

Gene Therapy Strategies in Prostate Cancer

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Abstract: Androgen ablation is the choice of treatment for patients with advanced prostate cancer. Although untreated tumors are mostly androgen-dependent, hormone withdrawal is only palliative. The major problem in prostate cancer treatment represents the progression to androgen-independent growth during therapy, rendering current strategies inefficient. Thus, there is an urgent need to develop novel treatments to combat therapy-resistant prostate cancer. Intensive research strongly improved the knowledge about the molecular changes, which are believed to occur during prostate carcinogenesis and progression to androgen-independence. This in turn led to the identification of several interesting genes, which may be useful as targets for prostate cancer gene therapy. In fact, there is a broad range of different gene therapy approaches in the field of prostate cancer, some of which have already progressed to clinical evaluation in patients. Promising data and best benefit for patients currently provide studies where gene therapy strategies are combined with conventional treatments like chemotherapy or radiation.

In this review we will give an overview of several interesting gene therapy concepts and delivery systems in prostate cancer and discuss their usefulness in the clinic.

Keywords: Gene therapy, prostate cancer, antisense, viral vectors, ultrasound, microbubbles.

INTRODUCTION

Surgery is the first choice of treatment for patients with prostate cancer, one of the most frequently diagnosed malignancies in men. However, surgical removal of the prostate is only successful for organ-confined disease. Tumors which already grow beyond the prostate capsule or which recur after surgery, can be treated with androgen ablation. This is mostly successful, since the majority of untreated prostate tumors are androgen-dependent [Brinkmann, A.O., *et al.*, 1999]. A major problem in prostate cancer treatment is that prostate tumor cells are able to adapt to their androgen-deprived environment and become androgen-independent during hormone ablation. Since chemotherapy or radiotherapy have only limited success in patients with advanced prostate cancer, there is currently no efficient therapy available.

Gene therapy may therefore, be a potential tool to treat patients in this stage of the disease. There is a panel of different gene therapeutic approaches, which either encompass to abrogate the expression of tumor growth-promoting genes, or to restore the "normal" status of genes, which are mutated, down-regulated or lost in tumor cells. Other strategies include inhibition of tumor neovessel formation, induction of apoptosis, or stimulation of the immune system.

Intensive research strongly improved our knowledge about the molecular changes, which are thought to be associated with prostate cancer development and progression to therapy resistance. Nevertheless, the right choice of target is thought to be anything but trivial, especially in terms of

the strong heterogeneity of prostate tumors. There is a large number of genetic alterations, which have been associated with prostate cancer, and which may be exploited to develop a gene therapeutic approach (Table 1). Gain or loss of specific alleles [Nupponen, N.N., *et al.*, 1998] but also overexpression of tumor growth-promoting genes, so-called oncogenes have often been described to stimulate proliferation and tumor progression, respectively. On the other hand, tumor suppressor genes may be downregulated, lost or inactivated by mutations. Some of the most intensively studied molecules in prostate cancer are p53, the retinoblastoma gene (RB), PTEN, ras, and HER-2 [Lara, P.N. Jr., *et al.*, 1999, Shi, X.B., *et al.*, 2002]. Moreover, antiapoptotic genes like bcl-2 [Miyake, H., *et al.*, 1999], testosterone-repressed prostate message-2 (TRPM-2) [Miyake, H., *et al.*, 2000], and members of the IGF signaling pathway [Djakiew, D., 2000] have been associated with prostate cancer.

A considerably important role in tumor progression and metastasis plays the extracellular matrix (ECM), which represents a natural barrier to tumor cells. Its main components laminin and collagen, but also non-collagenous proteins such as osteopontin, fibronectin, and thrombospondin and cell adhesion molecules like integrins, cadherins, and selectins are thought to be essential for migration and attachment of disseminating cancer cells. Moreover, several proteases (matrix metalloproteinases, cathepsins) strongly influence the organization of the ECM and are likely to contribute to metastasis (for review see: [Stewart, D.A., *et al.*, 2004]). Multiple studies have demonstrated differential patterns of expression of ECM-associated molecules, which may be useful gene therapy targets.

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Table 1. Possible Molecular Changes Associated with Prostate Cancer

Molecular alterations	Possible targets
Gain or loss of specific alleles	
Altered expression of tumor growth-promoting genes	ras, HER-2, IGF signaling molecules
Inactivation of tumor suppressor genes	p53, RB, PTEN
Up-regulation of anti-apoptotic genes	bcl-2, TRPM-2
Hypermethylation of GSTP-1	GSTP-1
Increased AR activation and/or expression	AR
Cell cycle regulatory molecules	p21, Ki-67, c-myc
Changes in ECM organization	collagen, laminin cell adhesion molecules (integrins, cadherins, selectins) proteases (matrix metalloproteinases)
Evasion from immunosurveillance	IL-2, IL-12

One event that may cause expression changes is hypermethylation. Hypermethylation of the glutathione-S-transferase (GSTP1) promoter was found in prostate tumor specimens and also in very early prostate tumor lesions of intraepithelial neoplasia (PIN lesions), considering that this is one of the very early events in prostate cancer [Brooks, J.D., *et al.*, 1998, Lee, W.H., *et al.*, 1997, Lee, W.H., *et al.*, 1994].

An important key regulatory role in prostate cancer seems to play the androgen receptor (AR). This intracellular steroid receptor mediates the action of androgens and thereby regulates growth, function, and differentiation of prostate cells [Grino, P.B., *et al.*, 1990, Wilding, G., 1995, Wilson, J.D., *et al.*, 1995]. It is expressed in primary prostate cancer as well as in advanced hormone-refractory tumors [Ruizevelt de Winter, J.A., *et al.*, 1994, Van der Kwast, T.H., *et al.*, 1991] and in metastatic lesions [Hobisch, A., *et al.*, 1995, Hobisch, A., *et al.*, 1996]. Furthermore, AR gene amplifications may occur in tumors, which recurred after androgen ablation therapy [Palmberg, C., *et al.*, 1997, Visakorpi, T., *et al.*, 1995] and mutations in the AR gene can result in a broad ligand binding activation spectrum. The importance of the AR in prostate cancer is further outlined by the occurrence of ligand-independent activation through various growth factors like the epithelial growth factor (EGF), the insulin-like growth factor (IGF), and also through cytokines such as IL-6 [Culig, Z., *et al.*, 1993, Culig, Z., *et al.*, 1994, Culig, Z., *et al.*, 1996, Klocker, H., *et al.*, 1999, Veldscholte, J., *et al.*, 1990, Veldscholte, J., *et al.*, 1992, Yang, L., *et al.*, 2003, Zhao, X.-Y., *et al.*, 2000]. The AR can even be activated through cross-talk with proteins from other intracellular signaling pathways such as the mitogen-activated protein kinase (MAPK) [Yeh, S. and Chang, C., 1996] and the Rho effector protein kinase C-related kinase [Metzger, E., *et al.*, 2003], pointing out its potential use as a target for gene therapy (Fig. 1).

Another important point in tumorigenesis and progression to therapy resistance is the ability of tumor cells to escape immune surveillance. Although the precise mechanisms underlying the failure of the natural immune system to prevent tumor formation are still unclear, there are several gene therapeutic approaches, which tend to re-stimulate the patients immune responses against cancer cells.

GENE DELIVERY METHODS

One major question in gene therapy is the efficient and safe transfer of the genetic material into the target tissue.

Viral Vectors

Viral vectors represent potential gene delivery vehicles. Especially with respect to a use in patients, highly attenuated or non-replicative viruses represent good candidates for gene delivery. Among the broad range of viruses, retro-, adeno-, adeno-associated, and Herpes simplex viruses are the most frequently used in prostate cancer. According to the differences concerning infection strategies, the different viral vectors may have advantages as well as disadvantages.

Retroviral vectors induce the insertion of the therapeutic gene into the host genome, resulting in stable and efficient transfection. This makes their use in humans critical due to high risk of genetic instability and mutagenesis [Bonnet, M.C., *et al.*, 2000]. Vector systems developed from murine oncogenic retroviruses have been successfully used for gene transfer. Their use in the clinic, however, is limited by the high risk to induce genetic instability and mutagenesis and by their inability to infect non-dividing cells, a problem, which can be overcome by lentiviruses. Although lentiviral vectors obviously represent potential gene delivery tools, their pathogenicity is a major critical point with respect to their use in patients [Buchsacher, G.L. Jr. and Wong-Staal, F. 2000].

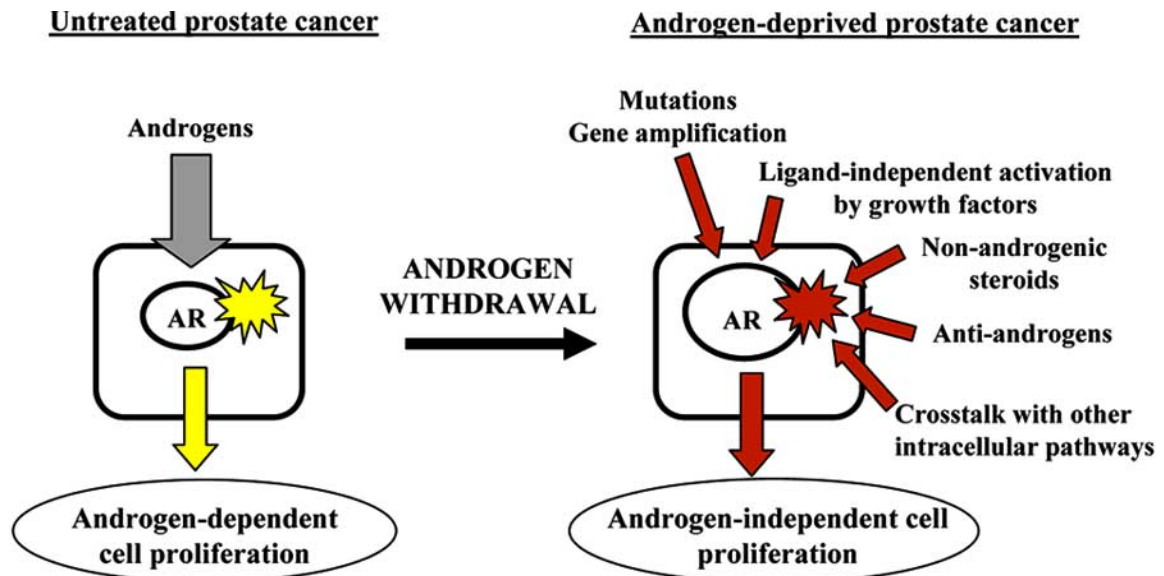


Fig. (1). Possibilities of AR activation in androgen-dependent and androgen-independent prostate cancer.

The androgen receptor (AR) mediates the action of androgens, which regulate growth and differentiation of prostate cells. Since the majority of untreated prostate tumors are androgen-dependent, androgen withdrawal results in apoptosis and tumor growth inhibition. However, prostate tumor cells are able to adapt to their androgen-deprived milieu. The AR and its activation in the absence of androgens thereby is believed to play a major role. Growth factors like EGF and IGF, non-androgenic steroids, and even antiandrogens are able to activate the AR. Ligand-independent AR activation may also occur through crosstalk with other intracellular signaling pathways. Moreover, mutations in the AR can result in a broad ligand binding activation spectrum. The importance of the AR in contributing to tumor cell survival and proliferation may be further strengthened by the fact that the AR gene is often amplified in advanced prostate cancer.

By contrast to retroviral vectors, adenoviral vectors can efficiently transfer genes into both, replicating and non-replicating cells. This may be advantageous for gene transfer in prostate tumor cells, which grow slowly [Ali, M., *et al.*, 1994]. In fact, several preclinical investigations and also clinical trials have been performed to study the use of replication-defective, replication-competent as well as conditionally replication-competent adenoviral vectors in prostate cancer [Herman, J.R., *et al.*, 1999, Hsieh, C.L., *et al.*, 2002, Matsubara, S., *et al.*, 2001, Satoh, T., *et al.*, 2003]. They are most frequently used in prostate cancer clinical trials and intensive research has resulted in improvement of safety, specificity, and cytotoxicity. Nevertheless, the use of adenoviral vectors in the clinic may be limited due to their strong immunogenicity [Bonnet, M.C., *et al.*, 2000].

Herpes simplex viral vectors are also frequently used in prostate cancer gene therapy. These viruses contain double stranded DNA and replicate in the nucleus of the host cell. They are commonly used vectors to induce the expression of prodrug activation genes [Kubo, H., *et al.*, 2003, Park, H.S., *et al.*, 2003]. Nevertheless, several studies have documented that they can cause strong immunological responses [Kaminski, J.M., *et al.*, 2003].

Other viruses, which were shown to be useful vector constructs in prostate cancer patients, are vaccinia viruses. These DNA poxviruses have a large genome and are therefore able to express large foreign proteins. Moreover, they are able to transfect dividing as well as non-dividing cells [Eder, J.P., *et al.*, 2000, Gulley, J., *et al.*, 2002].

Summarizing, viruses are obviously potential vectors yielding high transfection efficiencies. The major argument

against the use of viral vectors is the risk of increased genetic instability of transfected host cells. Moreover, they may induce strong immunological responses, which should be considered especially in terms of repeated drug administration [Baum, C., *et al.*, 2003, Ikegami, S., *et al.*, 2002, Isner, J.M., 2002]. Since tumor patients are often immune-suppressed, these side effects may have severe consequences. Safety testing and also technical improvements are inevitable and researchers should always keep "an open eye" when using viral vectors. Then they undoubtedly represent potential delivery vehicles for gene transfer.

Tissue-specific Promoters

The use of specific promoters allows to restrict gene activation to the target site and this in turn may contribute to reduced toxic side effects. Unfortunately, only a limited number of prostate-specific promoters such as PSA or rat probasin (rPb) have been identified so far [Latham, J.P., *et al.*, 2000, Pang, S., 2000]. PSA and rPb promoters are strong and efficient promoters, however, their activation is strongly regulated through androgens. In patients with hormone-refractory prostate cancer, who, in general, already underwent androgen ablation, androgen-regulated promoters may therefore have only limited efficacy. In order to overcome this problem, androgen-independent prostate-specific promoters like osteocalcin or the prostate-specific membrane antigen (PSMA) may be more useful [Ikegami, S., *et al.*, 2002, Matsubara, S., *et al.*, 2001]. Another possibility is to modify androgen-dependent promoters like rPb in order to render them active also in the absence of

androgens by introducing a retinoic acid-response element [Furuhata, S., *et al.*, 2003].

Another interesting strategy is the use of an osteocalcin promoter [Kubo, H., *et al.*, 2003]. Since osteocalcin is a major noncollagenous bone matrix protein, which is highly expressed in prostate cancer cells but also in osteoblasts, this osteocalcin-driven promoter allows a prostate tumor cell-specific activation of the therapeutic gene not only in the primary tumor but also in metastatic lesions, which are frequently found in lymph nodes and bone [Matsubara, S., *et al.*, 2001]. This treatment was shown to be well tolerated in patients with locally recurrent prostate cancer [Herman, J.R., *et al.*, 1999] and in patients with lymph node and bone metastasis of hormone-refractory prostate cancer [Kubo, H., *et al.*, 2003]. Osteocalcin promoter driven adenoviral-mediated gene delivery was also shown to be useful to co-target prostate tumor epithelial cells and bone stromal cells. This strategy allows to inhibit the intercellular communication between the epithelium and the stroma, which is thought to have a strong influence on prostate tumor growth and progression [Matsubara, S., *et al.*, 2001].

Recently, conditionally replication-competent vectors have been developed where the adenoviral replication is driven by a specific promoter such as PSA, vitamin D3, or osteocalcin to further increase cytotoxicity and acquire tissue specificity [Hsieh, C.L., *et al.*, 2001].

Pramudji *et al.* investigated the advantages to use a caveolin-1 promoter, which should not only be more active than PSA promoters but also additionally direct tumor-associated endothelial cells, thus producing a bystander effect [Pramudji, C., *et al.*, 2001]. Their results in fact supported the concept of targeting prostate tumor cells and tumor-associated endothelial cells simultaneously.

Similarly, the promoter of the DD3^{PCa3} gene, was described to be useful in gene therapy to specifically deliver therapeutic agents to prostate cancer [Verhaegh, G.W., *et al.*, 2000]. DD3^{PCa3} expression is restricted to prostate tissue and was found to be highly upregulated in prostate cancer [Bussemakers, M.J., *et al.*, 1999]. Preclinical experiments demonstrated that DD3^{PCa3}-based adenoviral constructs are attractive candidates to be used in prostate cancer gene therapy [Schalken, J.A., *et al.*, 2003].

Non Viral Delivery Methods

The simplest method of drug delivery is the injection of naked DNA. This however includes problems such as inefficient uptake of the therapeutic gene into the target cells or rapid clearance of the DNA from the circulation. Antisense oligodeoxynucleotides (ODNs) are most commonly administered like that. Numerous chemical modifications have been explored to improve the usefulness of antisense ODNs. These so-called "second generation ODNs" are more potent and very stable against exonucleases [Dean, N.M. and Bennett, C.F., 2003]. Nevertheless, administration of naked DNA often results in unsatisfying therapy responses.

Transfection efficiency can be increased with the help of liposomes [Caplen, N.J., 2000, Caplen, N.J., *et al.*, 1995, Galanis, E., *et al.*, 1999, Rini, B.I., *et al.*, 1999] but also

through physical methods such as electroporation [Bergan, R., *et al.*, 1993, Bergan, R., *et al.*, 1996, Eder, I.E., *et al.*, 2000, Flanagan, W.M. and Wagner, R.W., 1997]. Gokhale and coworkers recently reported on novel cationic liposomes for *in vivo* delivery of antisense ODNs against c-raf. They showed that antisense ODNs entrapped into liposomes were more stable in plasma and tissue and also improved the antitumor effect *in vivo* [Gokhale, P.C., *et al.*, 2002]. Several other lipid-based vectors, such as "Leuvectin" are currently under clinical evaluation (www.wiley.co.uk/genetherapy/ clinical).

Recently, ultrasound (US) has gained attraction for gene transfer. It is believed that treatment of cells with US increases the permeability of eukaryotic cell membranes to various agents including DNA. The major advantage of US is its easy and safe use in patients and the opportunity to deliver therapeutic agents repeatedly as well as site-specifically. US treatment was successfully used to transfect prostate cancer cells *in vitro* [Bao, S., *et al.*, 1997, Tata, D.B., *et al.*, 1997, Unger, E.C., *et al.*, 1997] and *in vivo* [Anwer, K., *et al.*, 2000, Huber, P.E. and Pfisterer, P., 2000, Miller, D.L., *et al.*, 1999]. Latest studies have shown that transfection efficiency can be further optimized by combining US treatment with the application of contrast agent microbubbles. Microbubbles are routinely used as diagnostic tools for the enhancement of US imaging of cardiologic diseases [Lawrie, A., *et al.*, 2000] and might also be considered for use in prostate cancer diagnosis [Frauscher, F., *et al.*, 2001, Halpern, E.J., *et al.*, 2000]. They consist of a membrane on which DNA or any other drug can be bound relatively easily [Srinivasan, S.K., *et al.*, 1995] and an interior void with a biocompatible gas, which allows their disruption by US exposure [Porter, T.R., *et al.*, 1996]. Similar to US, microbubble contrast agents are potential and safe tools for clinical use [Frenkel, P.A., *et al.*, 2002]. In terms of gene therapy, microbubbles have gained interest as efficient carriers, even if this possibility is still under laboratory research. The idea behind is, that the gene therapeutic agent is attached to the microbubble surface, delivered into the target tissue, and released site-specifically by destruction of the bubbles with US [Porter, T.R. and Xie, F., 2001, Skyba, D.M., *et al.*, 1998, Unger, E.C., *et al.*, 2001, Unger, E.C., *et al.*, 2002]. Recent studies have shown that US-targeted microbubbles can be successfully used to deliver an antisense ODN into coronary endothelial cells *in vitro* [Miura, S., *et al.*, 2002]. There are several other reports, which demonstrate the usefulness of this method for efficient gene transfer *in vivo* mainly for therapy of heart diseases [Azuma, H., *et al.*, 2003, Miller, D.L. and Song, J., 2003] and it was also used to deliver an adenoviral vector [Chen, S., *et al.*, 2003].

Similarly to tissue-specific promoters, microbubbles may be modified on their lipid surface with cancer-specific marker molecules in order to direct the drugs specifically to tumor cells. Modification of microbubbles with specific antibodies has already been shown to improve accumulation in tumor tissue [Friedlander, M., *et al.*, 1995, Leong-Poi, H., *et al.*, 2003]. Recently, a number of novel peptides, predominantly expressed in the prostate tumor vasculature, were identified and may be useful marker molecules *in vivo* [Arap, W., *et al.*, 2002]. To date, microbubble-enhanced US

is a rather novel technique which warrants further preclinical evaluation, but nevertheless there is great promise in its use as a drug delivery system in the future.

GENE THERAPY APPROACHES IN PROSTATE CANCER

It is important to note that the molecules listed above represent only a small portion of the genes which are actually considered to be important in prostate cancer. Novel high throughput techniques, which allow to investigate broad expression spectra of tumor cells, revealed a tremendous amount of potential target genes and therefore the list of gene therapeutic strategies is also increasing. Depending upon the kind of molecular alteration, the approaches to combat prostate tumor growth are correspondingly different.

Abrogating the Expression of Tumor Growth-Promoting Genes

One possibility to develop an "anti-cancer drug" is to abrogate the expression of genes, which significantly stimulate tumor growth. Most of these tumor growth-promoting genes are involved in cell cycle regulation, apoptosis, angiogenesis, tumor invasion, and signal transduction.

Gotoh and coworkers used an adenoviral vector to abrogate p21 expression in hormone-refractory prostate cancer, resulting in significant inhibition of tumor growth *in vitro* as well as *in vivo* [Gotoh, A., *et al.*, 2003]. p21 is a cyclin-dependent kinase inhibitor, which regulates cell cycle arrest in G1 phase. Its abnormal expression was associated with poor prognosis of prostate cancer patients [Aaltomaa, S., *et al.*, 1999].

A unique approach to inhibit gene expression represents the use of antisense ODNs. These short nucleotide sequences are able to inhibit the expression of one particular molecule by binding to complementary sequences in the mRNA of the

target gene. There is a panel of promising antisense approaches to inhibit prostate tumor growth, some of which have already reached clinical evaluation (Table 2). One of the most intensively studied antisense-targets in prostate cancer is the anti-apoptotic protein bcl-2. Bcl-2 is frequently overexpressed in hormone-refractory prostate cancer with considerable impact on progression to androgen-independence [Colombel, M., *et al.*, 1993, McDonnell, T.J., *et al.*, 1997]. Gleave and coworkers have demonstrated that downregulation of bcl-2 with an antisense phosphorothioate ODN significantly inhibits prostate tumor growth *in vitro* as well as *in vivo* [Gleave, M., *et al.*, 2003, Gleave, M., *et al.*, 1999]. The therapeutic potential of bcl-2 downregulation has already been evaluated in various clinical trials including prostate cancer patients. It was shown, for instance, that bcl-2 antisense treatment enhances the sensitivity of prostate tumors to the chemotherapeutic agent docetaxel [Chi, K.N., *et al.*, 2001, Tolcher, A.W., 2001]. In a similar study, Miyake *et al.* investigated the efficacy of targeting another anti-apoptotic protein. They demonstrated that the downregulation of clusterin with an antisense ODN also supports the tumor growth inhibiting activity of the chemotherapeutic agent paclitaxel [Miyake, H., *et al.*, 2003]. These data show that antisense ODNs directed against anti-apoptotic molecules like bcl-2 and clusterin are useful tools to enhance the effect of conventional chemotherapeutic drugs. Clinical studies are currently ongoing to test the efficacy and toxicity of this combined treatment strategy to treat prostate cancer patients.

There are several other antisense strategies in the literature targeting genes such as C-raf, protein kinase C, and protein kinase A. These genes are critical elements in mitogen and stress-induced signaling response, cell survival, and proliferation. Inhibiting their expression is therefore thought to have a strong impact on prostate tumor growth. C-raf, for instance, as part of the MAPK signaling pathway, has an essential role in cell survival by transducing the signals, which come from cell membrane receptors after binding of

Table 2. Antisense Approaches in Prostate Cancer

Target gene	Drug	Targeted biochemical	Current status of investigation
Bcl-2	G3139	apoptosis	Phase II/III clinical trial
Protein kinase C alpha	ISIS 3521	signal transduction	Phase II/III clinical trial
c-raf	LErafAON	signal transduction	Phase I/II clinical trial
Clusterin	OGX-011	apoptosis	Phase I clinical trial
myc	AVI 4126	cell cycle regulation	Phase I clinical trial
protein kinase A	GEM 231	signal transduction	preclinical studies
androgen receptor	ODN	signal transduction	preclinical studies
Ki-67	ODN	cell cycle regulation	preclinical studies
IGF receptor	ODN	signal transduction	<i>in vitro</i> studies
fatty acid synthase	siRNA	fatty acid metabolism	<i>in vitro</i> studies

growth factor ligands like EGF and IGF [Culig, Z., *et al.*, 1996, Zhu, X. and Liu, J.-P., 1997]. Preclinical studies in fact have demonstrated that abrogating C-raf expression results in significant reduction of prostate tumor burden [Geiger, T., *et al.*, 1997, Kasid, U., *et al.*, 1989]. Clinical trials were conducted to investigate the usefulness of antisense c-raf ODNs to treat prostate cancer, with best results as combinatorial therapy [Cho, Y.S. and Cho-Chung, Y.S., 2003, Kasid, U. and Dritschilo, A., 2003]. Furthermore, encouraging data were obtained with antisense ODNs targeting protein kinase C alpha, another essential molecule of cell signal transduction [Benimetskaya, L., *et al.*, 2001, Tolcher, A.W., *et al.*, 2002]. Again, response rates in patients were highest in combination with another antisense drug or a chemotherapeutic agent.

Other promising molecular targets to develop a gene therapeutic approach are the cell cycle regulatory molecules Ki-67 [Kausch, I., *et al.*, 2003] and c-myc [Iversen, P.L., *et al.*, 2003]. Overexpression of c-myc was associated with uncontrolled cell proliferation in androgen-refractory prostate cancer. An antisense oligomer against c-myc was shown to induce a significant reduction in tumor burden after intravenous administration in patients with androgen-independent prostate cancer [Iversen, P.L., *et al.*, 2003].

There are also several strategies to inhibit the expression of molecules, which are known to have a strong impact on prostate cancer cell signal transduction pathways. Blocking the expression of the IGF1 receptor or several IGF binding proteins were shown to inhibit prostate tumor growth [Hellawell, G.O., *et al.*, 2003]. In our own lab, we investigated the effects of an antisense ODN directed against the AR. We could show that AR downregulation results in a significant inhibition of prostate tumor growth *in vitro* as well as *in vivo* [Eder, I.E., *et al.*, 2002]. Inhibition of AR expression also inhibited an androgen-independently growing prostate cancer cell line, LNCaPabl, which was established by long-term culture in an androgen-deprived medium [Culig, Z., *et al.*, 1999]. Recently, another group reported on prostate cancer cell growth arrest through AR inhibition with antisense molecules [Hamy, F., *et al.*, 2003].

Antisense technology has recently been extended by the use of small interference RNA (siRNA) molecules. These short double-stranded RNA compounds are directed against a specific sequence within the target gene, resulting in mRNA degradation by forming a so-called silencing complex (RISC) [Lieberman, J., *et al.*, 2003, Zamore, P.D., *et al.*, 2000]. siRNA molecules targeting fatty acid synthase (FAS) were shown to inhibit prostate tumor cell growth *in vitro* [De Schrijver, E., *et al.*, 2003]. FAS is a key enzyme in fatty acid metabolism, which is frequently overexpressed in prostate cancer [Swinnen, J.V., *et al.*, 2002]. siRNAs are thought to have several advantages over antisense ODNs including higher stability and also higher activity, allowing to apply significantly lower drug concentrations. Their use in gene therapy holds great promise even if, to date, siRNAs have not been investigated in humans.

Re-expression of Tumor Growth-Inhibiting Genes

Another important and also commonly used approach in gene therapy is the possibility to restore the expression of

tumor suppressor genes, which are frequently downregulated, lost or mutated in tumor cells. This certainly requires that the genetic material is transferred efficiently into the tumor, most commonly achieved by using viral vectors, which have been described above. Maspin, for instance, a potential tumor suppressor gene, was overexpressed in prostate cancer cells with the help of a retroviral vector. This overexpression was shown to decrease tumorigenicity and metastatic potential of tumor cells [Abraham, S., *et al.*, 2003].

One of the most intensively studied tumor suppressor genes is p53. It was found to be abnormally expressed in prostate cancer cells [Stackhouse, G.B., *et al.*, 1999, Tamboli, P., *et al.*, 1998] and correction of mutated p53 was speculated to inhibit prostate cancer cell proliferation. In fact, adenoviral-mediated transfection with wild-type p53 resulted in prostate tumor growth retardation regardless of p53 mutation status [Ko, S.C., *et al.*, 1996]. Another study reported on the introduction of wild-type p53 into androgen-independently growing prostate tumors with the help of an adenoviral vector.

There are also approaches to restore the expression of pro-apoptotic molecules like caspase-7. Overexpression of caspase-7 in prostate cancer cells through an adenoviral vector resulted in induction of apoptosis and significant inhibition of tumor cell growth [Marcelli, M., *et al.*, 1999].

Prodrug Gene Activation

A particularly interesting strategy with promising efficacy is the so-called prodrug gene therapy, or suicide gene therapy. The principle behind is that tumor growth arrest is achieved indirectly through a vector-mediated enzyme, which is able to convert a relatively nontoxic prodrug into a biologically active cytotoxic agent [Hall, S.J., *et al.*, 1997, Park, H.S., *et al.*, 2003]. This approach is often used to improve the response of tumor cells to chemotherapeutic agents. Freytag *et al.* transferred the herpes simplex virus thymidine kinase (HSV-tk) in combination with cytosine desaminase into prostate tumors resulting in increased sensitization of tumor cells to chemotherapy and radiation, respectively [Freytag, S.O., *et al.*, 2002]. Clinical trials, which were performed to evaluate efficacy and safety of this prodrug gene approach are quite promising.

Immunomodulatory Strategies

There are several immunotherapeutic studies, which focus on the stimulation of the patient's own immune system to combat prostate tumor growth for review see [Kaminski, J.M., *et al.*, 2003]. Some of these studies include the gene therapeutic delivery of cytokines such as IL-2 or IL-12 into immune cells to enhance immune responses [Beldegrun, A., *et al.*, 2001, Hull, G.W., *et al.*, 2000]. Another concept encompasses the transfer of tumor-associated antigens like PSA or PSMA. Altered antigen presentation on the surface of tumor cells prevents a recognition by the immune system and enables them to evade immunosurveillance. Genetic transfer of PSA or PSMA have been shown to stimulate immune responses in prostate cancer patients [Harris, D.T., *et al.*, 1999, Tasch, J., *et al.*, 2001]. Several immunomodulatory clinical trials are under way (<http://www.wiley.co.uk/genetherapy/clinical>).

CONCLUSION

Improved knowledge about the molecular biology of prostate cancer allowed to establish a number of novel gene therapy approaches. However, it became obvious that prostate tumor growth and the progression to androgen-independence, respectively, comprises a broad range of molecular alterations rather than the change of one single gene. This strong heterogeneity of prostate cancer may represent a major problem in response to gene therapy. Another intriguing problem is the lack of a potential and safe delivery system and intensive studies in this field are inevitably warranted. Technical improvements may hopefully help to be successful in the treatment of patients in the future. But in the meantime, current gene therapy strategies may be best used to overcome resistance to chemotherapeutic agents or radiation.

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