):2659-2664.
24. KATO H, KOSHIDA K, YOKOYAMA K, MIZOKAMI A, NAMIKI M: Potential benefits of combining cytosine deaminase/5-fluorocytosine gene therapy and irradiation for prostate cancer: experimental study. *Int. J. Urol.* (2002) 9(10):567-576.
25. MIYAGI T, KOSHIDA K, HORI O *et al.*: Gene therapy for prostate cancer using the cytosine deaminase/uracil phosphoribosyltransferase suicide system. *J. Gene Med.* (2003) 5(1):30-37.
26. CORBAN-WILHELM H, EHEMANN V, BECKER G, GREULICH D, BRAUN K, DEBUS J: Comparison of different methods to assess the cytotoxic effects of cytosine deaminase and thymidine kinase gene therapy. *Cancer Gene Ther.* (2004) 11(3):208-214.
27. LAWRENCE TS, REHEMTULLA A, NG EY, WILSON M, TROSKO JE, STETSON PL: Preferential cytotoxicity of cells transduced with cytosine deaminase compared to bystander cells after treatment with 5-flucytosine. *Cancer Res.* (1998) 58:2588-2593.
28. LOCKETT LJ, MOLLOY PL, RUSSELL PJ, BOTH GW: Relative efficiency of tumor cell killing *in vitro* by two enzyme-prodrug systems delivered by identical adenovirus vectors. *Clin. Cancer Res.* (1997) 3(11):2075-2080.
29. SEARLE PF, CHEN MJ, HU L *et al.*: Nitroreductase: a prodrug-activating enzyme for cancer gene therapy. *Clin. Exp. Pharmacol. Physiol.* (2004) 31(11):811-816.
30. LATHAM JP, SEARLE PF, MAUTNER V, JAMES ND: Prostate-specific antigen promoter/enhancer driven gene therapy for prostate cancer: construction and testing of a tissue-specific adenovirus vector. *Cancer Res.* (2000) 60:334-341.

31. DJEHA AH, THOMSON TA, LEUNG H *et al.*: Combined adenovirus-mediated nitroreductase gene delivery and CB1954 treatment: a well-tolerated therapy for established solid tumors. *Mol. Ther.* (2001) 3(2):233-240.
32. MARTINIELLO-WILKS R, GARCIA-ARAGON J, DAJA MM *et al.*: *In vivo* gene therapy for prostate cancer: Preclinical evaluation of two different enzyme-directed prodrug therapy systems delivered by identical adenovirus vectors. *Hum. Gene Ther.* (1998) 9(11):1617-1626.
33. WANG XY, MARTINIELLO-WILKS R, SHAW JM *et al.*: Preclinical evaluation of a prostate-targeted gene-directed enzyme prodrug therapy delivered by ovine atadenovirus. *Gene Ther.* (2004) 11(21):1559-1567.
34. LI X, MARANI M, YU J *et al.*: Adenovirus-mediated Bax overexpression for the induction of therapeutic apoptosis in prostate cancer. *Cancer Res.* (2001) 61(1):186-191.
35. LOWE SL, RUBINCHIK S, HONDA T, MCDONNELL TJ, DONG JY, NORRIS JS: Prostate-specific expression of Bax delivered by an adenoviral vector induces apoptosis in LNCaP prostate cancer cells. *Gene Ther.* (2001) 8(18):1363-1371.
36. HONDA T, KAGAWA S, SPURGERS KB *et al.*: A recombinant adenovirus expressing wild-type Bax induces apoptosis in prostate cancer cells independently of their Bcl-2 status and androgen sensitivity. *Cancer Biol. Ther.* (2002) 1(2):163-167.
37. ZHANG Y, YU J, UNNI E *et al.*: Monogene and polygene therapy for the treatment of experimental prostate cancers by use of apoptotic genes bax and bad driven by the prostate-specific promoter ARR(2)PB. *Hum. Gene Ther.* (2002) 13(17):2051-2064.
38. MARCELLI M, CUNNINGHAM GR, WALKUP M *et al.*: Signaling pathway activated during apoptosis of the prostate cancer cell line LNCaP: overexpression of caspase-7 as a new gene therapy strategy for prostate cancer. *Cancer Res.* (1999) 59(2):382-390.
39. SHARIAT SF, DESAI S, SONG W *et al.*: Adenovirus-mediated transfer of inducible caspases: a novel 'death switch' gene therapeutic approach to prostate cancer. *Cancer Res.* (2001) 61(6):2562-2571.
40. GRIFFITH TS, ANDERSON RD, DAVIDSON BL, WILLIAMS RD, RATLIFF TL: Adenoviral-mediated transfer of the TNF-related apoptosis-inducing ligand/Apo-2 ligand gene induces tumor cell apoptosis. *J. Immunol.* (2000) 165(5):2886-2894.
41. GRIFFITH TS, BROGHAMMER EL: Suppression of tumor growth following intralesional therapy with TRAIL recombinant adenovirus. *Mol. Ther.* (2001) 4(3):257-266.
42. KELLY MM, HOEL BD, VOELKEL-JOHNSON C: Doxorubicin pretreatment sensitizes prostate cancer cell lines to TRAIL induced apoptosis which correlates with the loss of c-FLIP expression. *Cancer Biol. Ther.* (2002) 1(5):520-527.
43. KALIBEROV SA, KALIBEROVA L, STOCKARD CR, GRIZZLE WE, BUCHSBAUM DJ: Adenovirus-mediated flt-1 targeted proapoptotic gene therapy of human prostate cancer. *Mol. Ther.* (2004) 10:1059-1070.
44. NAKANISHI H, MAZDA O, SATOH E *et al.*: Nonviral genetic transfer of Fas ligand induced significant growth suppression and apoptotic tumor cell death in prostate cancer *in vivo*. *Gene Ther.* (2003) 10(5):434-442.
45. HEDLUND TE, MEECH SJ, SRIKANTH S *et al.*: Adenovirus-mediated expression of Fas ligand induces apoptosis of human prostate cancer cells. *Cell Death Differ.* (1999) 6(2):175-182.
46. HYER ML, VOELKEL-JOHNSON C, RUBINCHIK S, DONG J, NORRIS JS: Intracellular Fas ligand expression causes Fas-mediated apoptosis in human prostate cancer cells resistant to monoclonal antibody-induced apoptosis. *Mol. Ther.* (2000) 2(4):348-358.
47. LEBEDEVA IV, SU ZZ, SARKAR D *et al.*: Melanoma differentiation associated gene-7, mda-7/interleukin-24, induces apoptosis in prostate cancer cells by promoting mitochondrial dysfunction and inducing reactive oxygen species. *Cancer Res.* (2003) 63(23):8138-8144.
48. SAITO Y, MIYAHARA R, GOPALAN B *et al.*: Selective induction of cell cycle arrest and apoptosis in human prostate cancer cells through adenoviral transfer of the melanoma differentiation-associated-7 (mda-7)/interleukin-24 (IL-24) gene. *Cancer Gene Ther.* (2005) 12(3):238-247.
49. SAUANE M, LEBEDEVA IV, SU ZZ *et al.*: Melanoma differentiation associated gene-7/interleukin-24 promotes tumor cell-specific apoptosis through both secretory and nonsecretory pathways. *Cancer Res.* (2004) 64(9):2988-2993.
50. RAMESH R, MHASHILKAR AM, TANAKA F *et al.*: Melanoma differentiation-associated gene 7/interleukin (IL)-24 is a novel ligand that regulates angiogenesis via the IL-22 receptor. *Cancer Res.* (2003) 63(16):5105-5113.
51. REN C, LI L, GOLTSOV AA *et al.*: mRTVP-1, a novel p53 target gene with proapoptotic activities. *Mol. Cell. Biol.* (2002) 22(10):3345-3357.
52. SATOH T, TIMME TL, SAIKA T *et al.*: Adenoviral vector-mediated mRTVP-1 gene therapy for prostate cancer. *Hum. Gene Ther.* (2003) 14(2):91-101.
53. WALKER JR, MCGEAGH KG, SUNDARESAN P, JORGENSEN TJ, RABKIN SD, MARTUZA RL: Local and systemic therapy of human prostate adenocarcinoma with the conditionally replicating herpes simplex virus vector G207. *Hum. Gene Ther.* (1999) 10(13):2237-2243.
54. NAKAMORI M, FU X, PETTAWAY CA, ZHANG X: Potent antitumor activity after systemic delivery of a doubly fusogenic oncolytic herpes simplex virus against metastatic prostate cancer. *Prostate* (2004) 60(1):53-60.
55. PHUANGSAB A, LORENCE RM, REICHARD KW, PEEPLES ME, WALTER RJ: Newcastle disease virus therapy of human tumor xenografts: antitumor effects of local or systemic administration. *Cancer Lett.* (2001) 172(1):27-36.
56. AHMED M, CRAMER SD, LYLES DS: Sensitivity of prostate tumors to wild type and M protein mutant vesicular stomatitis viruses. *Virology* (2004) 330(1):34-49.
57. RODRIGUEZ R, SCHUUR ER, LIM HY, HENDERSON GA, SIMONS JW, HENDERSON DR: Prostate attenuated replication competent adenovirus (ARCA) CN706: A selective cytotoxic for prostate-specific antigen-positive prostate cancer cells. *Cancer Res.* (1997) 57(13):2559-2563.
58. DEWEESE TL, VAN DER POEL H, LI S *et al.*: A Phase I trial of CV706, a replication-competent, PSA selective oncolytic adenovirus, for the treatment of locally recurrent prostate cancer following radiation therapy. *Cancer Res.* (2001) 61(20):7464-7472.
59. HSIEH CL, YANG L, MIAO L *et al.*: A novel targeting modality to enhance

adenoviral replication by vitamin D(3) in androgen-independent human prostate cancer cells and tumors. *Cancer Res.* (2002) 62(11):3084-3092.

60. YU DC, CHEN Y, SENG M, DILLEY J, HENDERSON DR: The addition of adenovirus Type 5 region E3 enables calydon virus 787 to eliminate distant prostate tumor xenografts. *Cancer Res.* (1999) 59(17):4200-4203.

61. FREYTAG SO, ROGULSKI KR, PAIELLI DL, GILBERT JD, KIM JH: A novel three-pronged approach to kill cancer cells selectively: Concomitant viral, double suicide gene, and radiotherapy. *Hum. Gene Ther.* (1998) 9(9):1323-1333.

62. HUANG Q, ZHANG X, WANG H *et al.*: A novel conditionally replicative adenovirus vector targeting telomerase-positive tumor cells. *Clin. Cancer Res.* (2004) 10(4):1439-1445.

63. PANG S: Targeting and eradicating cancer cells by a prostate-specific vector carrying the diphtheria toxin A gene. *Cancer Gene Ther.* (2000) 7(7):991-996.

64. YU D, CHEN D, CHIU C, RAZMAZMA B, CHOW YH, PANG S: Prostate-specific targeting using PSA promoter-based lentiviral vectors. *Cancer Gene Ther.* (2001) 8(9):628-635.

65. LI Y, MCCADDEN J, FERRER F *et al.*: Prostate-specific expression of the diphtheria toxin A chain (DT-A): studies of inducibility and specificity of expression of prostate-specific antigen promoter-driven DT-A adenoviral-mediated gene transfer. *Cancer Res.* (2002) 62(9):2576-2582.

66. BOLAND A, RICARD M, OPOLON P *et al.*: Adenovirus-mediated transfer of the thyroid sodium/iodide symporter gene into tumors for a targeted radiotherapy. *Cancer Res.* (2000) 60(13):3484-3492.

67. BLADES RA, KEATING PJ, MCWILLIAM LJ, GEORGE NJ, STERN PL: Loss of HLA class I expression in prostate cancer: implications for immunotherapy. *Urology* (1995) 46(5):681-686.

68. LIU QY, RUBIN MA, OMENE C, LEDERMAN S, STEIN CA: Fas ligand is constitutively secreted by prostate cancer cells *in vitro*. *Clin. Cancer Res.* (1998) 4(7):1803-1811.

69. ROKHLIN OW, BISHOP GA, HOSTAGER BS *et al.*: Fas-mediated apoptosis in human prostatic carcinoma cell lines. *Cancer Res.* (1997) 57(9):1758-1768.

70. SANDA MG, RESTIFO NP, WALSH JC *et al.*: Molecular characterization of defective antigen processing in human prostate cancer. *J. Natl Cancer Inst.* (1995) 87(4):280-285.

71. SANDA MG, AYYAGARI SR, JAFFEE EM *et al.*: Demonstration of a rational strategy for human prostate cancer gene therapy. *J. Urol.* (1994) 151(3):622-628.

72. NASU Y, BANGMA CH, HULL GW *et al.*: Adenovirus-mediated interleukin-12 gene therapy for prostate cancer: suppression of orthotopic tumor growth and pre-established lung metastases in an orthotopic model. *Gene Ther.* (1999) 6(3):338-349.

73. HULL GW, MCCURDY MA, NASU Y *et al.*: Prostate cancer gene therapy: comparison of adenovirus-mediated expression of interleukin 12 with interleukin 12 plus B7-1 for *in situ* gene therapy and gene-modified, cell-based vaccines. *Clin. Cancer Res.* (2000) 6(10):4101-4109.

74. DONG Z, GREENE G, PETTAWAY C *et al.*: Suppression of angiogenesis, tumorigenicity, and metastasis by human prostate cancer cells engineered to produce interferon-beta. *Cancer Res.* (1999) 59:872-879.

75. CAO G, SU J, LU W *et al.*: Adenovirus-mediated interferon-beta gene therapy suppresses growth and metastasis of human prostate cancer in nude mice. *Cancer Gene Ther.* (2001) 8(7):497-505.

76. KIM JJ, TRIVEDI NN, WILSON DM *et al.*: Molecular and immunological analysis of genetic prostate specific antigen (PSA) vaccine. *Oncogene* (1998) 17(24):3125-3135.

77. ELZEY BD, SIEMENS DR, RATLIFF TL, LUBAROFF DM: Immunization with Type 5 adenovirus recombinant for a tumor antigen in combination with recombinant canarypox virus (ALVAC) cytokine gene delivery induces destruction of established prostate tumors. *Int. J. Cancer* (2001) 94(6):842-849.

78. OH YT, CHEN DW, DOUGHERTY GJ, MCBRIDE WH: Adenoviral interleukin-3 gene-radiation therapy for prostate cancer in mouse model. *Int. J. Radiat. Oncol. Biol. Phys.* (2004) 59(2):579-583.

79. HEIDENBERG HB, SESTERHENN IA, GADDIPATI JP *et al.*: Alteration of the tumor suppressor gene p53 in a high fraction of hormone refractory prostate cancer. *J. Urol.* (1995) 154(2 Pt 1):414-421.

80. BROOKS JD, BOVA GS, EWING CM *et al.*: An uncertain role for p53 gene alterations in human prostate cancers. *Cancer Res.* (1996) 56(16):3814-3822.

81. EASTHAM JA, HALL SJ, SEHGAL I *et al.*: *In vivo* gene therapy with p53 or p21 adenovirus for prostate cancer. *Cancer Res.* (1995) 55(22):5151-5155.

82. YANG C, CIRIELLI C, CAPOGROSSI MC, PASSANITI A: Adenovirus-mediated wild-type p53 expression induces apoptosis and suppresses tumorigenesis of prostatic tumor cells. *Cancer Res.* (1995) 55(19):4210-4213.

83. KO SC, GOTOH A, THALMANN GN *et al.*: Molecular therapy with recombinant p53 adenovirus in an androgen-independent, metastatic human prostate cancer model. *Hum. Gene Ther.* (1996) 7(14):1683-1691.

84. NIELSEN LL, LIPARI P, DELL J, GURNANI M, HAJIAN G: Adenovirus-mediated p53 gene therapy and paclitaxel have synergistic efficacy in models of human head and neck, ovarian, prostate, and breast cancer. *Clin. Cancer Res.* (1998) 4(4):835-846.

85. ROY I, HOLLE L, SONG W, HOLLE E, WAGNER T, YU X: Efficient translocation and apoptosis induction by adenovirus encoded VP22-p53 fusion protein in human tumor cells *in vitro*. *Anti-Cancer Res.* (2002) 22(6A):3185-3189.

86. JARRARD DF, BOVA GS, EWING CM *et al.*: Deletional, mutational, and methylation analyses of CDKN2 (p16/MTS1) in primary and metastatic prostate cancer. *Genes Chromosomes Cancer* (1997) 19(2):90-96.

87. ITOH N, KAKEHI Y, AKAO T, KINOSHITA H, OKADA Y, YOSHIDA O: Concomitant presence of p16/cyclin-dependent kinase 4 and cyclin D/cyclin-dependent kinase 4 complexes in LNCaP prostatic cancer cell line. *Jpn. J. Cancer Res.* (1997) 88(3):229-233.

88. LEE CT, CAPODIECI P, OSMAN I *et al.*: Overexpression of the cyclin-dependent kinase inhibitor p16 is associated with tumor recurrence in human prostate cancer. *Clin. Cancer Res.* (1999) 5(5):977-983.

89. HALVORSEN OJ, HOSTMARK J, HAUKAAS S, HOISAETER PA, AKSLEN LA: Prognostic significance of p16 and CDK4 proteins in localized

prostate carcinoma. *Cancer* (2000) 88(2):416-424.

90. GOTOH A, KAO C, KO SC, HAMADA K, LIU TJ, CHUNG LW: Cytotoxic effects of recombinant adenovirus p53 and cell cycle regulator genes (p21 WAF1/CIP1 and p16CDKN4) in human prostate cancers. *J. Urol.* (1997) 158(2):636-641.
91. STEINER MS, WANG Y, ZHANG Y, ZHANG X, LU Y: p16/MTS1/INK4A suppresses prostate cancer by both pRb dependent and independent pathways. *Oncogene* (2000) 19(10):1297-1306.
92. ALLAY JA, STEINER MS, ZHANG Y, REED CP, COCKROFT J, LU Y: Adenovirus p16 gene therapy for prostate cancer. *World J. Urol.* (2000) 18(2):111-120.
93. LIU G, LOZANO G: p21 stability: linking chaperones to a cell cycle checkpoint. *Cancer Cell* (2005) 7(2):113-114.
94. AALTOMAA S, LIPPONEN P, ESKELINEN M, ALA-OPAS M, KOSMA VM: Prognostic value and expression of p21(waf1/cip1) protein in prostate cancer. *Prostate* (1999) 39(1):8-15.
95. FIZAZI K, MARTINEZ LA, SIKES CR *et al.*: The association of p21((WAF-1/CIP1)) with progression to androgen-independent prostate cancer. *Clin. Cancer Res.* (2002) 8(3):775-781.
96. STEINER MS, ZHANG X, WANG Y, LU Y: Growth inhibition of prostate cancer by an adenovirus expressing a novel tumor suppressor gene, pHyde. *Cancer Res.* (2000) 60(16):4419-4425.
97. KLEINERMAN DI, ZHANG WW, LIN SH, NGUYEN TV, VON ESCHENBACH AC, HSIEH JT: Application of a tumor suppressor (C-CAM1)-expressing recombinant adenovirus in androgen-independent human prostate cancer therapy: a preclinical study. *Cancer Res.* (1995) 55(13):2831-2836.
98. LIN SH, PU YS, LUO W, WANG Y, LOGOTHETIS CJ: Schedule-dependence of C-CAM1 adenovirus gene therapy in a prostate cancer model. *Anti-Cancer Res.* (1999) 19(1A):337-340.
99. HE D, MU ZM, LE X *et al.*: Adenovirus-mediated expression of PML suppresses growth and tumorigenicity of prostate cancer cells. *Cancer Res.* (1997) 57(10):1868-1872.
100. BALAJI KC, KOUL H, MITRA S *et al.*: Antiproliferative effects of c-myc antisense oligonucleotide in prostate cancer cells: a novel therapy in prostate cancer. *Urology* (1997) 50(6):1007-1015.
101. STEINER MS, ANTHONY CT, LU Y, HOLT JT: Antisense c-myc retroviral vector suppresses established human prostate cancer. *Hum. Gene Ther.* (1998) 9(5):747-755.
102. GLEAVE M, TOLCHER A, MIYAKE H *et al.*: Progression to androgen independence is delayed by adjuvant treatment with antisense Bcl-2 oligodeoxynucleotides after castration in the LNCaP prostate tumor model. *Clin. Cancer Res.* (1999) 5(10):2891-2898.
103. MIYAKE H, TOLCHER A, GLEAVE ME: Antisense Bcl-2 oligodeoxynucleotides inhibit progression to androgen-independence after castration in the Shionogi tumor model. *Cancer Res.* (1999) 59(16):4030-4034.
104. MIYAKE H, MONIA BP, GLEAVE ME: Inhibition of progression to androgen-independence by combined adjuvant treatment with antisense BCL-XL and antisense Bcl-2 oligonucleotides plus taxol after castration in the Shionogi tumor model. *Int. J. Cancer* (2000) 86(6):855-862.
105. GEIGER T, MULLER M, MONIA BP, FABBRO D: Antitumor activity of a C-raf antisense oligonucleotide in combination with standard chemotherapeutic agents against various human tumors transplanted subcutaneously into nude mice. *Clin. Cancer Res.* (1997) 3(7):1179-1185.
106. GLEAVE ME, MIYAKE H, ZELLWEGER T *et al.*: Use of antisense oligonucleotides targeting the antiapoptotic gene, clusterin/testosterone-repressed prostate message 2, to enhance androgen sensitivity and chemosensitivity in prostate cancer. *Urology* (2001) 58(2 Suppl. 1):39-49.
107. MCELENY K, COFFEY R, MORRISSEY C *et al.*: An antisense oligonucleotide to cIAP-1 sensitizes prostate cancer cells to fas and TNFalpha mediated apoptosis. *Prostate* (2004) 59(4):419-425.
108. WANG H, YU D, AGRAWAL S, ZHANG R: Experimental therapy of human prostate cancer by inhibiting MDM2 expression with novel mixed-backbone antisense oligonucleotides: *in vitro* and *in vivo* activities and mechanisms. *Prostate* (2003) 54(3):194-205.
109. GEIGER T, MULLER M, DEAN NM, FABBRO D: Antitumor activity of a PKC-alpha antisense oligonucleotide in combination with standard chemotherapeutic agents against various human tumors transplanted into nude mice. *Anti-Cancer Drug Des.* (1998) 13(1):35-45.
110. MIYAKE H, POLLAK M, GLEAVE ME: Castration-induced up-regulation of insulin-like growth factor binding protein-5 potentiates insulin-like growth factor-I activity and accelerates progression to androgen independence in prostate cancer models. *Cancer Res.* (2000) 60(11):3058-3064.
111. LEE CH, LIU M, SIE KL, LEE MS: Prostate-specific antigen promoter driven gene therapy targeting DNA polymerase-alpha and topoisomerase II alpha in prostate cancer. *Anti-Cancer Res.* (1996) 16(4A):1805-1811.
112. EDER IE, CULIG Z, RAMONER R *et al.*: Inhibition of LncaP prostate cancer cells by means of androgen receptor antisense oligonucleotides. *Cancer Gene Ther.* (2000) 7(7):997-1007.
113. CHEN S, SONG CS, LAVROVSKY Y*et al.*: Catalytic cleavage of the androgen receptor messenger RNA and functional inhibition of androgen receptor activity by a hammerhead ribozyme. *Mol. Endocrinol.* (1998) 12(10):1558-1566.
114. XU ZD, OEY L, MOHAN S *et al.*: Hammerhead ribozyme-mediated cleavage of the human insulin-like growth factor-II ribonucleic acid *in vitro* and in prostate cancer cells. *Endocrinology* (1999) 140(5):2134-2144.
115. DORAI T, PERLMAN H, WALSH K *et al.*: A recombinant defective adenoviral agent expressing anti-bcl-2 ribozyme promotes apoptosis of bcl-2-expressing human prostate cancer cells. *Int. J. Cancer* (1999) 82(6):846-852.
116. COLLIS SJ, TIGHE A, SCOTT SD, ROBERTS SA, HENDRY JH, MARGISON GP: Ribozyme minigene-mediated RAD51 down-regulation increases radiosensitivity of human prostate cancer cells. *Nucleic Acids Res.* (2001) 29(7):1534-1538.
117. TEKUR S, HO SM: Ribozyme-mediated downregulation of human metallothionein II(a) induces apoptosis in human prostate and ovarian cancer cell lines. *Mol. Carcinog.* (2002) 33(1):44-55.
118. AIGNER A, RENNEBERG H, BOJUNGA J, APEL J, NELSON PS,

CZUBAYKO F: Ribozyme-targeting of a secreted FGF-binding protein (FGF-BP) inhibits proliferation of prostate cancer cells *in vitro* and *in vivo*. *Oncogene* (2002) 21(37):5733-5742.

119. ORLANDI L, BINDA M, FOLINI M *et al.*: Ribozyme-mediated inhibition of PKCalpha sensitizes androgen-independent human prostate cancer cells to cisplatin-induced apoptosis. *Prostate* (2003) 54(2):133-143.
120. PENNATI M, BINDA M, COLELLA G *et al.*: Ribozyme-mediated inhibition of survivin expression increases spontaneous and drug-induced apoptosis and decreases the tumorigenic potential of human prostate cancer cells. *Oncogene* (2004) 23(2):386-394.
121. KIM SJ, JOHNSON M, KOTERBA K, HERYNK MH, UEHARA H, GALLICK GE: Reduced c-Met expression by an adenovirus expressing a c-Met ribozyme inhibits tumorigenic growth and lymph node metastases of PC3-LN4 prostate tumor cells in an orthotopic nude mouse model. *Clin. Cancer Res.* (2003) 9(14):5161-5170.
122. KALIBEROV SA, KALIBEROVA LN, BUCHSBAUM DJ: Combined ionizing radiation and *sKDR* gene delivery for treatment of prostate carcinomas. *Gene Ther.* (2005) 12:407-417.
123. GOWARDHAN B, WEST AF, ROBSON CN, LEUNG HY: Adenovirus-mediated expression of a soluble fibroblast growth factor receptor inhibits *in vitro* growth of prostate DU145 cells. *Prostate* (2004) 61(1):50-59.
124. YASUMOTO H, MATSUBARA A, MUTAGUCHI K, USUI T, MCKEEHAN WL: Restoration of fibroblast growth factor receptor2 suppresses growth and tumorigenicity of malignant human prostate carcinoma PC-3 cells. *Prostate* (2004) 61(3):236-242.
125. KIM M, M. W, DESHANE J, ACCAVITTI MA *et al.*: A novel gene therapy strategy for elimination of prostate carcinoma cells from human bone marrow. *Hum. Gene Ther.* (1997) 8:157-170.
126. BAUERSCHMITZ GJ, BARKER SD, HEMMINKI A: Adenoviral gene therapy for cancer: from vectors to targeted and replication competent agents (review). *Int. J. Oncol.* (2002) 21(6):1161-1174.
127. ZAISS AK, LIU Q, BOWEN GP, WONG NC, BARTLETT JS, MURUVE DA: Differential activation of innate immune responses by adenovirus and adeno-associated virus vectors. *J. Virol.* (2002) 76(9):4580-4590.
128. MARCONI P, KRISKY D, OLIGINO T *et al.*: Replication-defective herpes simplex virus vectors for gene transfer *in vivo*. *Proc. Natl. Acad. Sci. USA* (1996) 93(21):11319-11320.
129. HWANG C, SANDA MG: Prospects and limitations of recombinant poxviruses for prostate cancer immunotherapy. *Curr. Opin. Mol. Ther.* (1999) 1(4):471-479.
130. HACEIN-BEY-ABINA S, VON KALLE C, SCHMIDT M *et al.*: A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. *N. Engl. J. Med.* (2003) 348(3):255-256.
131. BONNET MC, TARTAGLIA J, VERDIER F *et al.*: Recombinant viruses as a tool for therapeutic vaccination against human cancers. *Immunol. Lett.* (2000) 74(1):11-25.
132. GLOVER DJ, LIPPS HJ, JANS DA: Towards safe, non-viral therapeutic gene expression in humans. *Nat. Rev. Genet.* (2005) 6(4):299-310.
133. CHEN Y, DEWEESE T, DILLEY J *et al.*: CV706, a prostate cancer-specific adenovirus variant, in combination with radiotherapy produces synergistic antitumor efficacy without increasing toxicity. *Cancer Res.* (2001) 61(14):5453-5460.
134. COLLIS SJ, DEWEESE TL: Enhanced radiation response through directed molecular targeting approaches. *Cancer Metastasis Rev.* (2004) 23(3-4):277-292.
- This paper describes the molecular basis for using gene therapy in combination with ionising radiation for cancer therapy.
135. SENZER N, MANI S, ROSEMURGY A *et al.*: TNFerade biologic, an adenovector with a radiation-inducible promoter, carrying the human tumor necrosis factor alpha gene: a Phase I study in patients with solid tumors. *J. Clin. Oncol.* (2004) 22(4):592-601.

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