Peptide Growth Factors as Biomarkers of Prostate Cancer Risk

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INTRODUCTION

Cell growth in the human prostate is largely controlled by peptide growth factors acting through autocrine, paracrine, and even intracrine mechanisms. These growth factors respond to endocrine hormone signals and are capable of regulating proliferation, apoptosis, differentiation, and interaction with the extracellular matrix (1, 2). Early in cancer development, imbalances in stimulatory and inhibitory signals could create a "field effect", in which hyperproliferation leads to somatic mutation and clonal selection of malignant phenotypes. Measurement of growth factors and understanding of the environmental and genetic factors that up- or down-regulate their expression (or the expression of their receptors), therefore, should be of great value to investigators interested in the etiology and prevention of prostate cancer. However, because these growth factors operate in the local microenvironment, it is not easy to develop intermediate biomarker assays in accessible biologic fluids or tissues suitable for population studies. The purpose of this presentation is to review the rationale for measuring specific growth factors, the various methods that have been attempted to date, and the challenges currently faced in this area of research.

Valid biomarkers of growth factor expression would have several major applications in the domain of prostate cancer epidemiology and prevention. First, growth factor levels could provide important intermediate biomarkers in trials of lifestyle and chemopreventive agents. Second, growth factor patterns could predict the aggressiveness of prostate cancers before surgery—a crucial point given the variability in the behavior of prostate tumors and the number of men now diagnosed after prostate-specific antigen screening. Third, growth factor levels could clarify the prognosis in cases of resected prostate cancer. And fourth, growth factor levels could provide insights into the molecular etiology of prostate cancer and important links to genetic or environmental risk factors.

OVERVIEW OF VARIOUS GROWTH FACTORS IN PROSTATE CANCER DEVELOPMENT

Although prostate cancers arise from epithelial cells, there is considerable evidence for the importance of disturbances in normal stromal-epithelial cell communication. In fact, in some current models, dihydrotestosterone, the key intraprostatic androgen, is formed from testosterone in epithelial cells, but then interacts with stromal cells to regulate the release of paracrine growth factors (3). Cancer cells appear to develop alternative signaling pathways, including autocrine responses to androgen and androgen-independent pathways. Most of the data concerning the role of growth factors comes from studies involving cultured prostate cancer cells, a few relevant animal models, and analyses of human tissue samples. Several families of growth factors have been implicated in prostate cancer development, as shown in table 1.

All of these growth factors affect prostate cell growth in vitro and are present in human prostate tissue, as are their receptors. Epidermal growth factor and transforming growth factor-α, which are homologous and can both interact with the epidermal growth factor receptor, have been associated with mitogenic effects and possible local mediation of androgen action (4). The protein product of oncogene c-erbB-2 (p185erbB-2) is homologous with the epidermal growth factor receptor, and its overexpression has been implicated in the progression of prostate cancer. On the other hand, transforming growth factor-β, and in particular the transforming growth factor-β1 isoform, could have a bi-functional role in prostate cancer development (5). Transforming growth factor-β1 generally inhibits epithelial cell proliferation. However, in more advanced tumors in vivo it could promote tumor growth and metastasis by stimulating angiogenesis, inhibiting local immune response, or enhancing invasiveness through changes in the stromal matrix.

Several members of the large fibroblast growth factor family are expressed in the human prostate. Fibroblast growth factor-2 (basic fibroblast growth factor) is produced by stromal cells, and is only weakly mitogenic to normal epithelium. However, an autocrine loop can develop in prostate cancer cells, and fibroblast growth factor-2 could contribute to tumor progression through its effects on angiogenesis and alteration of the extracellular matrix. Fibroblast growth factor-7, also known as keratinocyte growth factor, and fibro-
TABLE 1. Growth factor families associated with prostate cancer development

<table>
<thead>
<tr>
<th>Family</th>
<th>Specific molecules*</th>
<th>Affected processes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermal growth factor</td>
<td>EGF, TGF-α, EGFR, p185ee2CONS</td>
<td>Proliferation</td>
</tr>
<tr>
<td>Transforming growth factor-β</td>
<td>TGF-β1, TGF-β2, TGF-β-RI, TGF-β-RIII</td>
<td>Proliferation, immunosuppression, differentiation, invasiveness</td>
</tr>
<tr>
<td>Insulin-like growth factor</td>
<td>IGF-1, IGF binding proteins, HGFR</td>
<td>Proliferation</td>
</tr>
<tr>
<td>Fibroblast growth factor</td>
<td>FGF-2, FGF-7, FGF-8, FGF-10, VEGF</td>
<td>Proliferation, angiogenesis, metastasis</td>
</tr>
<tr>
<td>Vascular endothelial growth factor</td>
<td>VEGF, NT-2, PDGF-α-R, c-met (HGFR)</td>
<td>Proliferation, motility</td>
</tr>
<tr>
<td>Nerve growth factor</td>
<td>NGF, p75NN, Trk receptor, NT-2, PDGF-α-R, c-met (HGFR)</td>
<td>Proliferation, motility</td>
</tr>
<tr>
<td>Other</td>
<td>PDGF-α-R, c-met (HGFR)</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: EGF, epidermal growth factor; TGF, transforming growth factor; EGFR, epidermal growth factor receptor; TGF-β1-R1 and R11, transforming growth factor-β-R1 receptors; IGF, insulin-like growth factor; IGF1, insulin-like growth factor receptor 1; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; NGF, nerve growth factor; Trk, tyrosine kinase; NT-2, neurotropin 2; PDGF-α-R, platelet derived growth factor α receptor; HGFR, hepatocyte growth factor receptor.

blast growth factor-8 have also been linked to abnormal prostate growth, but have been less extensively studied than fibroblast growth factor-2 (2, 3). Recent data suggest that dihydrotestosterone control over proliferation in normal prostatic epithelium could be mediated through expression of fibroblast growth factor-10 by stromal cells (6).

Vascular endothelial growth factor may play an angiogenic role in the prostate, as it does in other tissues. Adding vascular endothelial growth factor promotes the growth of human xenograft tumors in rodents, while anti-vascular endothelial growth factor antibody inhibits their growth and metastasis (7). Nerve growth factor was originally described as a positive regulator for the growth and maintenance of nerve cells. However, nerve growth factor and other neurotrophins are also produced in the prostatic stroma and exert a paracrine regulatory effect on epithelial cell growth. Increased expression of nerve growth factor, formation of an autocrine loop in cancer cells, and increased nerve growth factor activation of the tyrosine kinase, as opposed to the p75NN receptor, are all potential mechanisms for nerve growth factor promotion of prostate cancer growth (2). The protein product of the c-met proto-oncogene is hepatocyte growth factor receptor. Hepatocyte growth factor itself, which is produced in the stromal cells of the prostate, increases cancer cell proliferation and invasiveness. Quantitative expression of c-met increases progressively in human tissue with benign prostatic hyperplasia, prostatic intraepithelial neoplasia, localized cancer, and metastases (8).

**AVAILABLE MEANS FOR MEASURING GROWTH FACTORS IN EPIDEMIOLOGIC AND CLINICAL RESEARCH**

**Blood and urine**

Growth factors relevant to prostate carcinogenesis have been measured in serum (or plasma), urine, expressed prostatic fluid, and tissue samples. Even though growth factors arise and act primarily at the cell or tissue level, there are two reasons why serum or urinary growth factor levels could be considered significant. First, some growth factors, such as the insulin-like growth factors, are known to have endocrine functions, meaning that levels in the circulation could affect local tissue concentrations. Second, overexpression of growth factors or their receptors by occult tumors could be pronounced enough to raise systemic concentrations. Contemporary immunoassay techniques have become sensitive and specific enough to detect these peptides down to the picogram per milliliter level.

The insulin-like growth factors, that have been more extensively studied in blood samples than other growth factors, are discussed elsewhere in this issue of Epidemiologic Reviews. Two studies reported elevated plasma levels of transforming growth factor-β1 in men with prostate cancer (especially advanced cancer) compared with controls with no evidence of malignancy (9, 10). Three other studies failed to confirm this, although one reported elevated transforming growth factor-β1 in urine from prostate cancer cases and a positive association with disease stage (11-13). Fibroblast growth factor-2 and vascular endothelial growth factor, which are both associated with tumor angiogenesis, have also been compared in the sera of prostate cancer cases and controls. Serum vascular endothelial growth factor was not associated with the presence or stage of prostate cancer; however, two studies reported significantly higher serum fibroblast growth factor-2 in prostate cancer patients compared with healthy controls (14, 15). The extracellular domain of the c-erbB-2 protein (p105erbB-2) has also been measured in serum by immunoradiometric assays. Levels of this oncoprotein appear to be higher in advanced stage or more poorly differentiated prostate cancer compared with non-disease controls, and in one small follow-up study, patients with high serum c-erbB-2 protein had a shorter interval to disease progression (16).
Expressed prostatic fluid and seminal fluid

Expressed prostatic fluid, which can be readily obtained from most men by transrectal massage, provides a unique medium for non-invasively investigating the biochemical microenvironment of the prostate gland. It is produced by the apocrine and merocrine secretory activity of the prostate epithelium and, based on measurements of many biochemical constituents, it appears to reflect the metabolic status of the prostatic epithelium as a whole (17). In contrast, seminal fluid is a mixture of the secretory products of the prostate, testes, and seminal vesicles. Our group, and several other groups, have explored measurement of growth factors in both of these media. In one study, we measured concentrations of epidermal growth factor, transforming growth factor-α, and transforming growth factor-β1 in expressed prostatic fluid from men with untreated prostate cancer, benign prostatic hyperplasia, and no prostatic disease (18). Our results suggest that there are important associations between these concentrations and the presence of neoplastic or hyperplastic disease of the prostate. Concentrations of epidermal growth factor were 50 percent lower in expressed prostatic fluid samples from men with prostate cancer than in samples from age-matched men with normal prostates. Epidermal growth factor levels were approximately 25 percent lower in men with benign prostatic hyperplasia compared with the clinically normal group. Results for transforming growth factor-α were less clear; however, men with benign prostatic hyperplasia had lower concentrations in expressed prostatic fluid than in men with either prostate cancer or normal prostates. Transforming growth factor-β1 concentrations were more than 2.5 times higher among men with prostate cancer than among men with benign prostatic hyperplasia or normal prostates. The results for epidermal growth factor are shown in figure 1.

In the normal prostate, epidermal growth factor and transforming growth factor-α appear to be apocrine secretory products, with most immunostained material accumulating in ductal spaces (19). With dysplasia and further progression to carcinoma, the normal cell polarity is lost and immunostaining patterns are altered so that intracytoplasmic localization of epidermal growth factor and transforming growth factor-α is increased. This could explain our finding that epidermal growth factor levels in expressed prostatic fluid are lower in the presence of neoplastic disease. Only one previous study compared growth factor levels in expressed prostatic fluid across diagnostic groups (20). In that study, concentrations of epidermal growth factor were lower among men with benign prostatic hyperplasia (mean = 155 ng/ml) than among similar-aged men with clinically normal prostates (mean = 272 ng/ml). These results are similar to ours, and also suggest that benign prostatic hyperplasia might involve disruption of the normal secretory process for epidermal growth factor, or loss of epidermal growth factor required for maintenance of normal growth. Transforming growth factor-β and related peptides have been measured in

**FIGURE 1.** Epidermal growth factor (EGF) concentrations in prostatic fluid from prostate cancer (CaP), benign prostatic hyperplasia (BPH), and no prostatic disease (NPD) patients. The distributions of epidermal growth factor concentrations for patients in each diagnostic group and the median are displayed, with the number of patients per group shown beneath the group designation. In the CaP group, results for patients with stage A or B tumors are represented by circles, and those for patients with stage C or D tumors are represented by squares. The $p$ values are two-sided and were calculated by $t$ tests on log-transformed values: they are CaP vs. NPD, $p = 0.002$; CaP vs. BPH, $p = 0.17$; BPH vs. NPD, $p = 0.02$.
Prostate tissue samples

Growth factors in the prostate can be measured by immunohistochemical techniques in surgical specimens, core needle biopsy samples, and fine needle aspirates. The invasiveness of tissue sampling limits possible applications in clinical or epidemiologic work, but nonetheless, appropriate designs can be found. Numerous studies have examined concentrations of growth factors in tumor tissue as predictors of prognosis. For example, increased expression of transforming growth factor-β1 in tumors removed by prostatectomy has been associated with a higher risk of cancer recurrence (21). Prognostic studies could have limited relevance for clarifying the role of growth factors in cancer etiology, however. It is technically possible to measure growth factor expression by immunostaining of histologically normal prostate tissue from biopsy samples, although such studies have not yet been developed. New techniques for computer-assisted image analysis promise to surpass the accuracy and efficiency of measuring protein expression in tissue samples by visually counting stained bodies. Core needle biopsies using a spring-loaded gun are currently the standard method for obtaining tissue when prostate cancer is suspected. However, fine needle aspiration, which is less often used in clinical settings, could provide more epithelial cells with a lower rate of complications (22).

PROPOSED ITEMS FOR THE CURRENT RESEARCH AGENDA

Much work will have to be done before we can fully exploit growth factor measurements in human studies of prostate cancer. Areas for intensive work include the following:

1. Validating the relation between growth factor levels and prostate cancer risk. Although prospective, full-cohort analyses aimed directly at these issues are impractical; nested case-control studies can be conducted in defined cohorts with banks of frozen blood or urine samples. Archived biopsy samples could be quantitatively stained and used to determine whether growth factor expression in normal tissue is associated with prostate cancer incidence. High-grade prostatic intraepithelial neoplasia is most likely the precursor lesion for prostate cancer, which means that the spatial association of growth factor expression with high-grade prostatic intraepithelial neoplasia in tissue samples is of interest (23). Patients with high-grade prostatic intraepithelial neoplasia also constitute a very high risk group for diagnosis of prostate cancer, and, thus, could help define efficient cohorts for studying the links between potential biomarkers and cancer, including markers measured in expressed prostatic fluid.

2. Understanding the sources of intra- and interperson variability. A high ratio of intra- to interperson variability can render a potential biomarker useless in studies where the point is to explain differences between individuals in a population or to detect meaningful changes within an individual. Therefore, comparison of these sources of variation is often very important. For example, before studying differences between prostate cancer cases and controls, we measured the extent of consistency over time in epidermal growth factor and transforming growth factor-α levels in expressed prostatic fluid relative to differences between men (24). We found that two samples obtained from the same man 12 months apart correlated strongly, 0.89 and 0.71 for epidermal growth factor and transforming growth factor-α, respectively, and that between-man variability accounted for 84 percent of the total epidermal growth factor variance, as opposed to 61 percent of the total transforming growth factor-α variance. This could help explain why we observed a stronger association for epidermal growth factor than for transforming growth factor-α with disease status.

3. Identifying the key factors that modulate growth factor levels in the prostate. Once there is some evidence that a particular growth factor is implicated in prostate cancer development, it becomes reasonable to ask how local levels of that factor are determined. Endocrine hormones are obvious candidates for growth factor regulators, including the sex steroids and hormones of the insulin/insulin-like growth factor system. Although the effects of these hormones on growth factor expression in cultured prostate cells are quite well known, biomarker assay techniques in expressed prostatic fluid and tissue samples will have to be developed further before we are likely to have meaningful data on hormone-growth factor interaction in the human prostate. Ultimately, we hope to identify dietary changes or chemopreventive agents that interrupt the carcinogenic process very early, as indicated by molecular changes that might even precede histologic abnormalities.

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