Chapter 7

TRANSFORMING GROWTH FACTOR BETA AND PROSTATE CANCER

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1. INTRODUCTION

Prostate cancer is the most commonly diagnosed malignancy among American males and is the second leading cause of cancer-related death. It is estimated that over 230,110 men will be diagnosed with the disease in 2004 [1]. While substantial advances have been made towards the diagnosis and treatment of prostate cancer, the underlying molecular initiation events leading to prostate cancer development and progression to advanced metastatic disease remain elusive. Prostate specific antigen (PSA) screening has resulted in earlier disease detection, yet approximately 30% of men will die of metastatic disease. Slow progression, an aging population, and the associated morbidity strongly underscore the need for improved therapeutic strategies and prognostic markers. An array of growth factors is involved in the regulating normal prostate growth, including epidermal growth factor (EGF), transforming growth factor-α (TGF-α), keratinocyte growth factor, basic fibroblast growth factor (bFGF), insulin-like growth factor (IGF) and TGF-β families [2]. The TGF-β family is important for inducing differentiation and inhibiting prostate epithelial cell proliferation and for maintaining normal prostate homeostasis [3-5]. The first member of TGF-β superfamily of secreted polypeptide factors, TGF-β1, was discovered approximately 20 years ago [6]. This interesting growth factor family has grown considerably during the last two decades to a number of thirty distinct and yet structurally and functionally related members [7]. The present review will summarize the current acknowledge on the paradoxical roles of TGF-β1

and its signaling pathway in the regulation of prostate normal and tumorigenic growth and will highlight the significance of a defective TGF-β1 mechanism in the prognosis and treatment of prostate cancer.

2. THE TGF-β SUPERFAMILY HISTORY

TGF-β was originally named because of its ability to stimulate fibroblast growth in soft agar; but it can also serve as a potent inhibitor of epithelial cell proliferation [8]. The TGF-β superfamily includes the TGF-β family (TGF-β1 to β5), leading members of which are important in regulating the formation of extracellular matrix, and inhibiting cell proliferation and inducing apoptosis. The two major cell types, stromal and glandular epithelial cells from the normal human prostate and benign prostatic hyperplasia, express mRNA for TGF-β1 to β3, but the former primarily secreted TGF-β1, whereas the later secreted more TGF-β2, and β3 than TGF-β1 [9]. -TGF-β1 is important in regulating cellular growth, differentiation, and apoptosis [10-14]. TGF-β1, TGF-β2, TGF-β3, and TGF-β5 differentially enhance the expression of N-cadherin, N-CAM, fibronectin, and tenascin in precartilage condensations, suggesting that TGF-β isoforms play an important role in the establishment of cell-cell and cell-extracellular matrix interactions during precartilage condensations [15-17].

Other members of the superfamily include the activin family, the bone morphogenetic proteins (BMPs), the Vg1 family, Growth/differentiation factors (GDFs), glial-derived neurotrophic factor (GDNF), and Müllerian inhibitory factor (MIF). Significantly enough, activin inhibits androgen-responsive prostate cancer cell growth [18], and is important in apoptotic regulation of human prostate cancers [19]. Furthermore activin can be a physiological modulator of PSA gene transcription, secretion in the prostate, and may cooperate with androgen to up-regulate PSA in vivo, and can regulate prostate growth [20, 21]. BMPs are a family of growth factors, which may play a role in the formation of prostate cancer osteoblastic bone metastases. BMP-6 mRNA expressed strongly in prostatic adenocarcinomas, both in the primary tumor and in bone metastases. Evidence pointing to BMP-6 as a potential attractive marker and possible mediator of skeletal metastases in prostate carcinoma [22, 23]. Prostate-derived factor (PDF), a member of BMPs [24], involved in differentiation of the prostate epithelium [25], may also be important in the progression of prostate cancer [26]. GDFs like other members play an important role in cell growth and differentiation. GDF-15/MIC-1 is widely distributed in adult tissues including those of the prostate, being most strongly expressed in epithelial cells and macrophages [27]; The Vg1 cell-signaling pathway plays a central

role in left-right coordinator function [28]; while GDNF regulates apoptosis in epithelial cells [29]; and MIF is an essential factor for male sexual differentiation [30].

TGF-ß family ligands are translated as prepropeptide precursors with an N-terminal signal peptide followed by the prodomain and the mature domain, which is responsible for activation. Six to nine conserved cysteine residues in the mature domain form intra- and intermolecular disulfide bonds characteristic of this family of proteins [31]. Several members of the family (i.e., GDF-9, BMP-15, GDF-3) have a substitution of a serine for the cysteine normally involved in intermolecular disulfide bond formation [32]. TGF-β1 is the best-studied isoform; it is a disulfide-linked homodimer of a 112-amino acid peptide (25 kDa) derived from a 2.4-kb mRNA transcript; TGFβ1 mRNA is translated into a 390-amino acid precursor with a 29-amino acid N-terminal signal peptide. The precursor is dimerized, glycosylated, and cleaved at amino acid 278 to yield an N-terminal latency-associated peptide (LAP) and a C-terminal mature TGF-β1 peptide which remain complexed with each other as latent TGF-β1; the latent TGF-β complex is secreted [33]. The active form of TGF-β is a dimer stabilized by hydrophobic interactions, which are further strengthened by an intersubunit disulfide bridge [34].

There are three major classes of TGF-β receptor proteins TGFβ receptor types I–III (abbreviated as TβRI, TβRII, and TβRIII, respectively)[35]. TβRI and TβRII are serine-threonine protein kinases that contain an extracellular ligand-binding domain, a single transmembrane domain, and a cytoplasmic serinethreonine kinase domain. Only TβRI has a GS domain that precedes the kinase domain; the GS domain contains the sequenceTTSGSGSG, a cluster of glycines (G), serines (S), and threonines (T). Compared to TβRIIs, TβRI has a shorter C-terminal tail at the end of the kinase domain, and an extracellular domain that is shorter and has a different distribution of conserved cystines [36]. The activation of the TβRI involves the phosphorylation of its GS domain by the TβRII; hence an active receptor-signaling complex comprises both types of receptors bound to the ligand. Several receptor variants have N-terminal or C-terminal extensions, most of them with as yet unknown function [31].

The TβRIII, also known as β-glycan, is thought to have a biological function distinct from the other two receptors TRI and TRII [37-39]. The TβRIII functions by selectively binding the autophosphorylated TβRII via its cytoplasmic domain, thus promoting the preferential formation of a complex between the autophosphorylated TβRII and TβRI, and then dissociating from this active signaling complex [40], elucidate important functional roles of the cytoplasmic domain of the TβRIII and demonstrate that these roles are essential for regulating TGF-β signaling.

2.1 The Major Players: TGF-β Intracellular Signaling

The current knowledge of the potential mechanism of intracellular TGF-β signaling is summarized in **Figure 1**. The biological action of this fascinating growth factor is primarily regulated by the Smad family of proteins [41]. Indeed Smads represent another intriguing and functionally connected family of structurally related signaling effectors, which like TGF-β family itself, is rapidly growing.

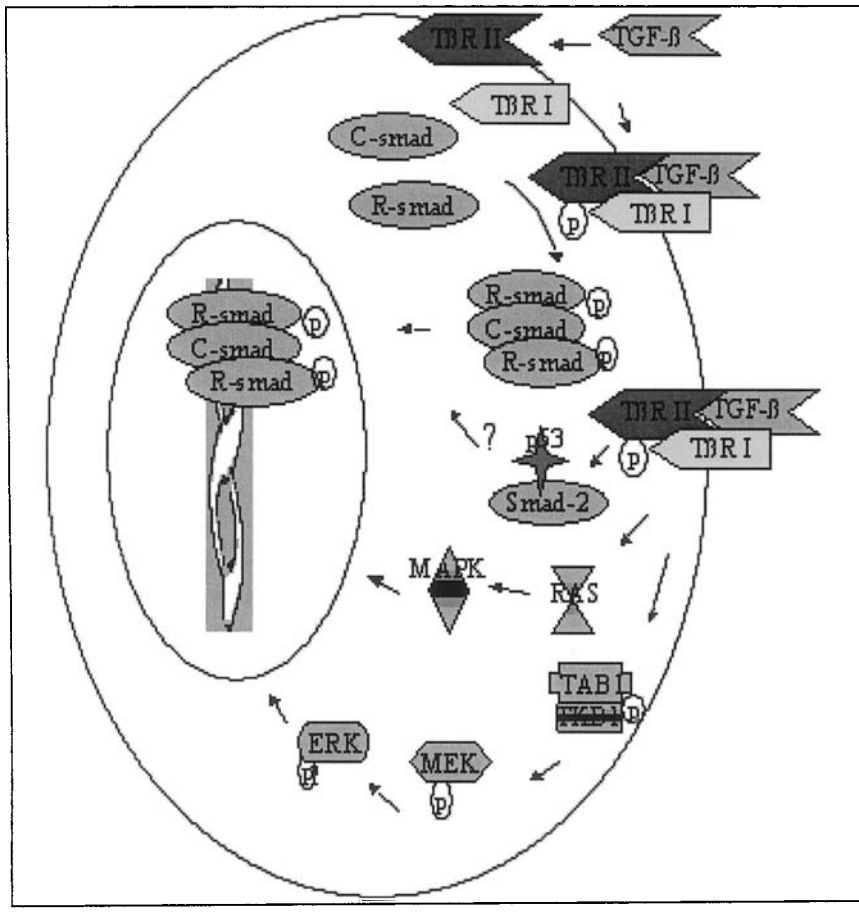

Figure 1. TGF-beta signaling in Prostate Cells

There are eight vertebrate Smads, Smad1 to Smad8, with a small number of amino acid differences between two very similar Smads in the same species confering distinct activities [42]. Smad2 and Smad3 are activated through

carboxy-terminal phosphorylation by the TGF-β receptors TβRI and ActRI β, whereas Smad1, Smad5 and Smad8 are activated by ALK-1, ALK-2, BMP-RIA/ALK-3 and BMP-RIB/ALK-6 in response to BMP1–4 or other ligands. These receptor-activated Smads (R-Smads) are released from the receptor complex to form a heterotrimeric complex of two R-Smads and a common Smad4 (CO-Smad), and translocate into the nucleus; Smad6 and Smad7 act as 'inhibitory' Smads [41]. The R-Smads contain two conserved structural domains, the N-terminal MH1domain, and the C-terminal MH2 domain; their C termini contain a characteristic SXS motif. The MH1 (MAD-homology 1) domain of Smad4 and most R-Smads exhibits sequence-specific DNA binding activity, may play a role in nuclear import, and negatively regulates the function of the MH2 domain [35]. Generally, the ligand binds a complex (types I and II) and induces transphosphorylation of the GS segments in the TβRI; the activated TβRI complex phosphorylates R-Smads at C-terminal serines, forming a complex with Smad4. Activated Smad complexes translocate into the nucleus, where they regulate transcription of target genes.

While TGF-β receptors remain active for at least 3–4 h after ligand binding, and continuous receptor activation maintains the Smad complexes in the nucleus, where they regulate gene expression [41,43]. Nuclear import of a Smad complex follows 'classical' nuclear translocation paradigms, established through studies of other proteins. Without ligand stimulation, R-Smads localize in the cytoplasm, whereas Smad4 is distributed in the nucleus and cytoplasm [43]. In the nucleus, R-Smads are constantly dephosphorylated, resulting in dissociation of Smad complexes and export of inactive Smads to the cytoplasm [41,43]. There is growing evidence to suggest that SMAD-independent pathways also exist, TGF-β activates other signaling cascades, including MAPK PP2A/p70S6K, RhoA and TAK1/MEKK1pathways [41, 44, 45].

2.2 A Prostate Insight of TGF-β Signaling

The paradoxical role of TGF-β in the regulation of malignant prostate growth can be attributed to a change in the expression of TGF-β receptors and the response of the host to TGF- β. Normal prostate epithelial cells exhibit relatively high levels of the ligand TGF- β [46]. On the other hand, TGF-β 1-2 is overexpressed in human prostate cancer, resulting in elevated levels of both urinary TGF-β1 and plasma TGF-β in prostate cancer patients [47]. However, even though cancer cells exhibit upregulated expression of TGF-β, the down-regulated expression of TβRI and TRII abrogates the autocrine growth inhibitory effects of the TGF-βs. This is most convincingly demonstrated by the observation that restoration of TRII expression in the TGF-β-resistant human prostate tumor cell line LNCaP

inhibits the in vivo growth of cancer xenografts via induction of apoptosis and upregulation of the cell cycle inhibitor $p27^{Kip1}$ [10] In addition, prostate cancer cells that exhibit up-regulation of the TGF-β and downregulation of their receptors, can also locally inhibit immune surveillance of prostate tumor growth [48]. Several experimental and clinical studies documented that although human prostate cancer cell lines exhibit partial loss of their ability to secrete and activate TGF-β, androgen-sensitive prostate cancer cells can compensate for this loss within the context of apoptosis regulation, by hormonal "adjustment" [49]. In addition, other intercellular regulators in the regulation of apoptosis, such as p53, have also been intimately connected with the TGF-β mediated apoptotic signaling in several cellular system [50]. Interestingly enough, recent work in *Xenopus* embryos reveals an unexpected developmental role for the tumor suppressor gene p53. p53-deficient cells display an impaired cytostatic response to TGF-β signals. Smad and p53 protein complexes converge on separate cis binding elements on a target promoter and synergistically activate TGF-β induced transcription. p53 can physically interact *in vivo* with Smad2 in a TGF-β-dependent fashion. The results unveil a previously unrecognized link between two primary mediary tumor suppressor pathways in vertebrates [51]. This finding may have implications for the evolution of our understanding of p53, via its interaction with Smads in TGF-β dependent mesoderm specification.

In the normal and malignant prostate Androgens negatively regulate TGF-β1 ligand[52, 53]and receptor expression[54, 55], along with Smad expression and activation[56]. A series of elegant studies by several investigators documented the ability of dihydrotestosterone (DHT) to inhibit TGF-β signaling in prostatic epithelial cells through interaction of AR with Smad3. Of major mechanistic significance was the finding that the binding of ligand-bound AR to activated Smad3 inhibits TGF-β transcriptional responses by blocking the association of Smad3 with Smad-binding element (SBE) [57-59]. Moreover, another report provides strong evidence to suggest the existence of a dynamic cross-talk mechanism between the androgen axis and TGF-β signaling in prostate stromal cells that affects cell proliferation and myodifferentiation. [60] In addition, one has to also consider the complexity of this functional interaction as an array of other factors such as p21 (ras)[61,62], bcl-2[10,63] , E-box[64] have been implicated as players in TGF-β signal transduction. Expression of the ligand TGF-β is significantly higher in prostate cancer compared to the normal gland [65,66]. Furthermore in rat prostate adenocarcinoma cell lines a direct correlation between increased TGF-β expression and tumor aggressiveness was detected. The TGF-β1 overproducing Dunning R3327 MATLyLu rat prostate carcinoma tumors had a faster growth rate, and exhibited a considerably higher metastatic ability than the parental tumor [67].

Compelling evidence emerging from studies on experimental and clinical specimens provides strong proof-of-principle that malignant transformation of prostatic epithelial cells was associated with loss of expression of functional TGF-β receptors and overproduction of TGF-β in malignant cells [68,69]. A significant decrease in the expression of TβRI and TβRII mRNA, in primary prostatic tumors and lymph nodes positive for metastases, indicating that the decreased protein expression was due to down-regulation of gene expression for the two receptors [70]. In other human malignancies including lung and laryngeal cancer, TβRII mutations were detected at high frequency, although that was not the case in prostate adenocarcinoma [71-73]. Since bone metastases of prostate carcinoma is closely associated with osteoblastic metastasis, the evidence that a disruption of TGF-β signaling in prostate cancer plays a causal role in promoting tumor metastasis [74], has significance clinical dimensions. Mechanistically TGF-β1 may indirectly enhance the formation of osteoblastic metastatic lesions by regulating tumor-derived factors, such as parathyroid hormone-related protein (PTHrP), shown to be actively involved in the development of osteoblastic metastases. This concept gains support from evidence that TGF-β1 increased PTHrP mRNA expression in canine normal prostate epithelial cells and stromal while resulted in a downregulation of this factor in prostate carcinoma cells [75].

2.2.1 Targeting Prostate Growth: TGF-β as a Regulator of Cell Differentiation and Apoptosis

Evidence from experimental *in vitro* studies suggests that TGFβ1 may functionally contribute to the development of prostate cancer and BPH [76] (**Figure 2**) via its ability to regulate both the stroma cells and epithelial cells [3,10]. Treatment of rat prostatic epithelial cells with EGF or TGF-α resulted in a concentration-dependent increase in cell growth, whereas addition of TGF-β 1 into the culture resulted in an inhibition of cell proliferation that could be reversed with increasing concentrations of EGF. Addition of TGF-β 1 into the EGF-depleted medium caused a further increase of cell death [77].

Using a human papilloma virus 16 E6/E7 immortalized prostate epithelial cell line, HPr-1, Ling et al. [78] reported that TGF β1 suppressed the expression of Id-1, a helix-loop-helix protein, which plays a key role in inhibition of cell differentiation and growth arrest. Considering that up-regulation of $p21^{WAF1}$, one of the downstream effectors of Id-1, is an early induction during the apoptotic response to TGFβ1, indicates the involvement of Id-1 (transcription factor) in dictating the TGF β 1-induced growth arrest in human prostate epithelial cells.

TGF-β is found in high concentrations in prostatic fluid and benign glands in areas of pathologically characterized BPH[47,48]. Basal cell cultures established from prostate explants either grown into cellular senescence, or

stimulated with TGF-β1, β2 and β3.result showed TGF-β stimulation resulted in an increase of SA-β galactosidase (SA-β-gal) activity by supporting differentiation processes, but not cellular senescence [12].

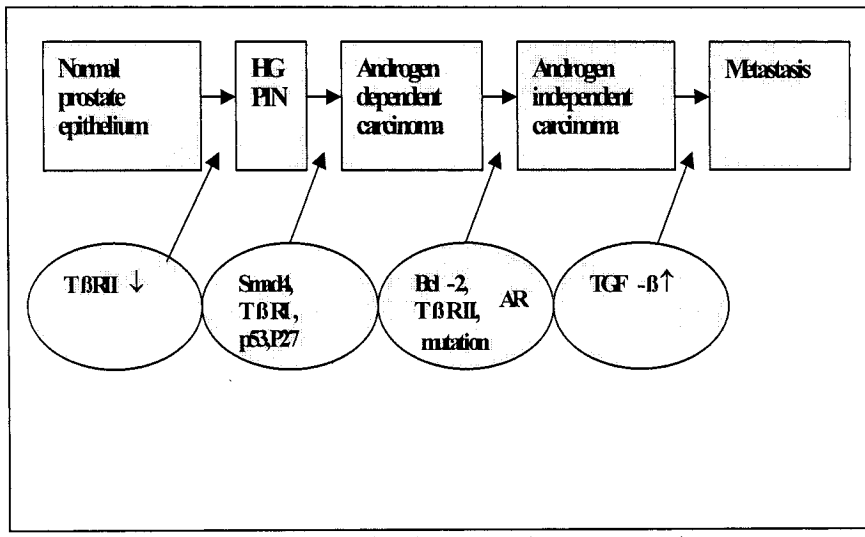

Figure 2. TGF-β signaling in Prostate Cancer Progression

It has been postulated that TGF-βs may induce human prostatic stromal cells to express the smooth muscle phenotype [79], an action that might contribute to the development of neoplastic growth in the aging gland.

Prostate-derived factor (PDF) is a member of TGF-β superfamily and has been directly implicated in differentiation of the prostate epithelium. Proprotein convertases (PCs), such as furin, are thought to mediate the processing of TGF-β superfamily. Human prostate cancer cell lines differentially synthesize and secret prostate PDF, and that PDF secreted by LNCaP is processed by PCs[25] and the causal contribution of both growth factors and their signal transduction mechanisms in prostate tumorigenesis awaits further investigation. TGF-β has been shown to exert the role of an apoptosis inducer in a variety of human cell lines including lens epithelial [80], liver [81], lung [82], and brain cells [83]. A significant down-regulation was detected in TβRII and Smad4 expression in high-grade prostate intraepithelial neoplasia (HGPIN) and prostate cancer compared with benign prostatic hyperplasia; Evaluation of the incidence of apoptosis revealed a significant decrease in the apoptotic index among the epithelial cell populations in HGPIN and a further decrease in prostate carcinoma [84]. These results further define deregulation of TGF-β signaling effectors as a molecular basis for loss of apoptotic control contributing to the development of prostate tumors.

In vitro studies from this laboratory demonstrated that the androgen-sensitive prostate cancer LNCaP engineered to overexpress TGF-β RII cells; undergo cell cycle arrest and apoptosis in response to TGF-β treatment in the presence of physiological levels of dihydrotestosterone [10]. This effect temporally correlated with an increased expression of the cell cycle regulator p21 and the apoptotic executioner, procaspase-1, with a parallel down-regulation of the antiapoptotic protein, bcl-2. Furthermore, apoptosis induction was suppressed by the caspase-1 inhibitor, z-YVAD, but not the caspase-3 inhibitor, z-DQMD [84,85]; thus TGF-β-mediated apoptosis in prostate cancer cells can actually be enhanced by androgens through specific mechanisms involving cell cycle and apoptosis regulators. Provocative as it might seem this evidence suggests the ability of androgens (at physiological levels) to stimulate the intrinsic apoptotic potential of prostate cancer cells. Driven by these findings one may speculate on the synthesis of a molecular basis for the priming of prostate cancer cells for maximal apoptosis induction potentially by TGF-β, during hormone-ablation therapy [85] of prostatic tumors.

2.3 *In vivo* Action of TGF-β: Lessons from Mice

Analysis of bcl2, bax, p53, and caspase knockout mice while establishing distinct role for each of these apoptotic players, they also provide valuable information for the design of specific inhibitors of apoptosis. Thus blocking one pathway, as in caspase knockout mice, what we observe is not a complete suppression of apoptosis but rather a delay in apoptosis induction [86]. A significant insight into the in vivo functional importance of TβRII was provided by Bhomwich et al. [5], who reported on the successful generation of mice conditionally inactive for Tgfbr2. Early development of the Tgfbr2fspKO mice appeared normal, but by 3 weeks of age, there was a rapid increase in the number of stromal fibroblasts in the prostate, followed by epithelial neoplasia. This evidence firmly supports the concept that a signaling pathway known to suppress cell-cycle progression when activated in epithelial cells, can also have an indirect inhibitory effect on epithelial cell proliferation when activated in the adjacent stromal fibroblasts in vivo. Loss of this inhibitory effect can result in increased epithelial proliferation and may even progress to invasive carcinoma in some tissues, highlighting the importance of a reactive stroma in determining the proliferative/apoptotic status of the glandular epithelium via TGF-β signaling. The transgenic Adenocarcinoma of Mouse Prostate (TRAMP) animal model [87] represents a powerful tool for studying the mechanism of prostate cancer initiation, progression as well as therapeutic, and chemoprevention targeting. In recent elegant studies Tu et al. [74] bred transgenic mice expressing the tumorigenic SV40 large T antigen in the prostate with transgenic mice expressing a

dominant negative TβRII mutant (DN II R) in the prostate, their findings clearly established that the loss of TGF-β signaling promotes prostate cancer metastasis. These findings confirmed the evidence reported in the clinical setting of prostate cancer that TβRII loss correlated with prostate tumor progression and increasing Gleason grade.

Transplantation of murine bone marrow (BM) expressing a dominant-negative TβRII (TβRIIDN) leads to the generation of mature leukocytes capable of a potent antitumor response in vivo; treatment of male C57BL/6 mice with TβRIIDN-BM resulted in the survival of 80% of recipients versus 0% in green fluorescent protein-BM recipients or wild-type controls [88], supporting the anti-tumor therapeutic potential of gene therapy-based approach to inducing TGF-β insensitivity in transplanted BM cells. Genetic studies based on targeted disruption of the key TGF-β signaling effectors, using the TβRII and p27 knockout mouse models provide exciting new insights into the functional contribution of both the TβRII and p27 gene and their products in estrogen-induced tumorigenesis [89]. TGF-β1 also plays an important role in regulating the survival and differentiation of other cell types such as the primitive proliferating hematopoietic progenitors via cell cycle-independent mechanisms [90].

2.3.1 TGF-β Signaling: Therapeutic Significance in Prostate Cancer

The current standard therapeutic approaches employed for the treatment of organ-confined prostate cancer include radiation or surgery, in some cases incorporating adjuvant hormonal therapy [91, 92]. While these therapies are relatively effective in the short-term, a significant proportion of patients initially presenting with localized disease ultimately relapse. Moreover, each of these therapies may incur unwanted side effects. As a result, there is a demand for new therapies that more specifically target the cellular events involved in the development of malignancy. Gene therapy has been introduced into prostate cancer treatment recently [93, 94]. The knowledge of dysfunctional apoptosis pathway in cancer development and progression provides a molecular base for therapeutic targeting and apoptosis-based prevention approaches [95, 96]. The complexity of death signaling pathways suggest that apoptosis is not a single-lane, one-way street. Signals transduction from the cell surface to the nucleus that regulate cell growth, differentiation and survival and become subverted during the multistep processes of carcinogenesis and tumor progression provides a particularly attractive target and better diagnostic markers [97].

3. SUMMARY

The TGF-β superfamily is the most versatile considering the ability of its members to regulate proliferation, growth arrest, differentiation, and apoptosis of prostatic stromal and epithelial cells as well as the formation of osteoblastic metastases. TGF-β mediated action in prostate cells follows a complex signaling pathway from binding and phosphorylation of receptor type II to the TβRI kinase to Smad activation, resulting in ligand-induced transcription. TGF-β as an indirect tumor suppressor, its role of regulating tumor induction, as well as tumor suppression depending on the tissue microenvironment merits further exploration. The rationale for targeting growth factors and their receptors for therapeutic intervention is based upon the fact that these proteins represent the most proximate component of the signal transduction cascade. The alternate targeting of intracellular effectors in the signal transduction may be thwarted by cross talk between signaling pathways (such as the Smads in a dynamic interplay with the androgen receptor). TGF-β within the context of its well-documented apoptosis regulatory actions in the prostate and the significance its key receptor TβRII as a potential tumor suppressor, provides a highly attractive candidate for such targeting with high clinical significance for the treatment and diagnosis of prostate cancer.

Abbreviations: TGF-β, transforming growth factor-β; PSA, Prostate specific antigen; EGF, epidermal growth factor; bFGF, basic fibroblast growth factor; IGF, the insulin-like growth factor; BMPs, bone morphogenetic proteins; GDFs, Growth/differentiation factors; GDNF, lial-derived neurotrophic factor; MIF, Müllerian inhibitory factor; PDF, Prostate-derived factor; TβRI, TβRII, and TβRIII, TGFβ receptor types I, II, and III respectively; HGPIN, high-grade prostate intraepithelial neoplasia; BPH, benign prostatic hyperplasia.

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