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Functions and regulation of transforming growth factor-beta (TGF-β) in the prostate

David Danielpour *

Case Comprehensive Cancer Center and Department of Pharmacology, Case Western Reserve University, Wolstein Research Building, Room 3-532, 2103 Cornell Road, Cleveland, OH 44106, USA

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Abstract

The prostate is a highly androgen-dependent tissue that in humans exhibits marked susceptibility to carcinogenesis. The malignant epithelium generated from this tissue ultimately loses dependence on androgens despite retention or amplification of the androgen receptor. Accumulating evidence support that transforming growth factor-β (TGF-β) plays key roles in the control of androgen dependence and acquisition of resistance to such hormonal control. Although TGF-β functions as a key tumour suppressor of the prostate, it can also promote malignant progression and metastasis of the advanced disease, through undefined mechanisms. In addition to giving an overview of the TGF-β field as related to its function in prostate cancer, this Review focuses on novel findings that support the tumour suppressor function of TGF-β is lost or altered by changes in the activity of the androgen receptor, insulin-like growth factor-I, Akt, and mTOR during malignant progression. Understanding the mechanisms of cross-talk between TGF-β and such growth modulators has important implications for the rational therapeutics of prostate cancer.

Keywords: Prostate; TGF-beta; Smad3; Androgen; Carcinogenesis; IGF-I; Akt; Rapamycin; Therapeutics

1. Introduction

In the past 15 years, numerous reports have supported that transforming growth factor-beta (TGF-β) may be critical to involution and apoptosis of the prostate upon androgen withdrawal, and aberrations in TGF-β signalling may control the development and progression of prostate cancer. There are three mammalian isoforms of TGF-β, each of which occurs as a 25 kDa protein, predominantly in covalently-linked homodimers of 12.5 kDa peptides, and regulate many critical cellular functions, particularly growth arrest, differentiation, and apoptosis [1–5]. TGF-β signals through an interaction with two transmembrane serine/threonine kinase receptors, TβRI and TβRII. The best-documented intracellular mediators of these receptors are a family of proteins known as Smads [6]. Smads 2 and 3 are activators, Smad4 is a mediator, and Smad7 is an inhibitor of TGF-β responses [5,7,8]. However, many of the mechanisms by which TGF-β controls apoptosis and the growth of epithelial cells remain poorly understood, partly because of the substantial complexity and diversity in TGF-β signalling mechanisms among various systems, tissues, and cell types. Exceptionally striking are the opposing functions of TGF-β, promoting tumour

Abbreviations: TGF-β, transforming growth factor-β; TβRII, TGF-β1 receptor II; TβRI, TGF-β1 receptor I; TβRIII, TGF-β1 receptor III; IGF-I, insulin-like growth hormone factor-I; IGF-IR, IGF-I receptor; PKB/Akt, protein kinase B; AR, androgen receptor; SARA, Smad anchor for receptor activation; Hrs, hepatic growth factor-regulated tyrosine kinase substrate; Dab-2, disabled-2; MAPK, mitogen-activated protein kinase; FKHR, forkhead in rhabdomyosarcoma; mTOR, mammalian target of rapamycin; PTEN, phosphatase and tensin homologue deleted on chromosome 10.

* Tel.: +1 216 368 5670; fax: +1 216 368 8919.
E-mail address: david.danielpour@case.edu.

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suppression at one end of the spectrum and tumour promotion at the other end. TGF-β signalling mediators, particularly Smads 2, 3, and 4 selectively associate to and cross-talk with a highly complex network of signalling molecules, many of which, such as the androgen receptor (AR), are expressed in a tissue-specific manner [9,10].

Prostate cancers invariably lose their dependence on androgen, with patients ultimately failing on hormonal therapy [11]. During carcinogenesis, prostate epithelial cells also uniformly gain resistance to growth suppression and induction of apoptosis by TGF-β [12–14]. *In vivo* studies support that TGF-β functions as a tumour suppressor of the prostate [12,13,15–18]. As AR responses are likely to be functionally coupled to suppression of TGF-β-induced cell death, such resistance to TGF-β during carcinogenesis may contribute to the loss of androgen dependence. A better understanding of the molecular basis for the induction of apoptosis by TGF-β in prostatic epithelial cells will undoubtedly have an important impact on prostate cancer therapeutics.

2. Overview of signal transduction, growth suppression and control of apoptosis by TGF-β

2.1. Activation of the pathway

TGF-βs 1 and 3 signal by first associating to TβRII that then recruits TβRI to form a ligand-receptor heteromeric complex, consisting of 2 TβRIIs and 2 TβRIs [19–21]. TGF-β2 signals through a modified mechanism that requires betaglycan, formally called TβRIII, for its delivery to TβRII [22]. A constitutively active kinase in the cytoplasmic domain of TβRII then activates TβRI at a juxtamembrane site, known as the GS box. The activated TβRI, with the potential help of a couple of membrane anchor FYVE domain-containing proteins, known as SARA [23] and Hrs/Hgr [24], recruites and activates Smads 2 and 3 by phosphorylating their carboxyl SSXS domain. Presentation of Smads 2 and 3 to TβRI by SARA or Hrs/Hgr requires another TβRI-bound protein, Dab-2 [25]. Once activated, Smads 2 and 3 homodimerise and then enter the nucleus either with or without Smad4 [7,26,27]. Nuclear translocation of Smads 3 and 4 occur by exposure of a conserved nuclear localisation signal (NLS) motif which binds to the nuclear transport protein Importin-β [21]. Smads 3 and 4 then bind directly to consensus SBE sites in promoters, or indirectly to other promoter elements via association with a variety of transcription factors, leading to the transcriptional activation of target genes [28]. Smads are required for TGF-β to regulate the expression of numerous genes, arrest cell growth [29], promote differentiation, and induce apoptosis [30]. Members of the mitogen-activated protein kinase (MAPK) family are also activated during TGF-β signalling and may have interdependent functions with Smads [31], particularly in transcriptional activation [32–35].

2.2. Smads: structure and transcriptional control

Smads are characterised as a family of highly conserved N-terminal MH1, and C-terminal MH2 domain proteins that are separated by a poorly conserved middle linker region [36]. While the MH1 domain is involved in DNA binding, MH2 is the site of protein–protein interactions [36]. The L3 loop within the centre of the MH2 domain is the site of interaction with TβRI [37]. In the cytoplasm, non-activated Smads 2 and 3 are associated to microtubules [38], and are auto-inhibited through the associations between their N-terminal and C-terminal domains [39]. Phosphorylation of the C-terminal serines by an activated TβRI relieves this auto-inhibition, permitting their nuclear translocation, association to other proteins through the MH2 domain and the direct binding to DNA. The nuclear targeting sequence of Smads 3 and 4, KKLK, is in the N-terminus, and is required for the association of Smad3 to the nuclear transporter, importin-β [40]. The MH1 domain of Smad2 differs from those of Smads 3 and 4 in having a 30 amino acid (aa) insertion, believed to be responsible for its inability to bind directly to both the SBE consensus DNA binding motif and importin-β [39,41]. The nuclear targeting sequence of Smad2 is located in the C-terminus and is masked by its association to SARA [39,40,42].

SBE consensus binding motifs (i.e., GTCTAGAC), which Smads bind to with low affinity, but function as strong enhancers, act most often through cooperation with other response elements and transcription factors that associate with Smads [43]. Indeed, Smads have been shown to interact with many different transcription factors, transcriptional co-regulators such as P/CAF, MSG1, SNIP, p300/CBP, TGIF (TG interacting factor), HDAC (Histone deacetylase), Sno, Ski, AP1, and several steroid hormone receptors [28,44].

2.3. Role of non-Smad proteins that associate to TGF-β receptors

Although the mechanism of Smad activation by TβRI has been well studied, how TGF-β receptors activate the MAPK cascade remains largely unexplored, but likely requires their association with other accessory proteins. Indeed, Smads 2 and 3 comprise only two of many proteins shown to associate with TGF-β receptors and mediate or control TGF-β signals. The immunophilin, FKBP12, binds to the Leu–Pro sequence located near the phosphorylation sites of TβRI [45,46]. FKBP12

does not inhibit the binding of TβRI to TβRII, but prevents the ligand-independent phospho-activation of TβRI by TβRII [47,48]. Another protein that interacts with TβRI is the α-subunit of farnesyl transferase; however, its role in TGF-β signalling is unclear [49,50]. The cytoplasmic portion of TβRI also associates with the Bα subunit of protein phosphatase 2A, an association that depends on the activation of TβRI and that regulates TGF-β signalling [51]. Clusterin, also known as ApoJ or TRPM2, may control TGF-β responses by interacting with the cytoplasmic regions of both TβRI and TβRII [52]. Importantly, clusterin expression is induced following androgen withdrawal in rat prostates [53], and is also induced by TGF-β through the induction of c-fos [54]. TβRII also directly associates with other proteins such as the TRIP-1 (TGF-β-receptor interacting protein-1) [55] and Daxx [56]. TRIP-1, a WD-40 repeat domain protein, is phosphorylated in a ligand-independent manner by TβRII and inhibits both TGF-β- and Smad3-induced PAI-I promoter activity. Daxx, which associates directly to TβRII and not TβRI, is reported to be required for TGF-β-induced cJun-N-terminal kinase (JNK) activation and apoptosis [56]. Homeodomain interacting protein kinase-2 (HIPK2), which associates to Daxx, has been shown to mediate the activation of JNK by TGF-β through interaction with Daxx [57]. STRAP, another WD-40 domain protein that binds to both TβRI and TβRII, synergises with Smad7 to block TGF-β signalling [58].

2.4. Tumour suppression by tumour promotion by TGF-β

The relevance of TGF-β signalling to cancer is evident from the ubiquitous loss of TGF-β responsiveness during carcinogenesis, largely between adenoma to carcinoma transitions [59–62]. This is accompanied by either loss of receptor expression, inactivation of receptor function by mutations in these receptors, or loss of signal transduction by changes at post-receptor signalling steps, including inactivating mutations in Smad proteins [63,64]. Impressively, overexpression of the wild-type TβRII reverses the malignant phenotype of cell lines derived from colon [65,66], gastric [67], breast [68], and prostate cancers [16].

2.5. Cell cycle control and regulation of apoptosis by TGF-β

TGF-β inhibits growth and induces apoptosis through multiple mechanisms, that are often dependent on the cell-type. TGF-β has been shown to upregulate the expression of cyclin-dependent kinase inhibitors, $p15^{INK4b}$ and $p21^{CIP/WAF1}$, in keratinocytes via Sp1 binding sites activated by Smads [69,70]. In cell lines defective in $p15^{INK4b}$, growth inhibition by TGF-β is reported to occur via downregulation of cdc25A, through recruitment of HDAC by E2F-p130 [71]. Smads 3 and 4 have been shown to associate to the TIE (TGF-β inhibitory element) promoter element of the c-Myc proto-oncogene and downregulate *c-myc* expression [72]. Downregulation of c-myc frees its binding partners, Max and Miz-1, the latter of which then transcriptionally activates $p15^{INK4b}$ by binding to the transcriptional initiator (Inr) element and recruiting p300/CBP [73,74]. TGF-β-induced growth arrest has also been shown to occur by inactivation of the $p70^{S6K}$ kinase, through activation of the Bα subunit of PP2A by TβRI [75].

TGF-β induces apoptosis in a variety of cells, likely through multiple related mechanisms. The activation of both Smads and AP-1 has been shown to be essential for such apoptosis [30]. Evidence for the involvement of caspases in TGF-β-induced apoptosis comes from several studies that show caspases are activated by TGF-β, and that reactive peptide caspase inhibitors (BD-fmk, ZVAD-fmk, DEVD-fmk) block such apoptosis [76–80]. TGF-β has been shown to also induce the expression of pro-apoptotic BCL2 members, downregulate anti-apoptotic members of this family [81,82], and promote the release of cytochrome *c* from mitochondria [81]. The mechanism by which TGF-β regulates BCL2 proteins is not clear and may or may not involve Smads. Loss of protection by BCL2-related proteins may mediate apoptosis by TGF-β. For example, TGF-β downregulates the expression of the anti-apoptotic protein Bcl-xL in NRP-154 cells, and overexpression of Bcl-xL blocks cytochrome *c* release, caspase-3 and -9 activation, and the induction of apoptosis [81]. However, it is not clear whether loss of Bcl-xL alone induces apoptosis in these cells or requires other factors. Other effectors that seem to play a role in triggering apoptosis by TGF-β involve transcriptional activation of DAP (death associated protein) kinase through Smads [83], activation of a MAP kinase member (TAK-1) [84], Daxx [56], Nuclear factor-kB (NF-kB) [85], and the septin family protein ARTS (apoptosis-related protein in the TGF-β signaling pathway) [86,87]. How these effectors may cooperate to activate apoptosis remains to be determined.

3. Roles and functions of TGF-β in the prostate

Prostatic epithelium requires androgens not only for growth and development, but also for preventing the extensive apoptotic cell death that ensues without this hormone [88–90]. Involution of the prostate following castration is accompanied by rapid upregulation of TGF-β ligands and receptors, as well as the activation of Smads in the regressing tissue [91–93]. Moreover, TGF-β implanted in the prostate of intact rats induces apoptosis of this epithelium [94]. Androgens (dihydrotestosterone (DHT), testosterone, R1881) have been shown to suppress the expression of TGF-β on prostatic

cells in culture [95,96]. The induction of apoptosis in the prostate epithelium by TGF-β is likely to be direct and occur at physiological levels of this ligand, as showed with prostatic epithelial cell lines in culture [97]. In a recent study where dominant-negative TβRII (DN-TβRII) was targeted in transgenic mice to the prostate with a C3 promoter, loss of TGF-β signalling substantially lowered the levels of apoptosis and increased epithelial cell proliferation in the proximal duct of the prostate [98]. This study confirms key roles of TGF-β in the homeostatic control of apoptosis and growth of the normal prostate epithelium.

Acquisition of resistance to TGF-β during prostatic carcinogenesis in humans is accompanied by loss of TβRI and TβRII expression [12–14,99]. Restoration of TGF-β receptor function reduces malignancy in the human prostatic carcinoma cell line, LNCaP [16], inferring a tumour suppressor role for the TGF-β receptors. The function of TGF-β as a tumour suppressor of prostatic epithelial cells was also tested by overexpression of DN-TβRII in the prostatic cell line, NRP-152, derived from the preneoplastic dorsal–lateral prostate of the Lobund–Wistar rat. NRP-152 cells are non-tumorigenic and have many properties of normal prostate epithelial cells, including androgen receptor expression, growth responsiveness to androgens, and high sensitivity to a variety of growth factors and TGF-βs [100]. Most unique about NRP-152 cells are their stem cell properties *in vitro* and *in vivo* [101,102]. TGF-β promotes growth arrest [100], apoptosis [97] and basal to luminal transdifferentiation [101] of this cell line. Importantly, expression of DN-TβRII in these cells inhibits TGF-β– induced apoptosis *in vitro* and triggers their malignant transformation [30]. These results were recently reproduced in another non-tumorigenic rat prostatic epithelial line named DP-153, which was developed from the dorsal prostate of a Lobund–Wistar rat. Like NRP-152 cells, DP-153 is a spontaneously immortalised basal epithelial cell line that is highly responsive to numerous hormones, growth factors and TGF-β. DN-TβRII blocks essentially all growth inhibition by TGF-β on DP-153 cells, and promotes their malignant transformation, inducing carcinomas as early as 4 weeks in athymic mice [103]. Clear demonstration that loss of TGF-β signals promotes malignant progression and metastasis of prostate cancer was provided in a study where transgenic mice crosses of Simian Virus 40T antigen (SV40T) with DN-TβRII targeted to the prostate showed enhanced metastasis [104].

Stromal cells are believed to be critical in promoting and maintaining malignant transformation of prostatic epithelial cells [105–107]. However, the mechanism by which they promote tumour development is poorly studied. Recent studies strongly suggest that TGF-β may have an important role in stromal cell function and in controlling stromal–epithelial cell interactions. Normal adult prostatic fibroblasts have TGF-β receptors and are highly responsive to this modulator, as indicated by their by ability to transdifferentiate to smooth muscle cells following TGF-β treatment [108]. The role of TGF-β in stromal–epithelial interactions is more clearly illustrated by a recent study, where knocking out TGF-β signalling in the stromal compartment led to malignant transformation of the prostatic epithelium [109]. In this study, TβRII was selectively knocked-out in fibroblasts by *cre-lox* targeted with a fibroblast-specific promoter, causing intraepithelial neoplasia of the prostate [109]. Thus, TGF-β indirectly establishes negative control on growth of prostate epithelium through adjacent fibroblasts. TGF-β may exert this influence on prostatic stroma by suppressing the production of hepatocyte growth factor [109] or other growth factors induced by androgens through the AR in these fibroblasts. This may certainly be true for the developing prostrate where the AR is shown to reside in stroma, the compartment which also mediates androgen-dependent proliferative signals to the adjacent epithelium [110]. Clear mechanistic support for a role of TGF-β on AR-dependent cell–cell interactions is provided in a study in which TGF-β was shown to block androgen receptor signalling in prostate fibroblasts, likely through its effects on promoting translocation of AR from the nucleus to the cytosol [111].

Another important cell-to-cell communication that TGF-β may mediate in the prostate is that between basal and luminal epithelial cells. The rat prostatic basal epithelial cell line, NRP-152, acquires a luminal phenotype upon growth factor-depleted culture conditions which also induces the expression of autocrine TGF-β along with TβRII [101]. The autocrine TGF-β activity, at least partially, mediates luminal differentiation, since TGF-β neutralising antibodies reverse some of this luminal phenotype [101]. Moreover, it has been suggested that basal epithelial cells may control the growth of luminal epithelial cells through a paracrine pathway by providing active TGF-β to luminal cells that are highly sensitive to growth suppression by TGF-β, but do not by themselves produce active TGF-β [112]. Thus, a disruption in the ability of TGF-β to control such cell–cell interactions may promote uncontrolled proliferation and lead to malignant transformation.

4. Cross-talk of TGF-β with the androgen receptor axis

The androgen receptor signalling pathway has been shown to cross-talk with the TGF-β signalling pathway on multiple levels. The most pronounced effect shown in whole animal studies for the connection between TGF-β and androgen signals is the robust induction of TGF-β1 mRNA levels that occur in rat prostate following androgen ablation, temporally correlating with the onset of

apoptosis in this tissue [113]. The activation of TGF-β signals following androgen withdrawal are further supported by elevation in the expression of TGF-βs 2 and 3, and TGF-β receptors [114], as well as both the elevation and activation of Smads 2 and 3 [115]. These results together with those from *in vivo* [94] and *in vitro* [15,97] studies suggest an important role for TGF-β signals in the cell death response of normal prostatic epithelium following androgen withdrawal. Although it is now well established that androgens control TGF-β responses, the mechanisms for such cross-talk remain an important and active area of investigation. A seminal study using the preneoplastic prostatic cell line, NRP-152, showed that androgens can suppress the production of TGF-βs 1, 2, and 3 under serum-free conditions [95]. In this study, either monoclonal or polyclonal antibodies that neutralise autocrine TGF-βs stimulated cell growth, while damping the overall growth by androgens. This study suggested that androgens promote proliferation of prostatic epithelial cells partly through reversing growth inhibition by autocrine TGF-β [95]. Another study established that androgens can also suppress the expression of TGF-β1 in LNCaP cells [96].

Smad3 has recently been shown to function as a molecular interface to relay cross-talk between androgens and TGF-β. Smad3 binds directly to the AR [96,116,117] and either blocks [116,118] or enhances [117] the regulation of gene expression by androgens. Interestingly, Smad4 appears to control the ability of Smad3 to suppress AR-mediated gene induction [118]. The physical interaction of AR with Smad3 may be the basis for the ability of TGF-β to block responses of androgens in prostatic stroma cells by suppressing androgen-driven translocation of AR from the cytoplasm to the nucleus [57]. In addition, the direct association between AR and Smad3, at least partially, mediates the ability of androgens to suppress TGF-β responses [96]. Evidence from electrophoretic mobility shift assays using purified Glutathione-*S*-Transferase (GST)-AR and GST-Smad3 bacterially expressed fusion proteins suggests that the association of the ligand binding domain of AR to the MH2 domain of Smad3 prevents the interaction of Smad3 to the SBEs of TGF-β-responsive genes [96].

The biological effects and implications of this cross-talk need further investigation, since most of the biological response data have been generated by promoter reporter assays. Does TGF-β suppress or activate AR-induced cell growth or do androgens block growth arrest and apoptosis by TGF-β through the AR-Smad3 complex? In view of these conflicting endpoints, the final response will most likely depend on the levels of Smad3 and AR, the intensity of each of these signals and the temporal order of their activation. Included in this complexity are numerous proteins that associate with either the AR or Smad3 that may influence the pattern of their cross-talk. Using LNCaP cells that were made highly responsive to growth suppression by TGF-β with enforced overexpression of TβRII, Dr. Kyprianous's laboratory has demonstrated that androgens can actually enhance the cell death pathway induced by TGF-β [119,120]. In this case, overexpression of TβRII most likely blocked the pro-growth and pro-survival effects by androgen. Our preliminary results on LNCaP cells suggests that androgen may block the ability of TGF-β to induce cell death only when TβRII is moderately overexpressed, more likely within physiological levels (Song, data not shown). A similar protection of TGF-β-induced cell death by androgens was observed in NRP-154 cells overexpressing AR (Song, data not shown). These latter results are consistent with AR-mediated protection of activin-induced death in LNCaP cells without enforced overexpression of the activin receptor [121]. It is likely that TGF-β and activin, both of which activate Smads 2 and 3, induce cell death through similar downstream signals.

Recent studies show that acquisition or androgen autonomy occurs despite retention or elevated expression of AR in most prostate tumours [122], consistent with a role of AR in the loss of tumor supression by TGF-β. AR, a 110-kDa zinc finger transcription factor that belongs to the nuclear receptor superfamily, is activated by dimerisation upon ligand binding [123]. This promotes nuclear localisation and binding of AR to androgen-response elements in the promoters of androgen-regulated genes. AR-mediated transcription is regulated by many AR interacting proteins such as ARA24, ARA54, ARA55, ARA70, ARA160, ARA267 [10,124–128], along with cAMP response element binding protein [129], AP-1 [130], Ets [131], glucocorticoid receptor interacting protein-1 (GRIP1) [132], F-SRC-1 [133], RIP-140 [133] and Smad3. Some of these proteins share common motifs such as RING finger B-Box (i.e., ARA54), LIM motif (i.e., ARA55), WxxLF or FXXLF motifs (i.e., ARA70). To date, there are over 70 proteins shown to bind to or interact with AR. Although most of these associations are non-covalent, some such as such as UBC9 [134] are covalent and increase the activity of the AR, and others, such as Akt, phosphorylate the AR and inhibit its activation [135]. Most AR-associated proteins appear to interact with other steroid hormone receptors, although the specificity of interaction for many of these binding partners is still undefined. The growing list of recently discovered AR transcriptional co-regulators supports the notion that a complex network of signals tightly regulates transcription by androgen. Understanding how these molecular interactions cooperate to regulate growth and maintain cell viability will certainly impact on the therapeutic strategies for the prevention and cure of prostate cancer. For further review of AR co-regulators, a comprehensive list of these co-regulators

can be accessed on-line at the McGill Androgen Receptor Database: http://ww2.mcgill.ca/androgendb/.

5. Cross-talk of TGF-β with IGF-I, Akt, and mTOR

Insulin-like growth factor-I (IGF-I) is known to play a key role in promoting the growth and survival of epithelial cells, and the deregulation of IGF-I levels has been intimately tied to the control of carcinogenesis [135–139]. Early studies correlating serum IGF-I levels to the incidence of prostate cancer in humans have suggested that high serum levels of IGF-I may be a predictor of prostate cancer [140,141]. Indeed, enforced elevation of IGF-I in transgenic mice promotes prostate carcinogenesis [142], suggesting perhaps a causal link of high IGF-I and the induction of prostate cancer in humans. This is not a startling discovery as IGF-I signalling has also been proposed to be necessary for the development of a variety of other cancers [143–146]. The oncogenic effect of IGF-I is likely attributed not only to its ability to stimulate cell growth, but, more importantly, to its function as a potent suppressor of the cell death. Akt, also known as protein kinase B, is a potent apoptotic inhibitor that is effectively and rapidly activated by IGF-I through the generation of phosphotidylinositol-3-phosphate (PI3P) (phosphatidylinositol-3) by PI3-kinase [147]. Akt has been reported to be constitutively activated in a variety of cancers including prostate cancer [147–151]. The elevated levels of activated Akt in prostate cancer relative to the normal prostate may occur both through the activation of IGF-I receptors, and by mutational inactivation of the tumour suppressor PTEN found in 25–50% of patients with prostate cancer [148,152–157]. PTEN is a PI3P phosphatase that suppresses Akt activation through suppressing the levels of PI3P [158]. Significantly, activation of the IGF-I receptor blocks the induction of apoptosis by TGF-β in prostatic epithelial cells [97].

Although IGF-I blocks apoptosis induced by a variety of stimuli through inactivation of late signals such as inactivation of Bad, caspase-9, and FOXO [159], a recent report from my laboratory shows that IGF-I also functions through an Akt-dependent mechanism to inhibit transcriptional responses of TGF-β in NRP-152 prostate epithelial cells by specificially suppressing the TGF-β receptor activation of Smad3 [160]. Impressively, in this study rapamycin reversed the IGF-I suppression of TGF-β-induced transcriptional responses and Smad3 activation, suggesting that IGF-I's effects on Smad3 activation are, at least partly, mediated through the mammalian target of rapamycin, mTOR. However, rapamycin may also function to enhance TGF-β signals [161] by blocking the FKBP12-mediated suppression of TβRI activation [46,162]. We have observed that Akt can associate with Smad3 in an Akt-independent manner in NRP-152 cells (Song, data not shown), similar to the association of Akt to Smad3 recently observed in other cell lines [163]. The role of mTOR on this rapamycin effect, and the association of Smad3 to Akt [160,163,164], FOXO [165,166], or TSC2 [167] in mediating TGF-β suppression by IGF-I is currently being explored in our laboratory.

6. Potential therapeutics of TGF-β in the control of prostate cancer

Although TGF-β functions as a potent tumour suppressor of prostatic cells, at least in normal and preneoplastic cells, the direct therapeutic use of TGF-β to prevent or cure prostate cancer is greatly limited by problems associated with its delivery, adverse side-effects, such as sclerosis, fibrosis, immunosuppression, and enhanced tumour growth and metastasis in late-stage disease [168]. The active TGF-β ligand is very short-lived in blood, being cleared from the general circulation within minutes of entry, which is likely through its association to and clearance by plasma alpha-2 macroglobulin [169–172]. Systemic administration of TGF-β would therefore require a suitable carrier to prevent its rapid clearance, and would also require measures to prevent its adverse effects throughout the body. Thus, selective targeting of this ligand to tumour or pre-neoplastic epithelium would be essential for the therapeutic use of TGF-β in early-stage disease. Another effective strategy would be the use of agents that selectively enhance TGF-β expression or/activation in the tumour epithelium. Ionising radiation is one such agent that can induce the expression and activation of TGF-β in tumours [173,174]. Recent studies using knock-out animals show that low-dose radiation-induced cell killing requires TGF-β1 or Smad3 [175,176], suggesting that TGF-β may play a role in mediating the killing effect of radiation therapy, and that resistance to TGF-β receptor signalling may lead to resistance to cell death. A number of investigators suggest that retinoids, vitamin D compounds and the anti-oestrogen tamoxifen, used in the chemoprevention studies of prostate cancer, may prevent tumour growth by inducing the expression of TGF-β ligand, particularly TGF-β2 in the neoplastic epithelium [177–181]. The preferential induction of the TGF-β2 isoform would be less likely than the two other isoforms to have systemic effects on immunosuppression and fibrosis, as TGF-β2 is selectively neutralised by alpha-2 macroglobulin in the circulation [169,171,182].

In late-stage disease, the expression of TGF-β is greatly elevated in the tumours and in the circulation [18,183], and removal or neutralisation of TGF-β and its associated intracellular signals may prove beneficial for suppressing disease progression [184]. This may be achieved through the administration of TGF-β neutral-

ising antibodies, TGF-β LAP, or the soluble TβRII [185,186]. A fusion protein of the Fc portion of human IgG to soluble TβRII has proven to be a effective strategy to sustain the plasma levels of soluble TβRII [187]. An alternative strategy would be to block the intracellular signals that permit TGF-β-induced tumour growth and metastasis, while activating signals that promote cell death.

One potential drug that is under investigation for reversing the oncogenic or malignant properties of TGF-β is rapamycin. Rapamycin, which is being used clinically as a very useful immunosuppressant for organ transplantation [188], has recently been shown to be highly effective for killing cancer cells in combination with other therapeutic interventions, such as radiation and chemotherapy [188–191]. Rapamycin is believed to function by associating with the peptide FKBP12, which binds to mTOR and blocks the kinase activity of mTOR [190,192,193]. FKBP12 also binds to TβRI and prevents TGF-β-independent activation of TβRI by TβRII [47,194]. TGF-β is thought to displace FKBP12 from TβRII, thereby promoting the activation of TβRI by TβRII [162]. Consistent with this, rapamycin has recently been shown to enhance Smad activation in prostate epithelial cell lines [195]. Another study suggests that rapamycin synergises with TGF-β to kill tumour cells and this synergism cannot be fully explained by the ability of rapamycin to reverse the suppression of TβRI by FKBP12 [161]. Interestingly, similar to its ability to sensitise tumour cells to chemotherapy or radiation therapy, rapamycin has been reported to reverse the effects of oncogenic Ras on the resistance of cells to TGF-β-induced growth suppression [161].

A central underlying problem that remains in understanding the function of TGF-β during carcinogenesis and in determining the optimal use of TGF-β-based tools in cancer therapy is to identify the key molecular switches or targets that flip the behaviour of TGF-β from a tumour suppressor to an oncogene. Reversing those switches, even transiently, may effectively activate the cell death machinery in the tumour epithelium that is already primed with TGF-β ligand.

Conflict of interest statement

None declared.

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