Review

Gene therapy for prostate cancer

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The treatment options for the patient with advanced prostate cancer are limited. Due to the recent advances in the understanding of the molecular biology of prostate cancer, the easy accessibility of the gland for injection, and the availability of gene promoters that can provide tissue specific expression of therapeutic gene sequences, gene therapy for prostate cancer is rapidly advancing. Many potential approaches for prostate cancer gene therapy have been identified, with a few of these already entering Phase I clinical trials. This review will discuss the basis of prostate cancer gene therapy, look at the potential approaches, and will compare the different vectors that can be used to deliver the therapeutic genes to the prostate tumor.

Keywords: prostate cancer; gene therapy; review article

Introduction

The current treatment of organ confined prostate cancer involves radical prostatectomy, brachytherapy, or external beam irradiation. While these therapies are curative for the patient who has a tumor that is confined to the prostate itself, there is no definitive cure for patients who have metastatic disease. Additionally, surgical and radiation based treatments will fail in up to 30% of patients, despite the presence of what appears to be an organ-confined tumor.¹ For the patient who has metastatic or recurrent prostate cancer, anti-androgen therapy can be very effective at inducing apoptotic cell death of the tumor over the short term. Unfortunately, most of these patients will eventually develop hormone refractory disease, with the cancer progressing once it escapes hormonal control. Due to the limitations of these current therapies, there is a critical need to develop more effective treatments for both organ-confined and metastatic prostate cancer.

Cancer represents a loss of normal cellular regulation at the genetic level: cancer cells lose contact inhibition, proliferate in an uncontrolled manner, and fail to apoptose. All of these factors result in the growth of the tumor, eventually causing the demise of the host organism. Given that the primary lesions that cause malignancy occur at the DNA level, it would seem to be ideal that the treatment of cancer should also be at this molecular level. With recent advances in the understanding of the

molecular biology of prostate cancer, the complex interactions between growth factors, cell surface receptors, nuclear receptors, intracellular signaling pathways, and intercellular adhesion molecules are becoming clearer. Attempts to institute therapy that directly addresses these molecular changes in prostate cancer is slowly becoming a reality. Additionally, the alterations in local immune system function that occur in prostate carcinogenesis are also becoming clearer, with gene based tumor vaccine approaches for prostate cancer just now entering clinical evaluation. Finally, through identification and characterization of the gene sequences that are expressed only in the prostate, target-specific gene therapy may also become a possibility in the near future.

In this review, we will look at all of the potential mechanisms by which gene therapy may work to control prostate cancer. Additionally, we will discuss advances in the vectors that can be used to deliver the gene to the prostate, and the routes by which these genes can be administered. We will describe some of the chromosomal and oncogene changes that have been detected in prostate cancer, discuss methods by which the genes can be administered to the patient, and will examine the potential target specific gene therapies that are currently being developed.

Approaches for prostate cancer gene therapy

There are many ways that gene therapy can be used to treat prostate cancer. Due to the presence of immunosuppressive proteins such as TGF- β within the tumor, vaccine

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Received 17 September 1997; revised 5 February 1998; accepted 20 February 1998

approaches that stimulate a host antitumor response and overcome intratumoral immunosuppression may be promising.² Alternately, as the alterations in cellular regulation and gene expression that occur in carcinogenesis become defined, attempts to institute therapy that corrects these molecular defects have been developed.^{3,4} Finally, with the identification of regulatory gene sequences that are only activated within prostate cells, target-specific, cytotoxic gene therapy has also entered pre-clinical evaluation.⁵ At this time, it's still unclear which modality will be most effective; each approach has theoretical limits and advantages, with more studies needed before the best method (or combination of methods) are identified.

Gene replacement strategies for prostate cancer

The gene defects that are involved in prostate tumorigenesis are likely to be multiple and complex. Tumorigenesis is a multi-step pathway involving the initiation, proliferation, loss of contact inhibition, invasion, and metastasis of the cancer cell; multiple genes involving cell cycle regulation, angiogenesis, immunoreactivity, intracellular signal transduction, and cell adhesion are all probably involved. Furthermore, to date, no single gene defect has been consistently implicated in the tumorigenesis for all prostate cancers, with perhaps many different mutation pathways leading to the end-point occurrence of malignancy. Finally, the identification of a gene mutation in a tumor does not necessarily imply that it has a role in the initiation of the malignant process: the detected mutation may simply be the result of genetic instability and impaired gene repair mechanisms that are inherent in most tumors. As such, the selection of the most potent target for gene replacement therapy has been difficult.

Additionally, due to the heterogeneity of prostate cancer, the use of a single type of gene therapy may not work for all prostate tumors, with different gene therapies probably having different degrees of efficacy in different patients, depending on the individual characteristics of the given tumor. It also may be the case that combinations of gene replacement therapies, or approaches that use standard therapies, such as androgen deprivation therapy, in combination with gene therapy may improve efficacy.

Despite these complexities in defining the optimal target for gene replacement therapy, many gene replacement studies have already been initiated. Abnormal expression of p53, p21, PML, c-myc, bcl-2 and TGF- β have all been implicated in prostate carcinogenesis, and all are undergoing evaluation as potential targets for gene therapy.⁶⁻¹¹

The p53 protein exerts control on cellular proliferation because of its ability to block DNA polymerase binding.^{12,13} The p53 gene stops cell proliferation at the G1 checkpoint in the cell cycle if DNA damage has occurred. If the gene product is absent, cell proliferation continues in the presence of DNA damage, resulting in genetic instability and further tumorigenic changes.

Early studies found that approximately 60% of prostate

cancer cell lines had mutations in the p53 gene.¹⁴ Abnormal p53 expression has been found in more aggressive cancers, and it is an independent predictor of cancer recurrence following radical prostatectomy. Additionally, it has been shown that transfection of the normal p53 gene into p53 deficient prostate cancer cell lines can decrease the tumorigenicity of the tumor cells, and impairs the growth of prostate tumors in a mouse xenograft model.¹⁵ From these preliminary results, plans are underway at UCLA and at other centers to begin phase I clinical trials using p53 gene replacement to treat prostate cancer patients who have had cancer recurrence following radiotherapy.

Besides replacing the defective p53 gene, two alternate approaches have also been proposed to target the abnormal expression of p53 in prostate cancer. The first involves the transfection of a gene sequence that converts the abnormal p53 oncoprotein into a 'killer gene' activator.¹⁶ A gene is constructed that can bind p53 (the killer) to its promoter site (the trigger); the gene is then expressed in the presence of the abnormal p53 protein, producing a toxin (the weapon) that then results in cell death.¹⁶ The second approach that targets p53 mutated cells involves the creation of viruses such as the ONYX dl1520 group C adenovirus, which can preferentially infect and lyse tumor cells that are deficient in p53 gene expression.¹⁷

The p21 oncogene is another potential target for gene replacement therapy. The p21 gene appears to be activated by the p53 gene, with p21 serving as a cyclin-dependent kinase inhibitor that helps to arrest cellular proliferation in the presence of DNA damage.¹⁸ Both *in vitro* and *in vivo* studies have been done to compare the effects wild-type p53 gene and p21 gene transfection in Murine prostate cancer cell lines.¹⁹ The cell line that was used in the study, 148-1PA, was known to be deficient in p53 expression. Interestingly, this study suggested that gene replacement therapy with p21 may be more effective than treatment with p53. *In vitro* studies showed decreased proliferation of the cell line transfected with p21 when compared to cell lines transfected with p53. Furthermore, in animal studies using intratumoral, adenovirus mediated transfection of the p21 gene, significant growth suppression of subcutaneously implanted 148-1PA prostate tumors was seen in mice, with survival of those animals greater than those that were transfected with the p53 gene. From this work, it was concluded that p21 is a better target for prostate cancer gene replacement therapy than p53 in this model of prostate cancer, although the generalization of this finding to other prostate cancer cell lines or to human prostate cancer has yet to be implemented.¹⁹

Preliminary studies have also suggested that the promyelocytic leukemia (PML) gene might be an effective gene to transfect into prostate cancer.⁷ The PML gene codes for a protein that is a growth and transformation suppressor; mutations in this gene have been implicated in the development of leukemia, breast cancer, and prostate cancer. In a study using a recombinant adenovirus carrying the PML gene, an infection efficiency of 90% was achieved in LnCAP, DU145 and PC3 cell lines using relatively low viral counts. Furthermore, it was shown that the gene is expressed for up to 18 d after transfection

into DU145 and LnCAP, with these cells showing decreased proliferation rates and a decreased ability to form tumors upon injection into nude mice. Finally, an *in vivo* antitumor effect was shown, with transfection of the PML gene into pre-established tumors decreasing the growth rates of the tumors. From this data, it was suggested that transfection of the PML gene may be an effective treatment for prostate cancer.

Attempts have also been made to target the overexpression of the c-myc, bcl-2 and TGF- β genes.⁹⁻¹¹ All of these oncogenes appear to be overexpressed in prostate cancer, with the transfection of anti-sense DNA into the cancer cells hopefully resulting in the inactivation of these target genes.²⁰ Preliminary studies have used nude mice carrying pre-established DU-145 tumors. These mice underwent intra-tumoral injections with retroviruses carrying an anti-sense c-myc gene; transcription of the anti-sense mRNA then resulted in decreased translation of the c-myc sense mRNA. In this study, a 95% reduction in tumor burden was seen, with 20% of the animals transfected with anti-sense c-myc DNA actually having a complete remission of their disease.⁹ Anti-sense approaches have also been proposed for the bcl-2 and transforming growth factor beta (TGF- β) genes, since both bcl-2 and TGF- β overexpression have been found in aggressive cancers.^{2,8,10} The bcl-2 oncoprotein appears to confer androgen resistance to prostate cancer cells, and also makes the cells resistant to other apoptotic stimuli such as serum starvation.⁸ In contrast, TGF- β has multiple effects on prostate cancer, with high levels of TGF- β allowing prostate cancer cells to adhere to bone marrow stroma, while also decreasing the immune system response against the tumor.^{2,11}

Therefore, numerous genetic changes have been identified in both the oncogenes and tumor suppressor genes that are probably involved in the initiation and the metastatic spread of prostate cancer. However, despite the complexity of prostate cancer initiation and promotion, animal studies and phase 1 human studies looking at gene replacement therapies have been initiated in order to identify the best target for gene therapy.

Tumor vaccine approaches in prostate cancer

An alternative approach for gene therapy involves the use of cytokine genes transfected into tumor cells to create 'tumor vaccines.' In this 'indirect approach,' attempts are made to sensitize the host immune system against tumor specific antigens. It has been hypothesized that tumor cells escape surveillance and destruction by the immune system through down-regulation of cell surface antigens such as the Major Histocompatibility Complex (MHC).²¹ In the tumor vaccine approach to gene therapy, tumor cells are transfected with cytokine genes such as interleukin-2 (IL-2), tumor necrosis factor alpha (TNF), Granulocyte-Monocyte Stimulating factor (GM-CSF) and interferon gamma.²²⁻²⁷ The cytokines from the transfected cells stimulate expression of cell surface antigenic proteins such as B7, and the HLA Class I and Class II antigens, which then enhances their immunogenicity. With the

increased immunogenicity of the tumor, it's then hoped that either a local or systemic immune response against the cancer will be generated.¹⁰

There are two methods by which tumor vaccines have been generated: *ex vivo* and *in vivo*. The *ex vivo* strategy involves the transfection of harvested tumor cells in cell culture with a cytokine gene, growth expansion and irradiation of these cells, and re-infusion of the cytokine producing autologous tumor cells to the host. The expression of cytokines by these tumor cells then stimulates recognition of tumor antigens by cytotoxic T-cells. A clonal expansion of these tumor specific lymphocytes then occurs, resulting in the target specific killing of cancer cells throughout the body.

While effective for generating tumor vaccines, the *ex vivo* approach is complex. For *ex vivo* transfection, the tumor has to be surgically removed, harvested in a sterile manner, cultured, transfected with cytokine genes, irradiated, and then administered back to the patient. The use of antigen presenting cells, or dendritic cells, for the presentation of tumor antigens has also been proposed for the *ex vivo* method.²³

To counteract these difficulties, newer approaches have been devised that use simpler, *in vivo* approaches for gene transfection. This second generation of tumor vaccine technology use viruses or packaged segments of DNA that can deliver the cytokine gene directly into the tumor while it's still in the patient (Figure 1).

Both *ex vivo* and *in vivo* tumor vaccine approaches have been used for the treatment of prostate cancer. Using the Dunning rat R3327-MatLyLu cell lines, *ex vivo* retroviral transfections of the IL-2 gene were performed with prostate cancer cells. These cells were then irradiated and then injected into the rats at a site remote from the primary tumor. The IL-2 tumor vaccine was not only capable of curing rats that had subcutaneously implanted prostate tumors, but it was also able to induce immune memory that protected the animals against future tumor cell challenges.²⁹ The transfection of the GM-CSF gene was also effective at suppressing tumor growth and in inducing immune memory in the Dunning R3327 G model. These latter results formed the basis of the current clinical protocols that will use the GM-CSF gene to treat patients with prostate cancer.²⁶

Figure 1 Creation of *in vivo* tumor vaccine approach using a liposome/IL-2 gene complex injected intratumorally.

However, in multiple mouse tumor model studies, orthotopically implanted prostate tumors did not respond well to the immunotherapy in comparison to subcutaneously implanted tumors, possibly because the prostate is somehow protected from the immune response triggered by the tumor vaccine.^{24,25,30} While it's possible that direct, intra-tumoral injection of the vaccine in the prostate will have better efficacy in treating intraprostatic disease, the long term role that tumor vaccine therapies will have in the treatment of prostate cancer is uncertain.

A new approach for the immunologic treatment of prostate cancer involves the use of dendritic cells.²³ Dendritic cells have been shown to be the most potent antigen presenting cell of the body, capable of stimulating a cytotoxic T-cell response when grown in the presence of tumor proteins. Segments of the PSA protein have been found to be immunogenic, with a specific, cytotoxic T cell reaction generated when it is used as a tumor vaccine antigen.²² It has been proposed that prostate specific antigens, such as PSA or PSM, can be used in combination with dendritic cells to create a tumor vaccine. The dendritic cells present the prostate antigens to T cells as foreign proteins, therefore stimulating a systemic, immunologically mediated, anti-tumor response.²³ From the initial work that showed *in vitro* activity, clinical protocols are now underway where patients will be vaccinated against the PSA protein or other prostate specific antigens, using dendritic cells to present the antigens to the immune system, with the hope that a systemic immune response against prostate cancer will be initiated.

Gerald Murphy, MD, of the Pacific Northwest Cancer Foundation recently presented preliminary data from the first, clinical phase I/II trial that used dendritic cells pulsed with the PSMA peptide. After enrolling over 50 patients with progressive, hormone refractory prostate cancer, PSA based responses were seen in seven patients to date, with the responses lasting for more than 200 d in many of the patients studied. The treatments were well tolerated, and no complications were reported. Future trials that will transfect dendritic cells with tumor antigen genes are currently under development as well.⁵⁹

Target specific approaches with suicide genes

The insertion of 'suicide genes' is a novel approach that can selectively kill tumor cells. In this model, a gene is transfected into tumor cells that then converts an otherwise benign medication into an agent that is toxic to the cell. In prostate cancer, this approach has additional promise due to the recent discovery of a bipartite, PSA enhancer promoter sequence.^{31,32} Prostate specific antigen (PSA) is a protein that is found only in cells that come from the prostate gland; it has also been shown that PSA can be found in almost all types of prostate cancer cells in addition to normal prostate tissue. Pang *et al* have characterized a highly efficient gene promoter/enhancer sequence for the PSA gene, by extracting it from a patient who had metastatic prostate cancer and extremely high serum PSA levels.^{31,32} The expression of the PSA gene

enhancer/promoter has been shown to be androgen responsive, and its expression appears to be specific only to prostate cells as demonstrated in both *ex vivo* and *in vivo* experiments.^{33,34} Therefore, when combined with a suicide gene, the PSA promoter has the potential to provide prostate cell specific expression of the transfected gene, with cytotoxic effects limited only to the target tissue (Figure 2).

There are two suicide gene systems in widespread use: the herpes simplex-thymidine kinase system (HSV-TK) and the cytosine deaminase system. The HSV-TK gene is non-toxic by itself; however, in the presence of gancyclovir, it converts gancyclovir into a toxic agent that prevents DNA synthesis and therefore kills the host cell.^{35,36} The cytosine deaminase gene is also non-toxic by itself.³⁷ However, cytosine deaminase can convert the inert compound 5-fluorocytosine into the antimetabolite 5-fluorouracil (5-FU), which then results in the death of the transfected cell. In both systems, there is a significant 'bystander effect,' where the toxins diffuse into adjacent cells that were not transfected, resulting in killing of the tumor even if transfection rates are low.

Figure 2 Thymidine kinase/gancyclovir suicide gene combined with the PSA promoter sequence in order to induce tissue specific cell killing.

Besides coupling suicide genes to prostate tissue specific promoters, it has also been proposed that the direct, intra-tumoral injection of suicide genes without the aid of the PSA promoter can also achieve target specific, anti-tumor activity. Berman *et al*³⁸ demonstrated the efficacy of intra-tumoral injection of the HSV-TK suicide gene in a Dunning prostate cancer rat tumor model. In this study, the growth of subcutaneously implanted tumors was significantly inhibited when compared to control animals, with little systemic toxicity.

However, while the intra-tumoral injection route is used to minimize systemic effects, Hall *et al* recently demonstrated that the intra-tumoral injection of the HSV-TK gene can also have systemic anti-tumor effects. This group showed that growth inhibition of pulmonary metastasis can be seen after intra-tumoral HSV-TK gene treatment in a mouse model of prostate cancer.^{35,36}

From all of this preclinical data, Peter T. Scardino, MD, and colleagues from the Baylor College of Medicine, have initiated multiple clinical trials using suicide gene therapy. At the Fourth Annual CaP CURE Scientific Retreat Program, Dr Scardino presented the preliminary data from one of the largest clinical experiences using suicide gene therapy. Using an attenuated adenovirus carrying the thymidine kinase gene, intratumoral injections of the vector has been done in 18 patients so far. At lower dose levels, no toxicity or clinical responses were seen. However, at subsequent dose escalations using between 10^9 – 10^{11} particles, 20–25% response rates were seen. Unfortunately, the last patient who was treated at the highest dose level suffered from grade IV hematologic and hepatic toxicity, which might be attributable to either gancyclovir toxicity or to the nonspecific gene activity of this vector.³⁹

Other suicide gene approaches have been proposed for the treatment of prostate cancer. These include either the creation of a cytopathic adenovirus that can only replicate in prostate cells, or the use other gene promoters in combination with other gene sequences (such as the osteocalcin gene promoter) that may also have prostate cancer cell specificity.^{40,41} While these approaches have been effective against prostate cancer cell lines grown in tissue culture, *in vivo* studies will be needed before any conclusions can be made about their therapeutic potential or toxicity.

Potential vectors for prostate cancer gene therapy

One of the rate limiting steps for gene therapy is the development a safe, reliable vector that can get the desired gene into the target. Multiple pathways can be used to deliver the therapeutic gene to the target tissue. Each approach has advantages and disadvantages, as will be discussed below.

Plasmid DNA vectors

The administration of naked DNA to tumor cells has been attempted, with one of the first efforts showing efficient transfection of genes into smooth muscle.⁴² However, with systemic administration it has been shown that

naked DNA plasmids are rapidly cleared by the bloodstream, with degradation of the DNA usually seen within 5 min. Furthermore, overall transfection efficiency was very low. Attempts were subsequently made to enhance the stability and transfection efficiency of DNA by coating them with lipids (liposome-DNA complexes). Initial studies looking at plasmid DNA encapsulated in liposomes demonstrated an increase in transformation efficiency by up to 5000-fold when compared to naked DNA alone.⁴³ Intact plasmid DNA sequences can be detected for at least 24 h after injection, suggesting that a longer half-life will translate into higher transfection efficiency *in vivo*.⁴⁴

Liposome-based gene therapy involves the construction of a recombinant therapeutic gene enveloped by a cationic lipid shell. The liposome shell around the plasmid protects the DNA from degradation after systemic administration to the host. The liposome vector also provides for efficient gene transfer since the lipid envelope can fuse with membranes of the tumor cell, resulting in direct delivery of the therapeutic gene to the cytoplasm of the cell. Finally, additional advantages of liposome delivery vehicles are their safety, lack of immunogenicity, and lack of infectivity. Repeated intravenous administration of these complexes can be safely done to produce high levels of systemic transfection, without any decrease in efficacy seen due to formation of blocking antibodies.⁴⁵

However, it appears to be the case that the majority of intravenously administered liposome gene complexes are rapidly cleared from the circulation due to uptake by the liver. Up to 60% of the dose can be found in the liver after intravenous administration, thus making systemic dosing of liposomal agents an inefficient method to transfect therapeutic genes.⁴⁶

Due to this fact, preliminary studies have been performed that demonstrated the safety and efficacy of the intratumoral injection of liposome-DNA complexes. Studies of a new liposome/IL-2 agent (Leuvectin; Vical Inc, San Diego, CA) found that 74–78% of patients had successful transfection of their tumor cells after intratumoral administration of the IL-2/liposome complex.⁴⁷ No significant side effects were noted. Additional studies have also confirmed the safety and efficacy of liposome complexes in the delivery of therapeutic genes.⁴⁸ While liposome vectors show significant promise, more studies are needed regarding their safety and efficacy *in vivo* prior to widespread use.

Viral vectors

Many different viral vectors have been developed for delivering genes into cells (Table 1). Each one has advantages and disadvantages, although it appears to be the case that adenoviral vectors will have the greatest application in prostate cancer.

Adenoviral vectors can provide highly efficient transfection of therapeutic genes in cell culture. The virus has an affinity for infecting epithelial cells by binding at cell surface membrane receptor sites, and it does not depend on cell replication for transfer of or expression of its genetic material.⁴⁹ Furthermore, genomic integration rates are low, and consequently there is little risk for long term sequelae due to the administration of adenovirus.^{49,50} Safety of adenoviral gene therapy vectors has

Table 1 Characteristics of viral vectors

	Retrovirus	Adenovirus	Adeno-associated virus
Requires dividing cells for transfection	Yes	No	No
Maximum size of gene insert	8 kb	8 kb	4.5 kb
Genomic integration	Yes (random)	No	No
Potential oncogenesis	Yes	Yes	No
<i>In vivo</i> delivery	Poor	Good	Good
Length of expression	Stable (> 1 y)	Transient (2–6 weeks)	Stable (?)

already been shown in human trials involving cystic fibrosis, ornithine transcarbamylase deficiency, and factor IX deficiency.^{51,52}

Additionally, adenoviruses can kill tumor cells not only via the effects of the transfected gene, but also by direct viral oncolysis caused by the replicating virus. This virally mediated tumor cell rupture may further enhance the immune response against tumor antigens, thus making the use of adenoviruses desirable in tumor vaccine approaches.

However, adenoviral vectors themselves are immunogenic, with most adults already carrying humoral immunity to circulating adenovirus. Thus, in the living organism, adenoviral transfection rates to the target cells may be very low due to the existence of preformed antibodies to the vector. Furthermore, the size of the gene sequence that can be placed into the virus is less than 4.8 kb, with the potential (albeit small) risk that the virus can recombine with naturally occurring adenoviruses and result in uncontrolled replication in the host organism.^{53,54}

To limit these potential problems, newer approaches that use modified adenoviruses (or by using other DNA viruses such as the vaccinia virus) have been devised; these strains carry less viral genomic DNA, are less immunogenic, and may permit multiple administrations of the gene in order to maximize transfection.^{55,56} However, these adeno-related viruses are more difficult to grow, since they have segments of the viral genome that are deleted to prevent them from replicating in the patient. These replication deficient viruses can only be cultured in modified kidney cells that have DNA that is complementary to the missing viral genes (Figure 3). Eventually, it's hoped that the use of helper viruses will allow for the construction of adenoviruses that have no significant amounts of viral DNA at all, thus allowing these new vectors to carry larger DNA sequences while being less immunogenic at the same time (Figure 4).⁵⁷

Technique of intraprostatic injection

Multiple methods have been described to inject genetic material into the prostate. Gomella and others have employed a trans-perineal route.⁵⁸ A transrectal ultrasound probe guides needle placement as it is passed through the skin and into the prostate. The advantage of this approach is that the infectious complications that are associated with the passage of needles through the rectum are avoided, and the potential spillage of genetic material in the feces is prevented. However, due to discomfort, local anesthesia and intravenous sedation are often required.

Figure 3 Creation of a replication deficient adenovirus by growing the E1a/E1b deficient virus in 293 cells.

Figure 4 Growth of an adenoviral vector devoid of all native gene sequences by coinfecting vector with a helper virus in 293 cells.

We have preferred the use of a transrectal approach. The main advantage of this approach is that it's technically easy to do, since the skills required for it are very similar to those that are used during ultrasound guided biopsy. Additionally, there is less discomfort since the perineal skin is not traversed.

For the procedure, the patient must be off of all anticoagulant medications (aspirin or warfarin) for at least 10 d; the patient must take an enema and an oral antibiotic prior to the procedure. With the patient in the lateral decubitus position, the ultrasound probe is placed into the rectum and prostate measurements are

Figure 5 Technique of ultrasound guided, intraprostatic injection of the gene vector.

taken. Subsequently, a hollow core, biopsy needle is inserted into the needle guide on the ultrasound probe; the spring loaded biopsy gun (Biopsy gun) is then fired, which puts the needle tip into the prostate tumor. The internal obturator of the needle is then removed (with the resultant tissue biopsy core send to the lab for analysis), and the syringe with the genetic agent is injected into the prostate through the hollow biopsy needle (Figure 5). A 0.5cc saline flush is then used to clear the dead space within the needle, and the needle and ultrasound probe are then removed from the patient. The patient is evaluated for side effects, and is discharged to home when stable.

Conclusions

The reinsertion of inactivated tumor suppressor genes, the inactivation of oncogenes, the insertion of immunomodulatory genes, and the insertion of suicide genes have all been used to treat prostate cancer in cell culture experiments and in animal models. The prostate represents an ideal target for gene based therapy, since multiple target specific genes for the prostate have been identified, and since the prostate itself is relatively easy to access via a perineal or transrectal route. Progress is rapidly being made to better understand the complex genetic and cellular mechanisms that underlie prostate tumorigenesis; due to these advances, phase I human trials of gene therapy have already been initiated using

suicide gene approaches and tumor vaccine approaches (Table 2).

However, problems still have to be overcome before gene therapy goes into general use. Safe and effective gene vectors will be needed to carry the therapeutic gene to the cancer cell, and the therapy will have to be tailored so that the desired effects occur only in the tumor cells. However, due to the fact that gene therapy targets the primary causes of cancer initiation and growth, gene therapy offers the greatest opportunity to eradicate malignancy if the technical problems of gene delivery and expression can be overcome.

References

- 1 deKernion JB, Belldgrun A, Naitoh J. Radical prostatectomy for localized prostate cancer: indications, techniques and results. In: Belldgrun A, Kirby RS, Oliver T (eds). *New perspectives in prostate cancer*. Isis Medical Media: Oxford, 1998, pp 185–205.
- 2 Steiner MS, Barrack ER. Transforming growth factor beta 1 overproduction in prostate cancer: effects on growth *in vivo* and *in vitro*. *Mol Endocrinol* 1995; **6**: 15–20.
- 3 Naitoh J, Walzak MP. Hereditary aspects of urologic cancer. *Surg Oncol Clin North Am* 1994; **3**: 609–639.
- 4 Taneja SS, Pang S, Cohan P, Belldgrun A. Gene therapy: principles and potential. *Cancer Surv* 1995; **23**: 247–266.
- 5 Dannull J, Belldgrun AS. Development of gene therapy for prostate cancer using a novel promoter of prostate specific antigen. *Br J Urol* 1997; **79**: 1–7.
- 6 Viola M *et al*. Expression of ras oncogene p21 in prostate cancer. *N Engl J Med* 1986; **314**: 133–137.
- 7 He Dalin *et al*. Adenovirus mediated expression of PML suppresses growth and tumorigenicity of prostate cancer cells. *Cancer Res* 1997; **57**: 1868–1872.
- 8 Raffo AJ *et al*. Overexpression of bcl-2 protects prostate cancer cells from apoptosis *in vitro*, and confers resistance to androgen depletion *in vivo*. *Cancer Res* 1995; **55**: 4438–4445.
- 9 Steiner MS *et al*. Gene therapy for the treatment of advanced prostate cancer by *in vivo* transduction with prostate targeted retroviral vectors expressing antisense c-myc RNA. *J Urol* 1996; **155**: 340A.
- 10 McDonnell T *et al*. BCL-2 confers resistance to cell death induction by androgen-ablation and chemotherapy in prostate cancer cells. *J Urol* 1996; **155**: 527A.
- 11 Kostenuik PJ, Singh G, Orr FW. Transforming growth factor beta upregulates the integrin mediated adhesion of human prostatic cells to type I collagen. *Clin Exp Metastasis* 1997; **15**: 41–52.
- 12 Brewster SF, Gingell JC, Brown KW. Tumor suppressor genes in urinary tract oncology. *Br J Urol* 1992; **70**: 585–590.
- 13 Linehan WM. Molecular genetics of tumor suppressor genes in prostate carcinoma. *J Urol* 1992; **147**: 808–809.
- 14 Isaacs WB, Carter BS, Ewing CM. Wild-type p53 suppresses growth of human prostate cancer cells containing mutant p53 alleles. *Cancer Res* 1991; **51**: 4716–4720.
- 15 Ichikawa T, Ichikawa Y, Isaacs JT. Genetic factors and suppression of metastatic ability of prostate cancer. *Cancer Res* 1991; **51**: 3788–3792.

Table 2 Current prostate cancer gene therapy programs (as of July 1997)

Treatment approach	Gene	Vector	Site
Immunotherapy	Granulocyte-monocyte colony stim.	Retrovirus	Johns Hopkins University
Oncogene inhibition	Antisense c-myc	Retrovirus	Vanderbilt University
Immunotherapy	PSA gene	Vaccinia virus	National Naval Medical Center; Dana Farber; University of Michigan
Immunotherapy	IL-2 gene	Liposome	UCLA; Duke University
Suicide gene	Thymidine-kinase	Adenovirus	Baylor

- 16 Da Costa LT *et al.* Converting cancer genes into killer genes. *PNAS* 1996; **93**: 4192–4196.
- 17 Bischoff JR *et al.* An adenovirus mutant that replicates selectively in p53 deficient human tumor cells. *Science* 1996; **274**: 273–276.
- 18 Deiry EI *et al.* WAF-1, a potential mediator of p53 tumor suppression. *Cell* 1993; **75**: 817–825.
- 19 Eastham JA *et al.* *In vivo* gene therapy with p53 or p21 adenovirus for prostate cancer. *Cancer Res* 1995; **55**: 5151–5155.
- 20 Gnarr JR, Dressler GR. Expression of Pax-2 in human renal cell carcinoma and growth inhibition by antisense oligonucleotides. *Cancer Res* 1995; **55**: 4092–4098.
- 21 Garrido F, Ruiz-Cabello F. MHC expression on human tumors—its relevance for local tumor growth and metastasis. *Semin Cancer Biol* 1991; **2**: 3–10.
- 22 Xue BH *et al.* Induction of human cytotoxic T-lymphocytes specific for PSA. *Prostate* 1997; **30**: 73–78.
- 23 Tjoa B *et al.* Presentation of prostate tumor antigens by dendritic cells stimulates T-cell proliferation and cytotoxicity. *Prostate* 1996; **128**: 65–69.
- 24 Vieweg J *et al.* Immunotherapy of prostate cancer in the Dunning rat model: use of cytokine gene modified tumor vaccines. *Cancer Res* 1994; **54**: 1760–1765.
- 25 Yoshimura I *et al.* Cytokine mediated immuno-gene therapy in rat prostate cancer model. *J Urol* 1996; **155**: 510A.
- 26 Sanda MG *et al.* Demonstration of a rational strategy for human prostate cancer gene therapy. *J Urol* 1994; **151**: 622–628.
- 27 Lee CT *et al.* Genetic immunotherapy of established tumors with adenovirus murine granulocyte macrophage colony stimulating factor. *Hum Gene Ther* 1997; **8**: 187–193.
- 28 Ogasawara M, Rosenberg SA. Enhanced expression of HLA molecules and stimulation of autologous human tumor infiltrating lymphocytes following transduction of melanoma cells with gamma interferon genes. *Cancer Res* 1993; **53**: 3561–3568.
- 29 Parker SE *et al.* Cancer gene therapy using plasmid DNA: safety evaluation in rodents and non-human primates. *Gene Ther* 1995; **6**: 575–590.
- 30 Yoshimura I *et al.* Cytokine mediated immuno-gene therapy in rat prostate cancer model. *J Urol* 1996; **155**: 510A.
- 31 Pang S *et al.* Prostate tissue specificity of the prostate specific antigen promoter isolated from a patient with prostate cancer. *Hum Gene Ther* 1995; **6**: 1417–1426.
- 32 Pang S *et al.* Identification of a positive regulatory element responsible for tissue specific expression of prostate specific antigen. *Cancer Res* 1997; **57**: 495–499.
- 33 Rodriguez R, Schuur ER, Lim HY, Henderson CA, 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.
- 34 Dannull J, Belldgrun AS. Development of gene therapy for prostate cancer using a novel promoter of prostate-specific antigen. *Br J Urol* 1997; **79** (Suppl 1): 97–103.
- 35 Hall SA *et al.* Adenovirus mediated herpes simplex virus thymidine kinase gene transduction and gancyclovir treatment for prostate cancer: suppression of metastasis in an orthotopic model and synergism with androgen ablation. *J Urol* 1996; **155**: 528A.
- 36 Hall SA *et al.* Adenovirus mediated herpes simplex virus-thymidine kinase gene and gancyclovir therapy leads to systemic activity against spontaneous and induced metastasis in an orthotopic mouse model of prostate cancer. *Int J Cancer* 1997; **70**: 183–187.
- 37 Hirschowitz EA *et al.* *In vivo* adenovirus mediated gene transfer of the E coli cytosine deaminase gene to human colon carcinoma derived tumors induces chemosensitization to 5-fluorocytosine. *Hum Gene Ther* 1995; **6**: 1055–1063.
- 38 Berman CJ, Davidson B, Lubaroff D. Use of adenovirus delivered herpes thymidine kinase gene for cyto-reductive therapy of prostate cancer using the Dunning rat model. *J Urol* 1997; **157**: 37A.
- 39 Scardino PT. Prostate cancer gene therapy: anti-metastatic approaches. *Read at the Fourth Annual CaP CURE Scientific Retreat Program*, September 5, 1997.
- 41 Ko SC *et al.* Osteocalcin promoter based toxic gene therapy for the treatment of osteosarcoma in experimental models. *Cancer Res* 1996; **56**: 4614–4619.
- 42 Rodriguez R *et al.* Prostate attenuated replication competent adenovirus (ARCA) CN706: a selective cytotoxic for prostate specific antigen positive prostate cancer cells. *Cancer Res* 1997; **57**: 2559–2563.
- 43 Zhu N, Liggit D, Liu Y, Debs R. Systemic gene expression after intravenous DNA delivery into adult mice. *Science* 1993; **261**: 209–211.
- 44 Felgner PL *et al.* Improved cationic lipid formulations for *in vivo* gene therapy. *Ann N Y Acad Sci* 1995; **772**: 126.
- 45 Lew D *et al.* Cancer gene therapy using plasmid DNA: pharmacokinetic study of DNA following injection in mice. *Hum Gene Ther* 1995; **6**: 553–564.
- 46 Norman A *et al.* Preclinical pharmacokinetics, manufacturing and safety studies supporting a multicenter cancer gene therapy trial. *Hum Gene Ther* 1995; **6**: 549–550.
- 47 Mahato RI *et al.* Physicochemical and pharmacologic characteristics of plasmid DNA/cationic liposome complexes. *J Pharm Sci* 1995; **84**: 1267–1271.
- 48 Vical Inc. Personal Communication, 1996.
- 49 Nabel GJ *et al.* Direct gene transfer with DNA liposome complexes in melanoma: expression, biologic activity, and lack of toxicity in humans. *Proc Natl Acad Sci USA* 1993; **90**: 11307–11311.
- 50 Yei S *et al.* Adenoviral mediated gene transfer of human surfactant protein B to respiratory epithelial cells. *Am J Respir Cell Mol Biol* 1994; **11**: 329–336.
- 51 Ah M, Lemoine NR, Ring CJ. The use of DNA viruses as vectors for gene therapy. *Gene Ther* 1994; **1**: 367–384.
- 52 Stratford-Perricaudet LD *et al.* Evaluation of the transfer and expression in mice of an enzyme encoding gene using a human adenovirus vector. *Hum Gene Ther* 1990; **1**: 241–256.
- 53 Smith TA *et al.* Adenovirus mediated expression of therapeutic levels of human factor 9 in mice. *Nat Genet* 1993; **5**: 397–402.
- 54 Bett AJ, Prevec L, Graham FL. Packaging capacity and stability of human adenovirus type 5 vectors. *J Virol* 1993; **67**: 5911–5921.
- 55 Oualikene W, Gonin P, Eloit M. Lack of evidence of phenotypic complementation of E1a/E1b deleted adenovirus type 5 upon superinfection by wild-type virus in the cotton rat. *J Virol* 1995; **69**: 6518–6524.
- 56 Gomella LG *et al.* Phase I study of intravesical vaccinia virus as a vector for gene therapy of bladder cancer. *J Urol* 1997; **157**: 194A.
- 57 Roth JA, Cristiano RJ. Gene therapy for cancer: what have we done and where are we going?. *JNCI* 1997; **89**: 21–39.
- 58 Kochanek S *et al.* A new adenoviral vector: replacement of all viral coding sequences with 28 kb of DNA independently expressing both full-length dystrophin and beta-galactosidase. *PNAS* 1996; **93**: 5731–5736.
- 59 Gomella LG. Personal Communication, 1997.
- 60 Murphy GP. Overview and experience with vaccines and T cell therapy of prostate cancer using dendritic cells pulsed with PSMA. *Read at the Fourth Annual CaP CURE Scientific Retreat Program*, September 5, 1997.