

GROWTH FACTORS INVOLVED IN PROSTATE CARCINOGENESIS

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Prostate gland and growth factors
4. Insulin growth factor (IGF)
5. Vascular endothelial growth factor (VEGF)
6. Transforming growth factor- β (TGF- β)
7. Epidermal growth factor receptor (EGFR) signaling
 - 7.1. EGFR expression in prostate cancer
 - 7.2. Interplay between EGFR and androgens in prostate cancer
8. Conclusions and perspectives
9. Acknowledgements
10. References

1. ABSTRACT

Prostate cancer is the most common non-skin cancer affecting men in United States and the second leading cause of death after lung cancer. The clinical course of patients after given diagnosis of prostate cancer is highly variable and the underlying reasons for such variability remain elusive. To better understand the pathophysiology of prostate cancer, there has been a push to elucidate the molecular mechanisms that mediate the development and progression of prostate cancer. Recent literature has pointed that a complex interplay between various cytokines, growth factors, and androgen receptors regulate the growth and functions of the prostate gland. Amongst the currently implicated anomalous pathways involved in prostate oncogenesis, the IGF-IGFBP axis has been demonstrated to play a very important role, although the precise molecular events regulated by IGF remain to be elucidated. The tumor promoting functions of VEGF has been defined in tumor angiogenesis and currently remains the central focus of anti-angiogenesis therapy in prostate cancer. Another key cytokine, TGF- β has tumor-suppressor functions in normal prostate gland, but its pleiotropic functions in prostate cancer are influenced by the hormonal state of the disease. In partnership with other deregulated growth factor signaling, the TGF- β cascade has also been implicated in the spread of prostate cancer. Lastly, members of the EGFR family, particularly the HER2 receptor, have also been recognized as crucial elements of aberrant signal transduction pathways, which induce activation of downstream signaling, involved in cellular proliferation, cell survival, and angiogenesis. The abnormal function of a number of growth factors in prostate cancer biology explains the heterogeneity of its histologic grade, mode of presentation and disease prognosis. At the same time, continued research in this field allows for the potential

development of drug therapies against a diverse pool of cancer causing targets.

2. INTRODUCTION

Prostate cancer remains the most common malignancy in US males and is the second leading cause of malignancy related deaths. In the US, the incidence of this disease continues to increase as our population ages. It is estimated that in the year 2004, 230,110 new cases will be diagnosed and 29,900 deaths will occur secondary to prostate cancer (1). Although prostate cancer has been predominantly considered to be a disease of older men, it can also occur in younger men. In one study, when morphology of the prostate gland was studied at the time of autopsy, high-grade prostatic intraepithelial neoplasia (PIN) was identified in 5% of men in their 4th decade of life, 10% of men in their 5th decade of life and 63% of men in their 7th decade of life (2). The corresponding figures for invasive carcinoma were 29%, 32% and 64%, respectively (2). The incidence of low grade PIN is 9% in men who were in only their third decade of life (3). Clearly, factors that influence the development of PIN and the subsequent development of an invasive cancer occur early in life. Although this first premalignant change occurs at a young age, the majority of clinical cancers are not detected in most patients until after age 60. The clinical course after diagnosis is highly variable, with some patients having a more indolent course characterized by prolonged progression free survival even without a therapeutic intervention, while in others the clinical course is more aggressive with significant morbidity and mortality. Only recently have we begun to have a better understanding of the molecular events and growth factors involved in prostate cancer development and this disparate rate of progression.

Growth Factors in Prostate Cancer

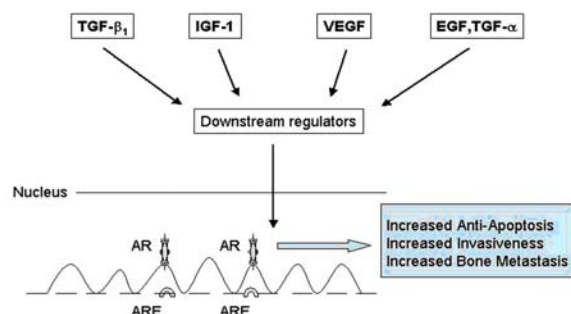


Figure 1. Overview of Growth Factor Signal Transduction in Prostate Cancer. Various growth factors (including TGF-β₁, IGF-1, VEGF, EGF, and TGF-α) activate numerous downstream regulatory proteins, which in turn modulate androgen receptors and androgen response elements (ARE) within the nucleus. When these pathways become deregulated, the resultant cells are conferred with immortality and increased invasiveness.

3. PROSTATE GLAND AND GROWTH FACTORS

The prostate gland is a sex steroid-dependent gland whose embryogenesis, adult development, and functions are modulated by testosterone and its active variant, 5α-dihydrotestosterone (DHT) (4, 5). Testosterone and its active metabolite, DHT, act by binding to androgen receptors within the prostate gland (4, 5). Androgen receptor (AR) belongs to the family of steroid receptors and like other nuclear receptors its protein structure incorporates a ligand-binding domain (LBD), DNA-binding domain (DBD), and an N-terminal domain (NTD) (4). The recent illustration of AR's LBD using 3-Dimensional crystallography has shed further light on the protein structure of the nuclear receptor and its ligand-mediated structural modulation (4). Based on such detailed analysis of the AR's structure, it is believed that androgens affix to a binding pocket within the LBD leading to activation of the inherently inactive receptors (4, 5). The androgen receptors, once released from their transcriptional-repressor state, recruit gene co-activators and activate transcription of target genes (4, 5).

Like any other human organ, the controlled growth and physiologic functions of the prostate gland are regulated by a complex interplay between various cytokines, growth peptides and androgen receptor signaling (5). The intercellular events regulating growth factor functions are triggered by ligand-mediated activation of their receptors, which subsequently relay downstream signals and regulate growth of the effector organ or cell in a positive or negative fashion (Figure 1). One of the crucial downstream targets involved in mediating the growth factors functions are the cyclin family of cell cycle proteins (6, 7). Cyclins are unstable proteins that activate cyclin-dependent kinases (CDK) involved in cell cycle progression (6, 7). Cyclin D, belonging to the cyclin family, is one such cell cycle regulator that acts in cohesion with cyclin E to drive cells into S-phase, by activating the CDK-kinases 2, 4, and 6 (6, 7). It is recognized that an array of mitogenic growth peptides converge to activate

cyclin D by different mechanisms (6, 7). Among which, the phenomenon of cyclin D1 induction by the PKC/Ras/Raf-1/Mek/ERK pathway is the best established (6, 7). On the contrary, lack of growth stimuli leads to proteosomal degradation of the cyclin D protein, thereby, inducing cell cycle arrest and apoptosis (6, 7).

Over the last decade there has been a growing wealth of evidence highlighting the molecular events involved in prostate morphogenesis and malignant transformation. Consequently, we now better understand the role of various signaling pathways involved in manipulating both the physiologic and pathologic functions of the prostate gland. From that pool of literature, it is now clear that the genesis of prostate cancer is a multi-step process involving heterogeneous molecular, biochemical, and genetic aberrations (4, 5). This chapter describes some of the important growth factors that are not only involved in the normal development of the prostate gland, but also have been implicated in prostate carcinogenesis, with added emphasis placed on targeted therapies against the tumor promoting pathways.

4. INSULIN GROWTH FACTOR (IGF)

Insulin growth factors are polypeptides that are structurally related to insulin and exhibit insulin-like mitogenic functions (8, 9). So far, two IGF peptides have been identified, IGF-I and IGF-II (8, 9). Liver secretes the IGF peptides upon stimulation by the growth hormone (8, 9). After synthesis, IGF-I, unlike insulin, has a widespread tissue distribution, where it regulates cell growth and differentiation (8, 9). To transmit their mitogenic functions, the IGFs bind to cell surface receptors, which include the insulin receptor, IGF-I receptor (IGF-IR), and IGF-II receptor (IGF-IIR) (8, 9). IGF functions are also regulated by a family of at least six clearly defined binding proteins, IGF-BP-1, 2, 3, 4, 5, and IGF-BP-6 (8, 9). At the tissue level, these binding proteins regulate IGF availability to its receptors while the IGF-binding proteins themselves are cleaved by enzymes known as IGFBP proteases, thus creating a finely tuned autoregulatory IGF-IGFBP circuit (8, 9). Several studies have implicated the IGF axis in cancer development (10-20), but the precise mechanism of action has not been elucidated.

Early studies looked to see whether IGF-R expression is under androgenic control. Results obtained from those studies indicate that androgens negatively regulate IGF-IR expression in non-cancerous prostate tissue (13). The investigators observed increased ligand binding capacity of both IGF-I and EGF in prostate tissue of patients with benign prostatic hypertrophy after they were medically castrated using a long-acting luteinizing hormone-releasing hormone (LHRH) analog (13). Subsequently, parallel experiments done on primary epithelial prostate cultures derived from different prostatic zones and from hyperplastic glandular tissue showed a variable distribution of IGFs and IGFBPs within the prostate gland (14). Compiled from the available evidence, the proposed IGF circuitry in place is that, in normal prostate tissue, the stromal cells produce IGF peptides,

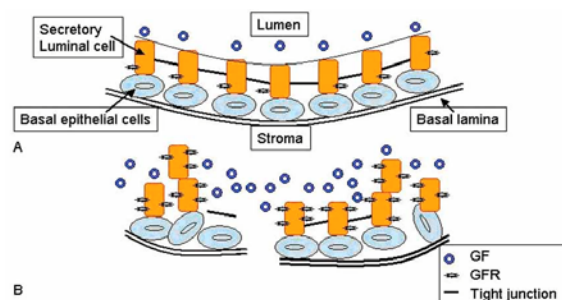


Figure 2. Interplay between growth factors (GF) and their receptors (GFR) in the prostate gland. Upper figure (A), in normal prostate gland tissue, GFR molecules are stimulated in a paracrine fashion by GF produced by the stroma. Lower figure (B), in malignant prostate gland tissue, disorganized luminal and basal anatomy allows GFR molecules located in the basolateral membrane to be exposed to GF secreted from the apical membrane resulting in autocrine proliferation.

which in turn bind to their epithelial target, IGFR, and regulate normal growth and differentiation of the gland (Figure 2; (13-17)). However, when such paracrine regulation gets disturbed, cancer develops within the ductal epithelial cells (Figure 2; (16, 17)). Besides the biologic evidence supporting the existence of autocrine IGF activity in prostate cancer evolution, recent epidemiologic nested case-control studies have also pointed towards an increased prostate cancer incidence risk in men with elevated serum IGF-I levels (18, 19). The most convincing evidence was provided by the prospective U.S. physician health study, which showed that in men within the highest quartile of serum IGF-I expression, the relative risk to develop prostate cancer was 4.3 (95 percent confidence interval 1.8 to 10.6) fold greater as compared to men in the lowest quartile (20).

Following early results, several studies have been done to unravel the role of growth factors, including IGFs role in prostate oncogenesis and, numerous such studies have demonstrated the existence of a mutually supportive nexus between the IGF axis, TGF- β and EGF/EGFR pathway, and AR (21, 90-95). Transfection experiments done in DU-145 cells, which inherently do not contain AR, showed that IGF-I, keratinocyte growth factor (KGF), and epidermal growth factor (EGF) positively regulate AR-mediated gene transcription, while the pure AR antagonist, casodex, inhibited ligand-dependent transcription in AR-transfected cells (21). Interestingly, constitutively active IGF-I receptors are recognizable not just in PC-3, DU-145 and LNCaP cells, but also in prostate cancer cells bearing the PTEN tumor-suppressor mutation, underscoring the presence of an exaggerated IGF-mediated signal transduction in prostate cancer (17, 22).

As previously mentioned, the IGF-binding proteins (IGFBPs) stringently control the tumor promoting mitogenic actions of IGF by regulating its tissue bioavailability, and an altered IGF/IGFBP ratio is considered to be tumor promoting (8-12). In a study done in patients with or without prostate malignancy, the serum

concentration of IGFBP-2 correlated directly with PSA levels, and was dramatically increased in patients with metastatic prostate cancer compared with healthy controls; suggesting that, perhaps like PSA, IGFBPs levels are also androgen-controlled (23). Tissue culture experiments, however, have portrayed a complex picture of ISBP modulation by the androgens in prostate cancer cells. Specifically, PC-3 cells express large amounts of IGFBP-2, 3, 4 and IGFBP-6 whereas the LNCaP cells exclusively express IGFBP-2 (16). Furthermore, AR-deficient PC-3 cells, when stably transfected with an AR-promoter construct, showed only inhibition of IGFBP-3 production even as the levels of IGFBP-2, -4, and -6 were equivalent in the conditioned medium of both AR-transfected and mock-control transfected cells (24). Therefore, in contrast to positive regulation of IGFBP-2 by androgens, the *in vitro* IGFBP-3 levels are negatively regulated by AR (23, 24). On the basis of these observations, an oblique postulate proposed is that the androgenic target, PSA, by proteolytically down-regulating IGFBP-3 levels, may invoke an unrestrained IGF-mediated mitogenic stimulation of the protease producing glandular cells (23-28). The human case-control studies also defend the hypothesis by demonstrating increased molar ratio of IGF/IGFBP or IGFBP-3/PSA in prostate cancer patients (25-27). Even though contentious, an altered IGF/IGFBP quotient is claimed as a surrogate marker for advanced prostate cancer (25, 26). As the debate about the use of prostate-specific antigen (PSA) testing and the optimal PSA-level threshold for biopsy continues, recently, serum levels of IGF-I, IGF-II, IGFBP-2 and IGFBP-3, taken either alone or as a combination, were evaluated to see whether the specificity of prostate cancer detection among men with a PSA level of 3 ng/mL or greater could be improved beyond that achieved by PSA fractionation (29). In that study, when the overall test performance was summarized using area under the receiver operating characteristic curve (AUC), the predictive performance of the free/total PSA index (AUC 0.73) as a cancer specific marker was significantly greater than that of IGF-I (AUC 0.59; $p < 0.0001$), IGF-I/PSA ratio (AUC 0.65; $p = 0.002$), IGF-I + IGFBP-3 (AUC 0.59; $p < 0.0001$), IGF-II (AUC 0.66; $p = 0.002$), and IGF-II + IGFBP-3 (AUC 0.67; $p = 0.05$), in the final analysis when all prostate biopsies were included (29). A similar trend was seen when the subset analyses was restricted to men with an initial PSA level of 3 to 10 ng/mL (29). In summary, even though present evidence supporting androgen modulation of IGF-IGFBP pathway continues to evolve, the oncogenic role of IGF axis seems to be more pertinent in metastatic prostate cancer than in early stage cancers (24-29). In advanced prostate cancer, an area where the role of IGFs is being keenly studied is in patients with bone involvement. This is because, IGF-I and IGF-II have previously been shown to stimulate osteoblastic activity leading to new bone formation (30-32). Therefore, by linking the osteogenic properties of IGFs to its pronounced mitogenic-signaling in advanced prostate cancers, one could speculate that IGFs promote development of bone metastases in prostate cancer, but the existence of such a scenario will have to directly test in future studies (24-32).

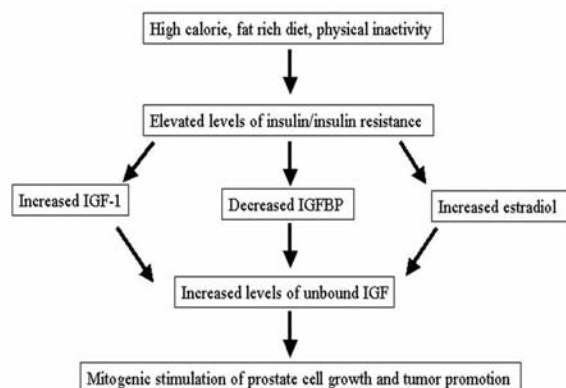


Figure 3. Model showing dietary modulation of the IGF-axis.

Increased incidence of prostate cancer in the western world has led many investigators to study the role played by diet and environment in the evolution of prostate cancer. Western diet, which typically is high-calorie, fat rich, and low in fiber content, has been shown to stimulate production of growth hormone, IGF-I and insulin, whereas caloric restriction has been shown to annul growth hormone and pro-mitogenic cytokine signaling (Figure 3; (8, 33-36)). After scientifically analyzing the emerging epidemiologic literature, nutrition researchers have started looking into dietary and life-style modifications as an adjuvant to improve the performance of established prostate cancer treatments and also study their role in chemoprophylaxis (34-37). Particularly appealing are the dietary supplements used in the eastern diet, namely, soy, green tea extract, and grape seed extract that are actively being investigated as anti-cancer agents (38-43). A shared theme in the dietary anti-cancer strategy is to study the effect of diet manipulations and nutritional supplements in reversing IGF-mediated signaling. Hence, after demonstrating anti-IGF properties in pre-clinical testing, many such agents are actively being studied in clinical setting, and are summarized in Table 1. Currently, questions in plentiful remain regarding the effectiveness of anti-IGF strategy in prostate cancer prevention and results from the ongoing clinical trials are eagerly awaited.

5. VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

Vascular endothelial growth factor (VEGF) plays a very important role in tumor angiogenesis and is currently the central focus of anti-angiogenesis therapy in cancer (44, 45). Recently, the Food and Drug Administration approved Bevacizumab (Avastin) as a targeted therapy to inhibit VEGF-mediated signaling in colon cancer (45). Bevacizumab is a VEGF-A-neutralizing monoclonal antibody, and its current indication is for use in combination with cytotoxic therapy in advanced colorectal cancer (45). Encouraged by the success of recently completed clinical trials using VEGF-inhibitors, future trials are being designed to redefine the treatment paradigm of various human malignancies by efficiently inhibiting VEGF-stimulated tumor growth.

VEGF-mediated signaling is involved in regulating embryogenesis, cell growth and differentiation,

as well as in tissue remodeling and healing (46-49). The mediators of VEGF actions are its receptors, which are subdivided into three subtypes, namely, VEGFR-1 (also known as Flt-1), VEGF-2 (identified as KDR in humans), and VEGF-3 (termed as Flt-4) (47-49). Like other tyrosine kinase receptors, an extracellular immunoglobulin homology domain and a tyrosine kinase intracellular domain characterize the VEGF-family of receptors, which upon ligand stimulation promote angiogenesis (47-49). Besides active VEGF-signaling, many other novel peptides that functions either independently or as auxiliary partners of VEGF have also been recognized as co-regulators of angiogenesis (Table 2; 47-49). Recent literature also supports neovascularization as one of the elemental defects in tumor vasculature resulting from a shift of balance favoring pro-angiogenic growth factors in proportion to the inhibitors of angiogenesis (47-49). Prior to that, some of the pioneering work done in tumor angiogenesis was presented by Judah Folkman in the early 1970's showing that active angiogenesis is required not only for continued tumor growth, but also for cancer cell metastases. Now almost three decades after that groundbreaking research, both laboratory scientists and clinicians have established inhibition of angiogenesis as a successful strategy in the fight against cancer.

In tumor vasculature, one of the most potent activators invoking angiogenesis is tissue hypoxia (48-52). Within the tumor microenvironment, oncogenic growth factors along with tumor hypoxia stimulate hypoxia-inducing factor 1- α (HIF-1 α) induction, which then heterodimerizes with its constitutive binding partner, the β subunits, and activate target genes regulating cellular growth, vascular remodeling, and cell survival (48-52). Enhanced angiogenesis thus adds another dimension to the aberrant functions of tumor promoting growth factors and oncogenic mutations, which then coalesce to induce malignant transformation of the normal cells (50-53). In an immunohistochemical analysis study done on clinical specimens containing high-grade prostate intraepithelial neoplasia (PIN), up-regulation of HIF-1 α was noted in majority of high-grade PIN lesions relative to the normal epithelium, stromal cells, and benign prostatic hyperplasia (BPH) tissue samples (54). The results, therefore, in parallel to demonstrating HIF-1 α up-regulation as an early event in prostate oncogenesis, also endorse HIF-1 α as a potential target for prostate cancer prevention and a proxy biomarker to recognize pre-malignant changes within the prostate gland (54). Like in the non-cancerous prostate tissue, HIF-1 α -induced VEGF up-regulation has also been demonstrated in LNCaP cells (55). The HIF-1 α -mediated VEGF induction in LNCaP cells was androgen-dependent and mediated through an autocrine loop involving EGF/phosphatidylinositol 3'-kinase/protein kinase B complex (55).

In line with the tissue culture results, in the *in vivo* setting also plasma VEGF levels are significantly elevated in patients with hormone-refractory prostate cancer when compared to patients with localized disease (56). Prognostically, in the androgen-independent setting, elevated VEGF levels inversely correlate with prostate

Growth Factors in Prostate Cancer

Table 1. Dietary agents currently being investigated in Prostate Cancer

| Pharmaceutical properties | Investigational Drugs | Phase of Development |
|---------------------------|------------------------|--|
| A. Antioxidants | Selenium and Vitamin E | Phase III (Closed to accrual) |
| | Lycopene | Phase II/III |
| | Pomegranate juice | Phase II |
| | Polyphenols | Pre-clinical |
| | Curcumin | Pre-clinical |
| B. Phytoestrogens | 2-Methoxyestradiol | Phase I/II |
| | Genistein, Daidzein, | Phase II |
| | Soy isoflavones | Phase I/II |
| C. Differentiating agents | Retinoids | <i>Cis</i> -retinoic acid (Phase II) |
| | | <i>All-trans</i> -retinoic acid (Phase II) |
| | | Targretin (Phase II) |
| | | Fenretide (Phase II) |
| | Vitamin D | Calcitriol (Phase II/III) |
| D. Modulators of IGF axis | Retinoids | Phase II |
| | Vitamin D | Phase II/III |
| | Lycopene | Phase II/III |
| | Low fat diet | Pre-clinical |
| | Exercise | Pre-clinical |

Table 2. Proangiogenic modulators of tumor vasculature in Prostate Cancer

| Ligand | Target Receptor |
|--|-----------------------------|
| Vascular endothelial growth factor | VEGFR-1, VEGFR-2, VEGFR-3 |
| Transforming growth factor- α and β | TGF- β -I, II and III |
| Angiopoietin-I | Tie-2 |
| Platelet derived growth factor | PDGFR |
| Tumor necrosis factor- α | EGFR |
| Epidermal growth factor | EGFR |
| Basic fibroblast growth factor | bFGFR-1, 2, 3, 4 |

Table 3. NCI conducted Anti-VEGF strategies currently being tested in Prostate Cancer

| Clinical Trial | Phase |
|--|-------|
| Study of Docetaxel, Estramustine, and Thalidomide in patients with androgen-independent metastatic adenocarcinoma of the prostate | II |
| Study of Docetaxel, Bevacizumab, Thalidomide, and Prednisone in patients with metastatic androgen-independent adenocarcinoma of the prostate | II |
| Leuprolide or Goserelin plus Thalidomide compared with Leuprolide or Goserelin alone in treating patients with nonmetastatic prostate cancer | III |

Derived from the National Cancer Institute (www.cancer.gov/clinicaltrials) website

cancer survival ($p = 0.002$) (56). Another recent study showed that plasma VEGF levels incrementally increased from healthy controls to patients with clinically localized disease to patients with more widespread lymph node and bone involvement (57). More significantly, a higher pre-operative level of VEGF was associated with lymph nodes involvement and an accelerated biochemical progression after surgery in both pre- and postoperative models (57). Even though prostate cancer is one of the most prevalent cancers in the western hemisphere, given the relatively recent recognition of VEGF and its regulatory proteins in prostate cancer development, it is no surprise that the clinical data on targeted anti-VEGF therapy in prostate cancer is lagging behind the results from colon cancer trials. However, the clinical interest derived from successful pre-clinical models has ensured continued investigation of anti-angiogenic therapy in prostate cancer. Some of the ongoing National Cancer Institute (NCI) sponsored studies exploring the efficacy of anti-VEGF

theme in prostate cancer are summarized in Table 3.

6. TRANSFORMING GROWTH FACTOR- β

Members of the TGF- β superfamily signal via membrane receptors bearing serine/threonine kinase activity, which in turn activate cytoplasmic effectors such as Smads and other non-Smad intermediary proteins to induce transcription of target genes (58, 59). Classically, TGF- β binds to the TGF- β 2 receptor, which sequentially heterodimerizes with the TGF- β 1 receptor to trigger downstream signaling cascade (58, 59). Of the many biologic functions of the TGF- β pathway, the regulation of cell proliferation and its action as a tumor-suppressor cytokine in the early stages of epithelial carcinogenesis that holds the most interest among prostate cancer researchers. TGF- β inhibits cell proliferation by repressing *c-myc* expression and by inhibiting the activity of cyclin-dependent kinases (CDKs) in part through transcriptional induction of CDK

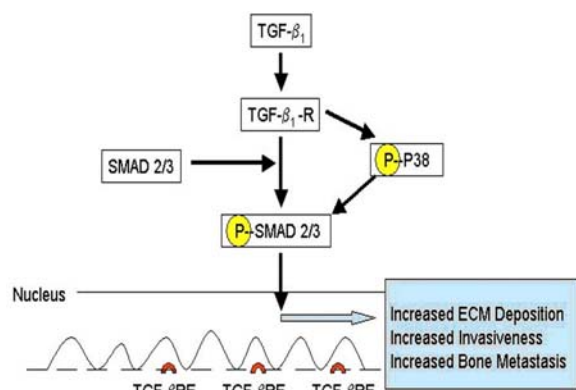


Figure 4. TGF- β 1 activates p38 MAPK. Once TGF- β 1 binds to its receptor, p38 is phosphorylated and subsequently activates SMAD 2/3. Phosphorylated SMAD 2/3 then, in turn, binds to TGF- β 1 response elements within the nucleus and augments ECM deposition and invasiveness, as well as increasing the likelihood of bony metastasis.

inhibitors, such as p15^{Ink4b} and p21^{Cip1} (58, 59). Even though TGF- β signaling recruits common elements including Smads and cell cycle modulators, its pleiotropic functions are cell-type specific (58-60). The role of TGF- β pathway in the development and progression of prostate cancer is poorly understood.

In the morphogenesis of the prostate gland, involvement of TGF- β -signaling mediated by all three isoforms comprising of TGF- β 1, TGF- β 2, and TGF- β 3, are considered to be obligatory (61). Studies performed in healthy rats have also shown that androgens positively control TGF- β expression in prostate tissue (62). Similarly, in prostate tumor models an induced anti-androgenic state also up-regulates TGF- β 1 expression; thereby suggesting that perhaps the anti-proliferative actions of TGF- β are needed to promote castration-mediated apoptosis of prostate cells (63). The tissue culture models of prostate cancer, however, have shown conflicting results, as the initial growth inhibition of monolayer formation by TGF- β was seen in a dose-response fashion in the two androgen-independent cell lines, PC-3 and DU145, but not in the androgen-dependent LNCaP cells (64). Such a disparity in TGF- β mediated growth inhibitory actions in prostate cancer cell lines was owing to secretion of TGF- β into the media of PC-3 and DU145 grown cells serving as an autocrine inhibitory factor (64). These results thus shed light on the androgenic modulation of TGF- β in both neoplastic and non-neoplastic prostate gland, but overall, the inconsistent trend seen in different model systems illustrates the complexity of TGF- β -mediated signaling in prostate cancer. To further obscure our current knowledge, the *in vivo* TGF- β signal transduction also appears to vary depending upon the hormonal status of the tumor (65). It is also likely that TGF- β may become deregulated due to the erroneous influence of other non-androgenic aberrant signaling pathways (65-67). Furthermore, loss of TGF- β 's growth-inhibitory effect, which has been linked to an aggressive tumor grade and a more advanced disease presentation in prostate cancer, has been attributed to insensitivity to TGF- β 's anti-proliferative actions due to

loss of its receptors, TbetaR-I and TbetaR-II (68, 69). In aggregate, the role reversal of TGF- β 's function from an anti-proliferative cytokine to a tumor promoting ligand in prostate cancer is intricate and needs further clarification in future studies.

Guided by results from other tumor systems, when clinical investigators studied the pre-operative concentrations of TGF- β in prostate cancer patients, they found elevated serum levels of TGF- β in patients with lymph node and distant metastases compared to those with localized cancer, thus implicating TGF- β in the spread of prostate cancer (70). Drawing from these results, the investigators advocate incorporating serum TGF- β levels along with other well-defined clinical variables, such as PSA level, primary and secondary Gleason's grade, and serum IL-6 soluble receptor (IL-6SR) levels, to predict the biologic behavior of clinically localized prostate cancer (71). Whether such biologic sub-classification of prostate cancer will change its disease-specific mortality or overall survival is unclear at this moment in time and needs further clarification before its outright application in the clinical setting.

After assimilating the mechanisms involved in TGF- β deregulation in prostate cancer, and then exploring for therapeutic benefits, one of the potential targets to challenge a dysfunctional TGF- β pathway would be either by reversal of TGF- β insensitivity or via inhibition of its tumor promoting actions. Using such an approach, racemic gossypol [(+/-)-GP], a naturally occurring polyphenolic yellow pigment present in cottonseed products, was recently shown to inhibit the *in vitro* proliferation of Dunning prostate cancer cells (MAT-LyLu), human prostate cancer cells derived from a bone marrow metastasis (PC-3), MCF-7 (breast cancer cell line) and primary cultured human prostate cells, by inducing TGF- β 1 expression, which in turn up-regulated cyclin D1 expression (72). Another study showed that TGF- β 2 secretion by tumor cells induced constitutive activation of NF- κ B transcription factor, thereby, promoting cell survival (73). Intriguingly, inhibition of NF- κ B activity using small interfering RNA against TGF- β 2 led to growth suppression of the neoplastic cells and improved their sensitivity to cytotoxic treatment (73). These findings are particularly important due to the newly defined role of chemotherapy in metastatic hormone-independent prostate cancer patients (74). Targeted therapy against deregulated TGF- β pathway could potentially enhance the anti-cancer performance of cytotoxic therapy in prostate cancer. Clinical testing of such a strategy in the future might be of immense value.

Apart from the growth inhibitory actions on epithelial cells, normal functions of TGF- β include stimulation of extracellular matrix (ECM) comprising of collagen, fibronectin, proteoglycans, and integrins, which are involved in regulating cell adhesion and motility (75, 76). It is this physiologic property of TGF- β that has led to the study of its role in the evolution of bone metastases due to disseminated prostate cancer. As TGF- β can modulate ECM deposition, it is hypothesized that TGF- β could

potentially promote tumor invasiveness and provide the necessary cellular environment to nurture metastatic tumor cells within the bony matrix, however, the molecular mechanisms remain unclear (65, 76). Study of the proximal signaling events regulating TGF- β mediated prostate cancer cell adhesion showed that the cytokine induces a dose-dependent increment in cellular adhesion of PC3-M cells, when compared to untreated controls, by activating its transcriptional target, the Smad family of cytosolic proteins (77). TGF- β treatment of metastatic prostate cells (PC3-M) activated Smad2 and -3 proteins by inducing phosphorylation and nuclear accumulation of the proteins (77). Furthermore, transfection experiments showed that dominant-negative Smad3A completely abrogated TGF- β -induced cell adhesion on prostate cancer cells (77). Finally, in a series of experiments done in PC3-M cells, it was demonstrated that participation of p38 MAP kinase is necessary to induce TGF- β -mediated phosphorylation and nuclear translocation of the Smad3; thereby, for the first time revealing existence of the cross-talk between the MAP kinase pathway and Smad transcription factors in transmitting the cell adhesive functions of TGF- β in prostate cancer (Figure 4; (77)). It is optimistically projected that forthcoming results from molecular research in this area will constitute a forerunner to develop novel targeted therapy against aberrant TGF- β signaling, which if successful, might potentially inhibit prostate cancer spread and decrease the morbidity associated with development of skeletal metastases.

7. EPIDERMAL GROWTH FACTOR RECEPTOR SIGNALING

Epidermal growth factor receptor (EGFR) belongs to the c-erbB family of receptor tyrosine kinases (78). EGFR, also known as HER-1 or c-erbB-1, is a 170-kd glycoprotein, which like many other tyrosine kinase receptors bears an extracellular ligand binding domain, a transmembrane domain, and an intracellular domain possessing tyrosine kinase activity (78). The other three identified members of the c-erbB group are HER2 (c-erbB-2), HER3 (c-erbB-3), and HER4 (c-erbB-4) (78). Of the known EGFR family members, the best studied is the HER2 receptor (79). HER2 has strong tyrosine kinase activity, but no associated ligand; therefore, on stimulation, it acts like a co-receptor and heterodimerizes with other EGFR subtypes (80, 81). It is estimated that HER2 is overexpressed in 25 to 30% of breast cancers and, more importantly, the enhanced expression of HER2 has been associated with an aggressive breast cancer presentation and shorter survival when compared to non-HER2 breast cancers (82, 83). The other non-breast tumors where EGFR overexpression has been identified are head and neck, lung, renal, colon, and ovarian cancers (78). More recently, the therapeutic targeting of deregulated receptor tyrosine kinases has been recognized as a successful anti-cancer tactic in a variety of human malignancies including prostate cancer (78, 84, 85).

The natural ligands for EGFR include epidermal growth factor (EGF), transforming growth factor- α (TGF), heparin-binding EGF, amphiregulin, and betacellulin (78).

Of the known ligands, epidermal growth factor (EGF) and transforming growth factor- α (TGF- α) are considered to be the most critical mitogenic mediators of EGFR-signal transduction (78). After ligand-binding, the EGFR subunits first dimerize, which brings together the catalytic domains of the receptors in close proximity, whereupon, signaling intermediaries, like adaptor proteins and downstream protein kinases are recruited to the phosphorylated tyrosine kinase domain of the receptor, ultimately resulting in activation of the transcriptional machinery (78). The two most well defined pathways activated by EGFR are the classical erbB1-Shc-Grb2/SOS-Ras-Raf-ERK1/2 signaling cascade pathway and the phosphatidylinositol (PI) 3'Kinase-Akt pathway (78, 79). These pathways along with numerous other receptor or non-receptor-mediated signaling cascades transcriptionally mediate the biologic functions of EGFR and their ligands, leading to cellular proliferation, angiogenesis, and anti-apoptosis (21, 78, 86, 87). Recent studies have also highlighted a link between EGFR-signaling and the VEGF pathway (86, 87). Such a cross-talk is responsible for EGFR-mediated up-regulation of VEGF, which in turn activates angiogenesis (86-89). Most interestingly, drawing a parallel to hormone-responsive breast cancer molecular biology, in prostate cancer, a multifarious relationship between the steroid receptor signaling and EGFR-mediated signal transduction is a realistic possibility and a topic to be researched.

7.1. EGFR expression in prostate cancer

EGFR-mediated signaling is indispensable to maintain the orderly growth and functions of the prostate gland in humans (90, 91). To defend this concept, when prostate epithelial cells were isolated in serum free conditions, mitogenic impulses mediated by EGF and a coalition of other growth peptides promoted the growth of epithelial cell cultures even without androgenic input (90). However, in the presence of male sex hormones, the same growth factors act in unison with sex steroids to shape the normal development of male accessory sex organs (91). Using immunohistochemical methods to study TGF- α and EGFR expression, it has previously been demonstrated that TGF- α expression occurs predominantly in the stroma, unlike its receptor, EGFR, which is mostly epithelial in distribution (92). Contrastingly, in the majority of the prostate cancer tissue samples, both EGFR and its ligand TGF- α were co-expressed (92). Therefore, equivalent to the IGF system, these findings underscore the presence of a paracrine or juxtacrine interaction between the stromal TGF- α and its epithelial receptor in normal and benign prostatic tissue, while in prostate cancer tissue an autonomously functioning pathway possibly accounts for the increased co-expression of TGF- α and EGFR (Figure 2; (92)). Various other groups have explored the link between natural EGFR ligands, EGF and TGF- α , and androgens in prostate cancer. Animal studies have shown that castration induces EGFR expression, which predictably, is reversed by exogenous testosterone administration (93). Likewise, androgen administration has also been shown to induce TGF- α mRNA in castrated rats, but unlike early EGF induction, this phenomenon was noted several days after androgen stimulation (93). In sum, based on these and many other studies done in both normal and cancerous

Growth Factors in Prostate Cancer

Table 4. Mechanisms involved in the development of Androgen-Refractory Prostate Cancer

| |
|---|
| Amplification of androgen receptor (AR) signaling a) AR gene amplification b) Increased sensitivity of AR |
| Non-specific activation of AR by non-androgenic molecules and growth peptides |
| Inactivation of tumor suppressor genes or gain of tumor promoting mutations |
| Activation of anti-apoptotic pathways |
| Activation of anti-apoptotic pathways |

Table 5. NCI conducted Anti-EGFR trials currently being tested in Prostate Cancer

| Clinical Trial | Phase |
|--|-------|
| To study the efficacy of ABX-EGF (a monoclonal antibody) given to patients with prostate cancer with or without tumor in other parts of the body | II |
| Docetaxel and Erlotinib in treating older patients with prostate cancer | II |
| Study of Everolimus and Gefitinib in patients with progressive metastatic prostate cancer | I/II |

Derived from the National Cancer Institute (www.cancer.gov/clinicaltrials) website

prostate tissue, EGFR and its ligand, TGF- α , are considered to be androgen-responsive targets, whose biologic activities are essential not only to fashion early glandular morphogenesis, but also to police cellular integrity, differentiation and functions of the developed gland (92-94).

In contrast to the growth inhibitory functions of EGFR and its ligands in a healthy prostate gland, in the setting of cancer there is evolving evidence to suggest that AR-mediated negative regulation of the TGF- α /EGF-EGFR axis may be disrupted leading to uninhibited EGFR stimulation by its natural ligands (21, 92, 94-97). Experiments done in tumor cells have shown that when LNCaP cells are engineered to an androgen-independent state or stimulated by androgens, EGFR expression is up-regulated (95). Accordingly, in humans, the hormone-independent metastatic prostate cancer cells also show increased expression of EGFR when compared to prostate tissue obtained after radical prostatectomy or after LHRH treatment (96). Another study showed synergistic colony forming effects of TNF- α and dihydrotestosterone when LNCaP cells were stimulated in soft agar (97). Moreover, like in breast cancer, aberrant EGFR signaling could also facilitate the development of prostate cancer metastases and impart cellular resistance to conventional cytotoxic therapy (98).

7.2. Interplay between EGFR and androgens and the role of Her-2 in prostate cancer

Irrespective of the factors responsible for enhanced EGFR expression or activity, the end result in hormone-independent prostate cancer is that the ligand-activated or constitutively active mutant growth factor receptor, operates as a surrogate pathway, to propagate the growth and spread of prostate cancer even when androgen stimulation is missing or is inconspicuous (21, 84, 92, 94-97). Substantiating evidence is provided by *in vitro* experiments showing that EGF and EGF-like mitogenic peptides induce activation of mutated androgen receptor via PI3K or the MAPK cascade (99, 100). Fittingly, the rising PSA level in the androgen-refractory clinical setting corroborates the existence of active non-androgenic signal transduction regulating AR-driven PSA production (4, 5, 84). However, an unanswered question that still remains is what factors lead to EGFR overexpression in prostate cancer, as unlike in breast cancer, c-erbB-2 gene amplification has not been demonstrated in

prostate cancer (101). Focused research in this subject might help us better understand the basis of EGFR deregulation in prostate cancer, nonetheless, taking into account the existing literature, the plausible molecular events responsible for exaggerated EGFR-signal transduction in prostate cancer are outlined in Table 4 (4, 5, 84).

Steered by Her-2-neu research in breast cancer and in other tumor systems, prostate cancer investigators are currently studying the oncogenic role of the receptor tyrosine kinase in prostate cancer. Early experiments showed that forced expression of the Her-2 gene into hormone-responsive LNCaP prostate cells leads to the development of a hormone-independent state (102). In the same study, the androgen-independent sublines expressed higher levels of the HER-2/neu receptor than their androgen-dependent counterparts (102). Consistent with the animal experiments, studies done in humans have also demonstrated similar findings; specifically, enhanced HER-2 expression has been demonstrated in tumor tissue exposed to androgen ablation, with the highest intensity measured in metastatic androgen-independent tumor tissue (101). This is an important observation, as overexpressed HER-2 in hormone-independent prostate cancer, like EGFR, has the affinity to induce AR-transactivation via MAPK signaling cascade even when androgen stimulation is absent (84, 103). Secondly, as EGFR has been identified as the natural dimerization partner of HER-2, it potentially gives the orphan receptor a binding partner to trigger signal transduction in hormone-independent prostate cancer (80, 81). Finally, Trastusumab (Herceptin), a monoclonal antibody against HER-2, has shown promising antitumor activity in prostate cancer xenografts, when used concomitantly with paclitaxel, in the setting of both hormonal-dependent and independent prostate cancer (104). Future clinical testing of Herceptin and other anti-EGFR strategies in prostate cancer are eagerly anticipated, because if successful, we might see a significant shift in the current treatment model being used for patients with prostate cancer. Some of the active anti-EGFR agents being tested in NCI supported clinical trials are summarized in Table 5.

8. CONCLUSION AND PERSPECTIVE

In summary, the abnormal function of growth factors in prostate cancer biology explains the heterogeneity of

its histologic grade, metastatic spread and androgenic transformation of the original tumor. Due to better understanding of the molecular events, we now have a broader hypothesis, which integrates together the clinical observations and the disease behavior at a cellular level. A general premise that emerges from the reviewed studies is that multiple mechanisms are involved in prostate cancer development and in maintaining its hormonal state. For a continued success and improvement in prostate cancer treatment, the identification of new oncogenic pathways is critical in this innovative era of targeted anti-tumor therapy to usher in novel and improved therapies, including chemoprevention strategies. Finally, over the last decade barring more availability in the choices of cytotoxic therapy, in the field of hormone-insensitive prostate cancer, therapeutic options remain limited and the overall prognosis grim, therefore, concerted efforts to identify new therapeutic targets holds the most interest amongst patients and their treating clinicians.

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