The University of California, Los Angeles/Jennifer Jones Simon Foundation Symposium on Prostate Cancer and Epithelial Cell Biology: Bringing Together Basic Scientists and Clinicians in the Fight against Advanced Prostate Cancer

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Abstract

Prostate cancer is the most common solid tumor in American men and is the second most common cause of cancer deaths. Although surgery and radiation therapy are effective for the treatment of organ-confined cancer, there is no effective treatment that is currently available for patients who have metastatic disease. Antiandrogen therapy is only palliative, and chemotherapy has largely been ineffective. However, recent advances in the understanding of the molecular biology of prostate cancer have lead to the development of new treatment strategies for metastatic cancer, including gene-based therapies, immunotherapies, and antiangiogenesis-based therapy. In association with the Jonsson Comprehensive Cancer Center and the University of California, Los Angeles Department of Urology, the Jennifer Jones Simon Foundation assembled 30 of the world's experts in prostate cancer research to review the most recent advances in the study of prostate cancer, with the hope that the resulting discussions would facilitate the rapid translation of new discoveries from the laboratory bench to the clinic.

Introduction

On January 30–31, 1998, over 100 students, clinicians, and basic scientists gathered on the campus of UCLA to discuss recent discoveries in the molecular biology of prostate cancer. Through the generous support of Jennifer Jones Simon and The Jennifer Jones Simon Foundation, the UCLA Prostate Cancer Program of the Jonsson Comprehensive Cancer Center hosted this symposium to bring together some of the leaders in cancer research in an attempt to identify new directions for prostate cancer research.

A faculty of 28 prominent researchers from throughout the United States and from four European countries presented their most recent discoveries regarding the initiation, proliferation, and spread of prostate cancer. The specific topics that were discussed included new gene discovery, animal models for the study of prostate cancer, function of the androgen receptor, identification of new tumor markers and antigens, immunotherapy, the mechanisms of hormone insensitivity, gene therapy, and treatment approaches for patients with advanced prostate cancer. The goals of this symposium were to bring together the different disciplines of cancer research to discuss common problems, forge new pathways, and establish a dialogue between the clinicians and basic scientists to facilitate the rapid translation of these new discoveries from the laboratory to the bedside.

Apoptosis and Other Intracellular Pathways

As a sign that increasing numbers of prominent basic scientists are becoming interested in the study of prostate cancer, Dr. David Baltimore, Nobel laureate and President of the California Institute of Technology, launched the meeting by reviewing his research on the molecular mechanisms of apoptosis. Because prostate cancer largely represents a failure of the tumor cells to undergo apoptosis, an increased understanding of the TRADD/FLICE/ICE intracellular signaling pathway that controls apoptosis will hopefully lead to the development of new treatments for prostate cancer (1, 2). Inhibitors of the TRADD pathway (such as p65 and p50) that can block apoptosis in certain circumstances (such as in the case of cancer, wound healing, or inflammation) have already been discovered; potentially, drugs that can block the actions of these inhibitors might be effective for the treatment of prostate cancer (3). Additionally, the direct transfection of the mediators that are downstream to the caspase enzyme is another route by which normal apoptotic mechanisms can be restored to cancer cells (4). However, although reactivation of normal apoptotic pathways represents a potentially effective cancer treatment, these pathways appear to be extremely complex, and it will be some time before they are completely delineated.

In contrast to these apoptosis pathways, other investigators have tried to identify the other errors of intracellular signal transduction that are involved in the development of prostate cancer. Dr. Don Tindall of the Mayo Clinic used differential display PCR, which compared the cDNA of LnCAP cells grown in normal serum to prostatic steroid-binding protein; IGF, insulin-like growth factor; IGFBP, IGF binding protein.

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1 This work was supported by Janssen Pharmaceuticals and Chiron Therapeutics. This report is from a symposium that was sponsored by the Jennifer Jones Simon Foundation and was held on January 30–31, 1998, on the campus of the UCLA School of Medicine in Los Angeles, CA. The participants included: Dr. David Baltimore from the California Institute of Technology; Dr. Neil H. Bander from the New York Hospital/Cornell Medical Center; Dr. Arie Belldegrun from the UCLA School of Medicine; Dr. Karen Bernstein from BioCentury; Dr. Marta A. Cheever from Corixa Corporation (Seattle, WA); Dr. Carlo Croce from the Kimmel Cancer Institute; Jean B. DeKernion from the UCLA School of Medicine; Dr. Robert A. Figlin from the UCLA School of Medicine; Dr. Oliva Finn from the University of Pittsburgh Cancer Institute; Dr. Norman Greenberg from the Baylor College of Medicine; Dr. Harvey Herschman from the UCLA School of Medicine; Dr. Guido W. Jenster from the M. D. Anderson Cancer Center; Dr. Phillip W. Kantoff from the Dana Farber Cancer Institute; Dr. Roger Kirby from St. George’s Hospital, London, United Kingdom; Dr. Paul Lange from the University of Washington Medical Center; Dr. Judi W. Moule from the Uniformed Services University of the Health Sciences; Dr. Leena Peltonen from the University of Helsinki; Dr. Michael Phelps from the UCLA School of Medicine; Dr. David Reese from the UCLA School of Medicine; Dr. Gert J. C. Rietjensmeller from the University of Munich; Dr. Neal Rosen from the Memorial Sloan-Kettering Cancer Center; Dr. Charles Sawyers from the UCLA School of Medicine; Dr. Peter Scardino from the Baylor College of Medicine; Dr. Jack A. Schalken from the University Hospital Nijmegen (Nijmegen, the Netherlands); Dr. Jan Schnitzer from the Beth Israel Deaconess Medical Center; Dr. Hans Schriever from the Beth Israel Deaconess Medical Center; Dr. Donald J. Tindall from the Mayo Clinic; Dr. Inder Verma from the Salk Institute (La Jolla, CA); Dr. Tapio Visakorpi from the Finland Institute of Medical Technology (Tampere, Finland); and Dr. Owen Witte from the Howard Hughes Medical Institute, UCLA.

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3 The abbreviations used are: UCLA, University of California, Los Angeles; EGR-α-early growth response α; AR, androgen receptor; PSA, prostate-specific antigen; PSBP, prostate-specific binding protein.

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LnCAP cells that were grown in testosterone-free serum (5). Through this study, he found that the EGR-α gene was differentially expressed between the androgen-dependent and -independent prostate carcinoma cell lines.

It has been shown that the EGR-α gene has significant homology to the Wilm’s tumor gene, with both proteins having “zinc finger” regions that can interact with the DNA and affect gene expression (6). Additionally, it has been shown that androgens and epidermal growth factor (both of which are important hormones for the growth of prostate cancer) both have profound effects on the expression of this gene, with EGR-α expression increased in cancerous versus benign tissue.

Unfortunately, the story isn’t so simple, given that it also has been shown that the EGR-α gene has the opposite effect in leukemia and lung cancer (7). Furthermore, the insertion of antisense DNA for this gene can actually result in the malignant transformation of certain cell types. Additional studies are currently ongoing to help researchers better understand this paradox, with the therapeutic applications of EGR-α being limited for the moment due to this contradictory data. However, another area of interest has been determining the method by which androgen increases the levels of EGR-α because the EGR-α gene itself has no regulatory region that would be directly controlled by androgens.

Besides EGR-α, the role of androgen receptor dysfunction in the development of androgen-resistant prostate cancer has been the subject of intense study. Dr. Guido Jenster of the Baylor College of Medicine and Dr. Tindall of the Mayo Clinic both presented their efforts to identify the cofactors of the androgen receptor that might be responsible for the occurrence of the androgen-independent state. One of the more interesting coactivators that was described was called AR-LBD; because this protein has an AR-binding domain and a domain that can bind to the DNA transcription complex, this protein appears to be an ideal effector protein for the AR to induce changes in gene expression. Furthermore, it has been shown that this protein can superactivate the function of the androgen receptor in normal cells and can convert a hormone-sensitive prostate cancer cell into a rapidly growing, highly tumorigenic cell line in vitro. Although these findings were intriguing, further study is needed to determine how often this cofactor is overexpressed in androgen-independent prostate cancer and whether specific inhibitors to this cofactor can be created to target this protein.

New Gene Discovery in Prostate Cancer Using Molecular Epidemiology

Besides the study of the androgen receptor and of intracellular signaling pathways, a significant effort has also been placed in new prostate cancer gene discovery. Because cancer itself represents the loss of normal gene regulation, the identification of the genes that are important in cancer growth and metastasis might be vital for the development of new ways to both cure and prevent cancer.

Recently, the identification of a highly penetrant but rare prostate cancer gene on chromosome 1q24–25 was reported by Smith et al. (8). Although this gene is believed to be involved in only about one-third of all familial prostate cancers, >80% of patients who carried this susceptibility gene will develop clinical prostate cancer versus the 5% incidence of prostate cancer in noncarriers of this gene. At the meeting, Dr. Roger Kirby reviewed the British experience to confirm the importance of this cancer susceptibility gene (9). Through the study of 136 familial clusters of prostate cancer, it was found that 20% of these families had a linkage to the proposed locus at chromosome 1q, with further studies narrowing the prostate cancer gene site to the chromosome marker DIS2882. However, despite the identification of this chromosomal locus, this area contains hundreds of genes, and a search is currently ongoing to identify the actual gene that causes prostate cancer.

Besides these familial studies, other investigators are also using epidemiological techniques to find candidate prostate cancer genes in men who have sporadic prostate cancers. In one such study, Dr. Judd Moul of the Uniformed Services University of the Health Sciences showed the importance of the apoptosis regulator proteins p53 and bcl-2 (10). In his study of patients who were treated with radical prostatectomy, overexpression of bcl-2 and p53 both correlated to the recurrence of cancer following surgery, independent of the Gleason score and pathological stage.

Dr. Phillip Kantoff of the Dana Farber Cancer Institute also screened tissue samples from large populations of men and found that decreased numbers of CAG nucleotide repeats in the AR gene sequence were associated with a higher risk of prostate cancer (11). There are usually between 6 and 30 CAG repeats in the normal AR gene; for every six CAG repeats that were lost, the risk of cancer was increased by 22%. Furthermore, a shorter GAG repeat was found in patients who had tumors that were of higher Gleason grade or of more advanced clinical/pathological stage; in particular, the risk of developing a lethal prostate cancer increased by 50% for every six CAG repeats that were lost.

Unfortunately, although the data were interesting, the clinical utility of these findings is limited to date, and no therapeutic avenue has yet been described that can correct these decreased CAG repeats. Furthermore, changes in the number of CAG repeats is not even specific for prostate cancer because diminished numbers of CAG repeats have also been seen in men who have benign prostatic hyperplasia and obstructive voiding symptoms. However, despite these limitations, in vitro studies that used a synthetic AR gene suggested that, if the number of CAG repeats could be increased, the hyperactive function of the testosterone receptor could be reduced (12).

New Gene Discovery Using Animal Models of Prostate Cancer

Dr. Charles Sawyers of the UCLA School of Medicine presented data that were based on the UCLA xenograft model for prostate cancer (13). In this model, tumors from patients with advanced prostate cancer are currently being harvested and implanted into immune-deficient (severe combined immunodeficient) mice (13). Of the 11 new xenograft models of prostate cancer that have been produced so far (designated LAPC-1–LAPC-11), the LAPC-4 cell line appears to be particularly interesting.

From studies of the LAPC-4 tumor line that used differential display technology to compare the androgen-dependent and -independent sublines, two candidate androgen independence genes have been identified so far: PTEN and cathepsin D (14). PTEN is believed to be a phosphatase, with deletions of this gene also detected in the PC3 but not in the LnCAP cell line. Preliminary results that looked at 10 of the LAPC xenograft tumor lines found that 4 of the tumor lines had a normal PTEN gene but had diminished PTEN mRNA and protein levels. Furthermore, it was shown that gene expression of PTEN can be restored to normal levels in the LAPC cell lines if methylation of the gene is inhibited. Thus, given that the gene dysfunction is due to abnormal DNA methylation, another route for anticancer therapy may someday be created if the factor that controls PTEN gene methylation can be determined.

Besides PTEN, it was found that the cathepsin D gene is also associated with the ability of LAPC-4 to grow in vitro, independently of androgens. In cancer cells, it has been hypothesized that cathepsin...
D is overproduced, gets secreted in the bloodstream, and then cleaves the serum protein IGFBP-3. Once cleaved, IGFBP-3 results in the release of IGF-1, which then binds to its receptor and results in the autocrine stimulation of tumor proliferation (15). This is of particular interest, given the recent report that showed that elevated serum levels of IGF-1 have been correlated to the later occurrence of prostate cancer (16).

Besides the severe combined immunodeficient mouse model for prostate cancer, Dr. Norman Greenberg of the Baylor College of Medicine has also created new mouse models to permit the discovery of novel prostate cancer genes (17). In his autochthonous transgenic mouse prostate cancer model (TRAMP, for transgenic adenocarcinoma mouse prostate), a specially bred lineage of mice was created that has a high incidence of spontaneously occurring prostate tumors.

Dr. Greenberg has shown that the TRAMP mice develop a distinct pathology in the epithelium of the dorsolateral prostate by 10 weeks of age. By 12 weeks of age, these mice have widespread infiltration of the prostate with well-differentiated tumor, with the cancer transforming to a poorly differentiated histology by 24 weeks. By 28 weeks of age, 100% of the mice will have developed metastatic deposits of prostate cancer in the lymph nodes or lungs (18). Thus, it has been shown that the TRAMP model can provide a consistent source of primary and metastatic tumor cells, with the hope that the study of these different cell populations will allow for the identification of the genetic changes that are associated with cancer initiation, promotion, invasion, and dissemination. Multiple sublines from the TRAMP model have already been established, with current ongoing studies to find the genetic changes that are involved in the development of prostate cancer.

Prostate Cancer Gene Discovery Using Cytogenetic Techniques

Earlier studies of prostate, breast, kidney, cervical, and lung cancer suggested that there was a nonrandom genetic loss in chromosome 3p. Through careful microdissection of prostate tumor tissue and focused cytogenetic study of chromosome 3p using the techniques of PCR and exon trapping, Dr. Carlo Croce of Jefferson Medical College found a candidate prostate tumor suppressor gene at the locus 3p1.42 (19). Called FHIT, this gene appeared to have sequence homology to a yeast gene that is involved in the cleavage of adenosine phosphate, although its function in humans is unknown.

Initial studies of microdissected tumors showed that the changes in the genetic sequence of FHIT in tumors resulted in the loss of protein production. Later, studies that used immunohistochemical techniques showed that almost 100% of lung and esophageal cancers made decreased levels of the FHIT protein, whereas 76% of cervical cancers, 60% of gastric cancers, 40% of colon cancers, and 30% of prostate cancers also showed loss of FHIT. These rates of gene loss are comparable to that for the p53 gene, a tumor suppressor gene with an importance in carcinogenesis that is well established. Furthermore, in studies of gastric cancer cell lines, replacement of the wild-type FHIT gene was able to slow down cell proliferation in vitro, and it was also able to reduce tumorigenicity and induce tumor regression in animal models (20). Thus, through the identification of a gross chromosomal change, a potentially important cancer-causing gene was identified, although more effort will be needed to understand the regulation and function of this gene.

In contrast to the search for a cancer-causing gene, Dr. Tapio Visakorpi from the Finland Institute of Medical Technology (Tampere, Finland) and Dr. Gert Riethmueller of the University of Munich (Munich, Germany) have used microdissection techniques to identify new cancer genes that are associated with cancer progression. Dr. Riethmueller has focused on the dissection of micrometastasis to identify the genetic changes that are involved in the ability of cancer to spread to sites that are distant from the primary tumor (21). Although this work has yet to be fully applied to prostate cancer, these techniques have been successfully used to identify metastasis genes in colon cancer, with these discoveries leading to new antibody-based treatments for patients with metastatic disease (22, 23).

In contrast, Dr. Visakorpi has used cytogenetic techniques to identify the chromosomal changes that are associated with the progression of prostate cancer from the androgen-dependent to the androgen-independent state. Through a comparison of chromosome structure of primary prostate tumors, metastatic tumors, and hormone-refractory tumors, Dr. Visakorpi found sites of gene loss in advanced prostate tumors at chromosome 6q, 8p, 10q, 13q, and 16q, whereas gains of genetic material were identified at chromosomes 7p, 7q, 8q, and Xq (24).

Interestingly, when attempts were made to identify the nature of the extra genetic material on the X chromosome, the main gene that appeared to be amplified at this site was the AR gene, located at Xq12 (25). Based on fluorescence in situ hybridization analysis, increased AR gene copy number was detected in over one-third of hormone-refractory tumors, with the mRNA levels of the AR gene correlated to the copy number of the gene.

However, although this result was intriguing, it also must be remembered that AR gene overexpression was not found in the majority of androgen-resistant tumors. Subsequent cytogenetic study found another gene that was overexpressed in the majority of androgen-resistant tumors. On the basis of the determination of mRNA levels and gene copy number, Dr. Visakorpi found that overexpression of the gene A1CI at chromosome 8q is correlated to the androgen-resistant phenotype (26). Although the function of this gene is still unknown, it has been suggested that this gene has homology to other proteins that activate gene translation, with ongoing research trying to determine the role that this protein has in the occurrence of androgen independence.

To wrap up the discussion regarding the search for new prostate cancer genes, Dr. Paul Lange of the University of Washington reviewed the efforts of the Genetics Consortium of CaPCURE (Association for the Cure of Cancer of the Prostate) to identify new prostate cancer genes. Through a multi-institutional cooperative effort, the prospective collection of large amounts of tissue sample and blood samples has been possible. By using high-density array hybridization and microchip technology that was developed by the Affymatrix Corporation in collaboration with Lee Hood of the University of Washington, screening of more than 6500 genes can be done in a single procedure (27). Thus far, over 100 candidate prostate cancer genes have been identified through the use of this microchip technology, with work currently ongoing to sequence and characterize these candidate prostate cancer genes.5

New Approaches in Therapeutics for Advanced Prostate Cancer

Besides the search for the molecular causes of prostate cancer, another significant focus of this meeting revolved around the development of new treatment strategies for the patient who has advanced prostate cancer. Some of these approaches included immunotherapy, gene therapy, and chemotherapy, for prostate cancer.

Immunotherapy. Although tumor immunologists have focused significant amounts of attention on kidney cancer and melanoma, prostate cancer has traditionally been ignored because it was generally believed that prostate cancer is a nonimmunogenic tumor. However, due to recent discoveries that were made regarding the mechanisms by

5 P. Lange, personal communication.
which prostate cancer evades surveillance by the immune system and due to the identification of new, potentially immunogenic prostate cancer antigens, the validity of this paradigm has come into question.

With recent advances in the understanding of the structure, synthesis, and antigenicity of the MUC-1 protein, Dr. Olivera Finn of the University of Pittsburgh, along with other investigators, has proposed that MUC-1 is a cancer-specific antigen that can be used for both diagnostic and therapeutic purposes (28). In its normal state, MUC-1 is a protein that sits on the luminal surface of glands, with its protein core covered by numerous carbohydrate residues. Interestingly, once a cell transforms to the malignant state, it has been shown that three things happen to the MUC-1 protein: (a) the MUC-1 protein is overproduced and it becomes deposited along the entire surface of the cell; (b) massive overproduction of MUC-1 results in underglycosylation of the protein core; and (c) the underglycosylated MUC-1 core protein becomes abnormally folded, given that the carbohydrate residues serve to fold the normal MUC-1 core protein into a helical configuration (28). Thus, with the MUC-1 core protein fully exposed to its new membrane location and due to decreased levels of glycosylation and with the protein folded in such a way that the protein forms new structures, it was subsequently hypothesized that the cancer-associated MUC-1 protein should be antigenic.

Initial studies showed that the MUC-1 tumor antigen was able to induce a T cell-mediated antitumor response and that cancer patients who were able to naturally generate antibodies to MUC-1 had a better prognosis than did the cancer patients who did not have the antibody (29, 30). On the basis of these results, work is currently underway to use pulse dendritic cells with the MUC-1 antigen to activate the immune system (31).

Although this approach is promising, a call for caution was raised by Dr. Hans Schreiber of the University of Chicago. Because MUC-1 and other “tumor-specific” antigens represent the abnormal expression of normal genes and because tumors routinely escape immune surveillance, despite the presence of these abnormal proteins (i.e., not all abnormal proteins can induce an immune response), Dr. Schreiber suggested that the breaking of immune tolerance to these antigens may not only be ineffective, but it may also be dangerous if there is any cross-reaction to normal cellular proteins (32).

An alternate approach to immunotherapy was proposed by Dr. Neil Bander of the New York Hospital/Cornell University Medical Center. In a preliminary study that used the prost30 monoclonal antibody to prostate-specific membrane antigen, 41% of patients who had advanced prostate cancer had a decrease in tumor size, with 23% of the patients having a >50% drop in the serum PSA (33). Furthermore, Dr. Bander suggested that these monoclonal antibodies could also be used as vehicles for the target-specific delivery of drugs or toxins to tumor cells (34). Kinetic studies of the prost30 antibody showed that: (a) the antibody binds specifically to an extracellular epitope of prostate-specific membrane antigen; (b) the antibody localizes to clathrin-coated pits on the cell surface; and (c) the antibody is rapidly taken up into the cytoplasm within 2 h of binding to the surface antigen. The effectiveness of the using monoclonal antibodies conjugated to bisnuth-231 has already been demonstrated in a LnCAP model of prostate cancer, and this antibody-based therapy has already been safely used in a clinical trial that studied patients who had acute myelogenous leukemia.

In addition to these antigen-antibody-based approaches, another immunotherapy approach that has been proposed for the treatment of prostate cancer involves the induction of an autoimmune reaction against the prostate and the tumor. Through the work of Dr. Martin Cheever and colleagues at the Corixa Corporation (Seattle, WA) experiments have been performed to see whether normal prostate proteins can be antigenic and whether immunization with these proteins can subsequently induce an autoimmune prostatitis (35). In their work using a rat model, a highly antigenic protein called PSBP was identified. Following systemic administration of the protein, vigorous antibody and T-cell responses were seen, with patchy areas of prostate inflammation also detected in some of the immunized animals. Furthermore, adoptive transfer of T cells that were sensitized to PSBP resulted in the induction of a destructive autoimmune prostatitis, as did gene therapy that used transfection of the PSBP gene into dendritic cells. Although these results demonstrated that a T cell–mediated, autoimmune prostatitis can be induced in a rat model, the human homologue to PSBP has yet to be identified. Furthermore, given the patchy and unpredictable nature of the prostatitis and given the lack of evidence that this immune reaction will cross over to either cancer cells or metastatic lesions, more work will be needed to define the mechanisms of immune system tolerance in the prostate and in prostate cancer.

Gene Therapy. The concept of gene therapy is simple: insert a gene sequence into cells that can replace a defective gene, or insert one that can shut down the function of an abnormally regulated gene (36). Although conceptually appealing, the efficacy of gene therapy has been limited by the inefficient vector systems that currently are being used to deliver genes to the cell (36). Due to these limitations, Dr. Inder Verma of the Salk Institute (La Jolla, CA) has put a significant amount of effort into the creation of new gene delivery vehicles (gene vectors).

Early gene therapy trials have used liposomes, retroviruses, and adenoviruses to deliver the genetic information. However, the utility of these vectors is limited by their low transfection efficiency, lack of target specificity, potential for random insertion of the gene into the genome, inability to induce long-term gene expression, and their immunogenicity, which interferes with their ability to deliver the gene to the target (36). To overcome these limitations, Dr. Verma has worked on the creation of new vectors that are based on the lentivirus family (37).

The lentiviruses (which include the HIV-1 virus) are RNA viruses that are unique in their ability to achieve stable, efficient incorporation into the host genome (38). However, the problems with wild-type lentiviruses are their selective infectivity for CD4+ cells and their potential to cause disease (such as AIDS). To circumvent these problems, Dr. Verma and his associates modified HIV by deleting almost all of the native viral genome and by replacing the capsular proteins on the surface of the virion with proteins that were derived from the vesicular stomatitis virus, which has a high affinity for a wide variety of epithelial cell types (38).

Preliminary animal studies demonstrated the “proof of principle” that this vector is able to induce efficient transfer of genes into multiple tissue types (37, 39). On the basis of these promising results, Dr. Verma has already tested the therapeutic efficacy of the lentiviral system in two different genetic disease processes: retinitis pigmentosa and hemophilia. Using animal models for these diseases, injection of the corrective gene into the mice has resulted in a reversal of these two disease processes. In the retina, gene replacement therapy appears to have restored the layers of the retina, although functional assessment still has not been done. Furthermore, in the factor IX-deficient mouse model, transfection using lentiviruses carrying the factor IX gene resulted in the long term production of factor IX and restoration of a normal phenotype (40).

Thus, although lentiviral vectors appear to have promise as a safe and effective gene therapy delivery vehicle, a method still needs to be developed to confer better target specificity for both gene delivery and

for gene expression. Along these lines, the use of a gene expression cassette that is controlled by the PSA promoter may represent one such way to provide target specificity (41). In addition, it will also be important to assess the safety of these vectors and to determine the biodistribution of the viruses following intratumoral or systemic treatment (i.e., it must be determined whether gene transfection results in incorporation of the vector into the germ cells).

**E-Cadherin and Vascular Caveolae Targeting.** Besides standard treatment approaches, the increased understanding of the molecular biology of cancer has led to the development of new treatment approaches. It has been shown that the E-cadherin cell surface protein is important in maintaining cell-cell contact, with loss of this protein correlated to a cancer’s ability to become invasive. Through a large study of patients who underwent radical prostatectomy, loss of E-cadherin correlated to the presence of a higher-grade cancer, with loss of the E-cadherin protein (as detected on immunohistochemical staining) also correlated directly to the risk of cancer recurrence following surgery (42). Due to these findings, Dr. Jack Shalken of the University Hospital Nijmegen (Nijmegen, the Netherlands) focused his studies on the ability of the normal E-cadherin protein to slow or even reverse the progression of cancer.

On the basis of his studies of kidney cancer and on PC3 prostate cancer cell line, he showed that correction of E-cadherin expression may not be enough because defects in many other cadherins are probably also involved in the progression of cancer, including cadherin 11 (43). Thus, although therapy based on the cadherin class of proteins is promising, more work will be needed so that all of the other cadherins that might be involved in carcinogenesis can be identified.

An alternate approach that can be used to generate an antitumor response was described by Dr. Jan Schnitzer and colleagues from Harvard University. Due to the development of new technology, these investigators have been able to isolate and study the microstructure of intact vascular endothelial membranes (44). Within these membranes, it was found that there are specialized, tissue-specific receptors (caveolae) on the surface of the membrane that facilitate the transport of substances across the vasculature and into the surrounding tissue. Thus, due to the need for tumors to develop a blood supply and due to the unique antigens that can be found on the caveolae of the tumor vasculature, in theory, antitumor therapy can be developed that is based on the targeting of these tumor-specific receptors. In a mouse lung cancer model, the specificity of anticaveolae therapy has already been demonstrated. Furthermore, the potential efficacy of this approach was shown in a mouse neuroblastoma model, in which the i.v. administration of monoclonal antibodies to a neuroblastoma-specific caveolar antigen was able to induce thrombosis and infarction of the tumor. Additionally, besides simple antibody-based treatments to induce thrombosis, these tumor-specific caveolar receptors could potentially be used to deliver medications, genes, or radioisotopes in a specific manner to tumor tissue. Although human trials have yet to be started using this new technology, work is currently ongoing to refine development of these conjugates in anticipation for human-based clinical trials.

**Chemotherapy.** Prostate cancer has been historically considered to be a chemoresistant tumor. Although androgen deprivation therapy represents the mainstay of treatment for the patient with metastatic prostate cancer, it is clear that hormonal therapy cannot provide long-term cancer remissions in the majority of patients. Although it is not completely effective, new pharmacological maneuvers have recently been discovered that can be used to achieve short-term responses in patients who have hormone-refractory prostate cancer.

Dr. David Reese of the UCLA School of Medicine reviewed some of these new advances. Once primary hormone therapy fails, it has been shown that combination therapy using ketoconazole and hydrocortisone can produce prolonged decreases in serum PSA and decreases in cancer symptoms in a small subset of patients. Although the primary mechanism of ketoconazole may involve the suppression of adrenal androgens, a direct cytotoxic effect may be important as well (45).

Other effective cytotoxic agents have also been recently developed: PSA-based response rates ranging from 50 to 60% have been seen following the administration of agents such as paclitaxel, docetaxel, and vinorelbine (46, 47). However, although objective PSA responses have been seen along with subjective improvements in pain secondary to cancer, most of these studies have failed to improve the duration of overall survival when compared to appropriate controls. Despite this fact, the value of these treatments should not be ignored because improvement in quality of life can represent a reasonable endpoint for therapy, even if overall survival is not affected.

Another new chemotherapy approach was discussed by Dr. Neal Rosen of Memorial Sloan-Kettering Cancer Center. Given that Ras is commonly overexpressed and mutated in many different types of cancer (disfunction of the ras gene can be found in approximately one-third of all cancers, including prostate cancer), it was hypothesized that finding a drug that could shut down the production or function of the abnormal Ras protein would lead to an effective treatment for cancer (48).

Recently, it was discovered that special lipid groups have to be attached to the mutated form of Ras for it to function (a process called farnesylation). As such, it was hypothesized that blocking the farnesylation of the Ras protein (as catalyzed by FPTase) might represent an effective way to inhibit Ras and inhibit tumor growth. Multiple FPTase specific inhibitors have already been identified and tested in cell culture and in animals models of prostate cancer. All of these agents have shown the ability to enter tumor cells, inhibit the function of farnesyl transferase, and prevent the farnesylation of the mutated Ras protein. Additionally, it has been shown that these new drugs are able to inhibit tumor implantation and growth in animal models of cancer and were able to shrink tumors that had preexisting ras gene mutations.

However, despite these promising results, clinical trials were delayed due to the subsequent finding that these FPTase inhibitors could also kill cells with K-ras mutations and that they were able to inhibit the posttranslational processing of the normal ras gene product in noncancerous cells. Due to the lack of significant toxicity, despite this relatively nonspecific mechanism of action, other routes of activity for these agents have been proposed, including an ability to directly induce apoptosis and an ability to destabilize the mitotic spindle in rapidly dividing cells. Although studies are now in progress to better understand the mechanism of action of this new class of drugs, Phase 1 human trials were recently initiated despite their unknown mechanism of action and despite the difficulties that will be encountered to determine the optimal dose, given their low toxicity.

**Summary**

Through the exhaustive work of many basic scientists, the molecular steps that underlie the growth and spread of prostate cancer are being uncovered. New understandings of the apoptosis pathway, the workings of the androgen receptor, and the ways that cancer evades the immune system will all hopefully lead to more effective and less toxic treatment regimens. Fortunately, in this time of increased public awareness and increased support of prostate cancer research through organizations like the Department of Defense, CaP Cure, and the Jennifer Jones Simon Foundation, the best and the brightest investigators are now focusing their intellects toward the problem of prostate cancer.  

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cancer. It is only through such a massive and comprehensive approach that the problem of prostate cancer can someday be understood and eliminated.

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References


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