

Symposium on Androgen Action in Prostate Cancer

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Introduction

The Symposium on Androgen Action in Prostate Cancer, held in Keystone, Colorado, March 4–6, 2004, was the organizational initiative of the investigators of the Specialized Programs of Research Excellence in Prostate Cancer. The Organ Systems Branch and the Division of Cancer Biology of the National Cancer Institute (Bethesda, MD) sponsored the symposium. The assembled experts reviewed recent findings on the central role of androgen action in prostate pathophysiology and defined future directions for translating findings from the laboratory to the clinic. Discussions focused on the androgen receptor and ways to exploit its properties to develop better strategies to prevent and treat prostate cancer.

Androgen Receptor Function

Many key aspects of the highly complex, yet poorly understood, functions of androgen receptor in prostate cancer were reviewed and analyzed. Dr. John Isaacs (Johns Hopkins University, Baltimore, MD) presented evidence that the androgen receptor serves as a switch from a paracrine growth mechanism in normal prostate epithelium to an autocrine growth mechanism in prostate cancer. Androgens suppress growth and promote differentiation in normal cells and stimulate growth in cancer cells. Also, expression levels of androgen receptor vary with the cell cycle. Symposium participants were presented the interesting concept that the androgen receptor is a tumor suppressor in normal prostate epithelium and an oncogene in prostate cancer.

Phosphorylation of the androgen receptor can regulate androgen receptor export from the nucleus in tissue culture cells (Dr. Bryce Paschal, University of Virginia, Charlottesville, VA). The ligand-bound states of nuclear receptors also influence their export. Binding of the antiandrogen bicalutamide to androgen receptor results in reduced levels of export compared with agonist-bound forms of the receptor. Thus, proteins involved in androgen receptor import and export are potential targets for drug development. Molecules that modulate androgen receptor effects on proliferation and differentiation, such as β -catenin (Dr. Steven Balk, Harvard University, Boston, MA), TCF4 (Dr. Balk), and Forkhead box A1 (FoxA1, Dr. Robert Matusik, Vanderbilt University Medical Center, Nashville, TN), are also potential targets. Other important aspects of androgen receptor function include cyclic loading of transcription factors (Dr. Myles Brown, Harvard University, and Dr. Olli Jänne, University of Helsinki, Helsinki, Finland), sumoylation of the androgen receptor (Dr. Jänne), and the role of androgen receptor in apoptosis.

Degradation of the androgen receptor is poorly defined. The androgen receptor is stabilized by androgen receptor-agonist complex

formation. In prostate cancer cells, however, the androgen receptor is more stable in the absence of added ligand. Efficient screening and testing schemes are needed to identify molecules that specifically promote androgen receptor degradation.

Coregulators of androgen receptor remain important, both for additionally defining androgen receptor function and as targets for drug development. Candidates include GRIP1/TIF2 (Dr. James Mohler, Roswell Park Cancer Institute, Buffalo, NY), Dr. Elizabeth Wilson, University of North Carolina, Chapel Hill, NC, and Dr. Jänne), SRC-1 (Dr. Nancy Weigel, Baylor College of Medicine, Houston, TX), NCoR (Dr. Balk), L-dopa decarboxylase (Dr. Paul Rennie, University of British Columbia, Vancouver, British Columbia, Canada), cyclin G-dependent kinase (Dr. Rennie), and CBP or its homologue, p300 (Dr. Zoran Culig, Innsbruck Medical University, Innsbruck, Germany).

Mutations remain useful in dissecting aspects of androgen receptor function. Studies in TRAMP mice indicate that hormonal status influences the location and nature of androgen receptor gene mutations. In intact animals, androgen receptor mutations localize predominantly to the COOH-terminal ligand binding domain, but in castrate animals, mutations conferring ligand independence lie in the NH₂-terminal region (Dr. Diane Robins, University of Michigan, Ann Arbor, MI, and Dr. Wayne Tilley, University of Adelaide, Adelaide, South Australia, Australia). In clinical prostate cancer, androgen receptor mutations colocalize to regions of the receptor distinct from inactivating mutations identified in inherited androgen insensitivity syndrome (Dr. Tilley). Similar to those seen in castrate TRAMP mice, mutations identified in tumors after combined androgen blockade predominantly colocalize to the NH₂-terminal region.

Efforts to identify downstream targets of the androgen receptor, using genomics (Dr. Zhou Wang, Northwestern University, Chicago, IL) and proteomics such as technology developed recently of isotope-coded affinity tags (Dr. Peter Nelson, Fred Hutchinson Cancer Research Center, Seattle, WA), have been informative. *In vivo* models are needed to monitor androgen action and to provide relevant end points in clinical trials involving novel compounds. Gene expression-based optical imaging (Dr. Michael Carey, University of California, Los Angeles, Los Angeles, CA), a noninvasive, repetitive means to visualize signaling pathways in a live animal, is one such model. Magnetic resonance imaging (Dr. John Kurhanwicz, University of California, San Francisco, San Francisco, CA), using surrogate markers such as citrate and zinc, is another model.

The Role of Androgen Receptor in Androgen-Refractory Prostate Cancer

Symposium participants considered whether the androgen receptor represents the “Achilles’ heel” of hormone-refractory prostate cancer. Levels of prostate-specific antigen (PSA), which is regulated by the androgen receptor, rise in the majority of recurrent cancers. Several lines of evidence point to a direct role for the androgen receptor in

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recurrent cancers (Dr. Rob Reiter, University of California, Los Angeles). The androgen receptor gene is amplified (Dr. Mohler), and the androgen receptor is overexpressed in the majority of recurrent cancers. Most patients failing hormonal therapy express PSA (Dr. Philip Kantoff, Dana-Farber Cancer Institute, Harvard University), and the androgen receptor binds the PSA promoter and enhancer in recurrent-cancer xenografts (Dr. Carey). In addition, previous work has shown that the androgen receptor drives PSA expression and is required for cell growth in the presence of castrate levels of ligand. Moreover, recent evidence suggests that prostate cells can synthesize enough androgen to transactivate the androgen receptor (Dr. Mohler). Therefore, symposium participants agreed that the androgen receptor is a valid target for drug development in prostate cancer.

Cross-talk with signaling pathways may sensitize the androgen receptor, allowing prostate cancer cells to adapt to an environment of low androgens. Proteins involved in these pathways thus present potential targets for drug development. The mitogen-activated protein kinase signaling pathway, for which the androgen receptor is a target, is up-regulated in prostate cancer, and some evidence suggests that mitogen-activated protein kinase inhibitors are effective against hormone-refractory cancers. However, no effect has been observed *in vivo*. Interleukin 6 may be another target. In LNCaP cells, interleukin 6 increases androgen receptor transactivation through the mitogen-activated protein kinase pathway (Dr. Marianne Sadar, British Columbia Cancer Agency, Vancouver, British Columbia, Canada), but it also inhibits proliferation and dihydrotestosterone-stimulated PSA expression (Dr. Gerhard Coetzee, University of Southern California, Los Angeles, CA). After longer periods of time, interleukin 6 induces a neuroendocrine cell phenotype similar to that observed after inhibition of androgen receptor expression by small interfering RNA, suggesting that interleukin 6 promotes cell differentiation by partially inhibiting androgen receptor activity at certain loci.

The Akt/phosphatidylinositol 3'-kinase pathway may be another area for cross-talk (Dr. Donald Tindall, Mayo Clinic, Rochester, MN). Cell survival depends on a balance between phosphatidylinositol 3'-kinase and PTEN, the latter often being mutated in advanced-stage cancers. Akt modulates several proteins involved in proliferation and survival, including FKHR/FoxO1. Activated FKHR/FoxO1 induces apoptosis, as does the withdrawal of androgens. Another potential area for cross-talk is prostatic acid phosphatase (Dr. Ming-Fong Lin, University of Nebraska Medical Center, Omaha, NE), which exists as both cellular and secretory forms. Before the PSA era, secretory prostatic acid phosphatase was used as a marker to monitor prostate cancer progression. Cellular levels of the enzyme are altered in inverse correlation with cell proliferation and tumorigenicity. In addition, prostatic acid phosphatase dephosphorylates the oncogene *ErbB2/neu/HER-2* in prostate cancer cells.

A second mechanism whereby tumor cells may adapt to a reduced-androgen environment is the development of a hypersensitive androgen receptor. This may result from changes in the phosphorylation state of the androgen receptor or its coactivators, altered shuttling of the androgen receptor between the cytoplasm and nucleus, increased androgen receptor expression, altered cellular signaling, or gain-of-function androgen receptor mutations. A hypersensitive androgen receptor may require lower androgen levels to function, coactivators may induce a greater transcriptional response, or the mitogen-activated protein kinase pathway may become hypersensitive, influencing coactivator function. In some cases, such as the LNCaP androgen receptor mutant (T877A), androgen receptor ligand-binding specificity changes, which may contribute to growth of recurrent cancer cell lines.

Other mechanisms, such as autocrine androgen biosynthesis (Dr. Isaacs), ligand-independent activation, or reactivation of the androgen

receptor for lipid metabolism, may be involved in the development of recurrent cancer. In addition, the low androgen levels that occur during androgen deprivation are sufficient to drive a wild-type androgen receptor (Dr. Mohler), suggesting that recurrent prostate cancer is not androgen independent. The current reliance on serum androgen in measuring response to treatment does not offer an accurate picture of androgen action during prostate cancer, treatment, and recurrence. Ultrasensitive serum androgen measurements and clinical assays measuring tissue androgen levels are beginning to be reported (Dr. Stephen Plymate, University of Washington, Seattle, WA). The idea of intermittent androgen ablation therapy, which may apply differentiation pressure while keeping the tumor intact, is attractive in light of concerns about recurrent cancers. However, although some studies suggest a benefit in terms of prolonged androgen sensitivity and slowed growth, no benefit has been observed in terms of overall survival. The combination of intermittent androgen ablation with other targeted agents may be promising.

Therapeutic Targets

Dr. Kantoff presented findings from several cohort studies at Harvard, including the Physicians' Health and Health Professional Follow-up Studies. He summarized clinical studies that focused on androgen-deprivation therapy, androgen signaling considerations for prevention and treatment, and evidence of a relationship between androgen action and risk for prostate cancer.

Symposium participants discussed the advantages of early therapy versus late therapy. The average response time for androgen-deprivation therapy in men with bone metastases is 18 months, but the duration of response has not been defined clearly for men with locally advanced disease or localized tumors (Dr. Kantoff). Furthermore, longer periods of androgen-deprivation therapy increase morbidity, and mounting evidence suggests that reduced or ablated androgen levels accelerate androgen independence and progression to hormone-refractory prostate cancer. Findings presented at the symposium suggest an ability of tumor cells to adapt to a reduced-androgen environment.

Studies have shown that monotherapy with antiandrogens, *i.e.*, either flutamide (250 mg three times a day) or bicalutamide (50 mg/day), whereas effective in the short term, are inferior to castration in terms of failure-free and overall survival. Bicalutamide at higher doses (150 mg/day) may be equivalent to castration when used in patients without metastases (Dr. Kantoff). Stronger antiandrogens should be developed. One target may be the interaction between the NH₂-terminal and COOH-terminal ends of the androgen receptor (NH₂-terminal and COOH-terminal interaction; Dr. Wilson), which is androgen dependent and required for the androgen receptor to activate androgen-responsive promoters. The NH₂-terminal and COOH-terminal interaction slows the dissociation rate of bound androgen and stabilizes the androgen-androgen receptor complex. Although the role of this is unclear, this remains an important area for research. The length of the polyglutamine tract, which resides in the NH₂-terminal region, may influence the risk for prostate cancer by affecting the NH₂-terminal and COOH-terminal interaction (Drs. Kantoff, Robins, and Tilley). However, several clinical studies examining relationships among androgen levels, the polyglutamine tract, and prostate cancer risk have yielded conflicting data, most likely because of differences in study size and population.

Activation function 2, which resides in the COOH-terminal ligand-binding domain and serves as the site for NH₂-terminal and COOH-terminal interaction and p160 coactivator binding, has long been of interest in drug development. Existing antiandrogens may fill the ligand-binding pocket and make it less flexible, thereby impeding the ability of cofactors to bind activation function 2. Small organic

molecules that perturb the activation function 2 surface have been screened for those that hinder activation function 2 accessibility (Dr. Robert Fletterick, University of California, San Francisco). However, efforts have been unsuccessful thus far. In addition, the function of activation function 2 is under debate. Most evidence suggests that androgen receptor transactivation relies primarily on the NH₂-terminal activation function 1 in normal prostate and shifts to include both activation function 1 and activation function 2 in prostate cancer (Dr. Wilson). Peptides have been recovered that bind activation function 2 with high affinity and do not block transcription (Dr. Donald McDonnell, Duke University, Durham, NC), suggesting that activation function 2 is dispensable for cell proliferation. Therefore, drug development efforts should include structural design and screening for molecules that enhance corepressor recruitment or target other surfaces in the androgen receptor, such as activation function 1 (Dr. Sadar).

Super antagonists, identified through their activities against recurrent prostate cancer, may represent one approach for drug development. A similar approach with the estrogen receptor yielded sequential endocrine therapies that are highly effective, although not curative, against metastatic breast cancer. However, it is not clear whether the super antagonist approach would be effective for the androgen receptor. Monotherapeutic use of antagonists, such as flutamide or bicalutamide, loses efficacy despite a castrate environment. Additional study is needed to determine why these antagonists are ineffective and how, under some conditions, they become agonists.

Additional exploration of androgen action in the prostate will pinpoint when disease begins and enable the development of compounds for preventive therapy. One trial under way is SELECT, which examines the preventive effects of selenium, which down-regulates androgen receptor expression and activation, and vitamin E. Clinical trials are under way to additionally evaluate 5 α -reductase inhibitors, which inhibit or prevent the conversion of testosterone to dihydrotestosterone. However, because the biology of advanced-stage cancers is still poorly understood, some lessons should be learned from the finasteride chemoprevention study (Dr. Kantoff), where the risk for prostate cancer decreased among men who received finasteride, but the likelihood of higher-stage cancers increased among finasteride recipients who did develop cancer.

Highlights

There appears to be a link between blood androgen levels and risk of prostate cancer; this has been supported by the finasteride chemoprevention trial wherein finasteride, a 5 α -reductase inhibitor, reduces the overall risk of prostate cancer by ~25%.

The finding from this trial that those treated with finasteride have a 20% increased likelihood of developing high-grade prostate cancer is supported by findings from the Health Professionals' Follow-up Study. This study suggests that individuals with lower testosterone levels years before diagnosis who develop prostate cancer are more likely to develop high-grade prostate cancer.

Androgen deprivation therapy remains the mainstay of therapy for men with advanced prostate cancer. The use of androgen-deprivation therapy in early prostate cancer has been established in men with locally advanced and high-risk prostate cancer when radiation therapy is used but not before surgery. The optimal duration of androgen-deprivation therapy has yet to be determined.

Whereas earlier androgen-deprivation therapy appears to prolong

survival, this has not been rigorously demonstrated in men with recurrent prostate cancer, nor has optimal timing been established. Because this is the case, one needs to consider the side effects of androgen-deprivation therapy as well in this population.

Whereas the androgen receptor appears to play a key role in all aspects of prostate and prostate cancer development, many fundamental aspects of its function and its interaction with other molecules are unknown but under study.

Of particular note is the continued presence of androgen receptor in the hormone-refractory state. Multiple mechanisms to support continued androgen receptor function can be demonstrated. This includes the persistence of low levels of androgen in the hormone-refractory prostate gland, the amplification of androgen receptor, the overexpression of androgen receptor, and in some cases, the mutation of androgen receptor giving rise to either constitutively activated androgen receptor or mutated androgen receptor that responds to nonandrogen ligands. There is evidence for androgen receptor activation through altered expression of coregulatory molecules and through other signaling pathways.

Given the persistence of androgen receptor function, the androgen receptor remains a target in hormone-refractory disease, and creative strategies to inhibit its function with improved antagonists or drugs that enhance its degradation are needed. These strategies should be facilitated through the recent crystallization of the molecule.

Appendix

The Symposium on Androgen Action in Prostate Cancer was sponsored by the Organ Systems Branch and the Division of Cancer Biology of the National Cancer Institute.

Presenters included the following researchers:

Steven Balk, M.D., Ph.D., Harvard University,
 Myles Brown, M.D., Harvard University,
 Michael Carey, Ph.D., University of California, Los Angeles,
 Gerhard Coetzee, Ph.D., University of Southern California,
 Zoran Culig, M.D., Innsbruck Medical University,
 Robert Fletterick, Ph.D., University of California, San Francisco,
 John Isaacs, Ph.D., Johns Hopkins University,
 Olli Jänne, University of Helsinki,
 Phillip Kantoff, M.D., Dana-Farber Cancer Institute, Harvard University,
 John Kurhanwicz, Ph.D., University of California, San Francisco,
 Ming-Fong Lin, Ph.D., University of Nebraska,
 Robert Matusik, Ph.D., Vanderbilt University,
 Donald McDonnell, Ph.D., Duke University,
 James Mohler, M.D., Roswell Cancer Institute,
 Peter Nelson, M.D., Fred Hutchinson Cancer Research Center,
 Bryce Paschal, Ph.D., University of Virginia,
 Stephen R. Plymate, M.D., University of Washington,
 Robert Reiter, M.D., University of California, Los Angeles,
 Paul Rennie, Ph.D., University of British Columbia,
 Diane Robins, Ph.D., University of Michigan,
 Marianne Sadar, Ph.D., British Columbia Cancer Agency,
 Wayne Tilley, Ph.D., University of Adelaide,
 Donald Tindall, Ph.D., Mayo Clinic,
 Zhou Wang, Ph.D., Northwestern University,
 Nancy Weigel, Ph.D., Baylor College of Medicine, and
 Elizabeth Wilson, Ph.D., University of North Carolina, Chapel Hill.

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