TGF-β signaling dictates therapeutic targeting in prostate cancer

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'The concept prevailing is that TGF-β1 has tumor-suppressor activity in the early stages of prostate tumorigenesis when the epithelial cell response is intact and, with progression to advanced disease, epithelial cell response is lost and the oncogenic activities dominate.'

One in six men will be diagnosed with prostate cancer in their lifetime, and one in 33 men will die of the disease, accounting for 10% of cancer-related deaths in American men. In 2006, an estimated total of 234,460 men were diagnosed with prostate cancer in the USA and 27,250 died of the disease [1]. Prostate cancer is a heterogeneous cancer with a natural history of progression from prostatic intra-epithelial neoplasia to locally invasive androgen-dependent to androgen-independent metastatic disease, which is associated with patient mortality [2]. Overcoming the androgen independence of prostate tumors is considered the most critical therapeutic end point for improving patient survival [3,4]. Development of androgen-independent state is a consequence of lack of an apoptotic response to androgen ablation [5], as androgen-independent tumors become ultimately resistant to hormone ablation and other chemotherapeutic modalities due to roadblocks in apoptosis rather than aberrant cell proliferation [6,7].

Transforming growth factor-β proceedings determine prostate apoptotic outcomes

Transforming growth factor-β (TGF-β1) is the quintessential negative growth factor via its ability to inhibit proliferation and induce apoptosis, regulate cell migration, adhesion, differentiation and the microenvironment. These signals are transduced via a signaling pathway from transmembrane type II (TβRII) to type I (TβRI) receptor kinase, to intracellular Smad activation resulting in ligand-induced gene transcription. The response is differentially read depending on the cellular context, and evidence-based framework indicates that TGF-β1-mediated growth inhibition and apoptosis in normal cells might be replaced by invasive and metastasis responses in cancer cells. The breadth of cellular responses is dictated by the numerous genes and their encoded proteins regulated by TGF-β [8,9]. Upon ligand binding, TβRII receptor phosphorylates and activates the TβRI receptor, which initiates the downstream signaling cascade by phosphorylating the receptor-regulated Smads (R-Smads) [9]. Other signaling pathways help to define the responses to TGF-β [10] and, in addition to Smads, TGF-β activates other effectors and Smad-independent pathways also exist for TGF-β signaling [11]. Deregulation of TGF-β signaling contributes to tumorigenesis due to either loss of expression or mutational inactivation of its membrane receptors or intracellular effectors, the Smads [7]. In advanced cancer, TGF-β promotes tumor progression and metastasis via induction of tumor-cell invasion, angiogenesis and immunosuppression [12,13].

By virtue of the central regulatory role played by TGF-β in coordinating cell growth and apoptosis during normal prostate homeostasis, an imbalance in either production of and/or response to TGF-β results in growth perturbation that contributes to prostate tumor development and progression [8]. Increased expression of TGF-β (ligand) is found in patients with advanced prostate cancer [14], and this elevated TGF-β is involved in promoting tumor suppression, primarily through paracrine effects on stromal elements such as increased angiogenesis and decreased immune surveillance (**Figure 1**). TGF-β may have direct tumor-promoting properties on the epithelium in advanced tumors through induction of the epithelial to mesenhymal transition, alteration in extracellular matrix, adhesion molecules and endothelial cell anoikis. Moreover, one also has to consider the contribution of stroma in promoting prostate tumorigenic growth and angiogenesis via targeting TGF-β effectors:

- Expression of the connective tissue growth factor, a key TGF-β signaling mediator in tumor-reactive stroma [15];

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- TGF-β-mediated increased caveolin-1 secretion into the microenvironment, inhibiting prostate cancer-cell apoptosis during perineural and endothelial invasion [16].

Thus, TGF-β not only takes a lead role in tumor-stroma interactions, regulation of anoikis and endothelial cell survival, but also in targeting prostate vascularity as a molecular therapeutic platform for the prevention and treatment of prostate cancer.

Dysfunctional TGF-β1 signaling: zeroing on TGFβ RII receptor

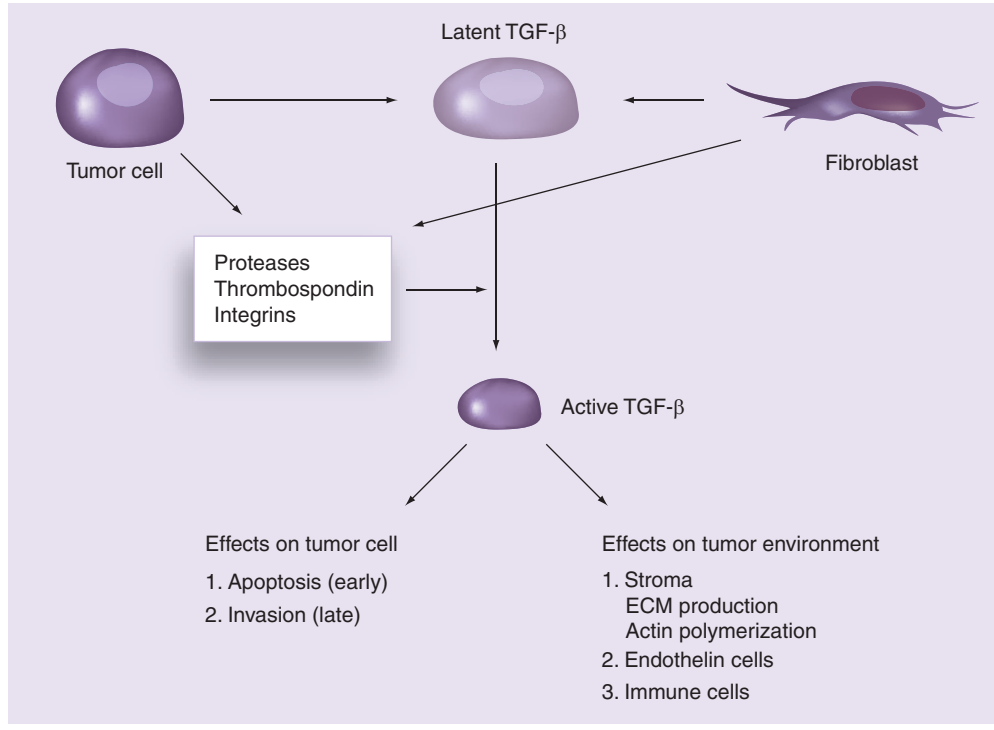
TGF-β1 directly activates apoptosis of normal prostate epithelial cells in the presence of a functional androgen axis, as well as tumorigenic prostate cells [17,18]. A dysfunctional TGF-β signaling mechanism, due to expression loss/defects in the transmembrane receptor and postreceptor effectors, results in loss of growth control contributing to prostate tumorigenesis (**Figure 2**). Although both transmebrane receptors TβRI and TβRII are essential for the apoptotic/antiproliferative signaling of TGF-β, a close association between functional inactivation/loss of expression of TβRII receptor and escape from TGF-β1-mediated growth inhibition has been documented in several human tumors [19,20]. Loss of responsiveness to TGF-β1 by introducing a dominant-negative mutant TβRII receptor induces malignant transformation of nontumorigenic rat prostate epithelial cells [21]. Overexpressing TβRII receptor in human LNCaP prostate cancer cells leads to suppression of prostate tumorigenic growth via caspase-mediated apoptosis [22,23], and androgens enhance the apoptotic effect of TGF-β1 [24]. This evidence implicates a role for the TβRII receptor as a tumor suppressor via its apoptotic action, and its functional loss can directly confer a survival advantage on prostate cancer cells. While human prostate tumors exhibit loss of TβRII expression [8,25], advanced prostate tumors have increased TGF-β1 (ligand) expression [26], and patients with invasive advanced disease have significantly elevated plasma TGF-β1 levels [14]. In experimental models of prostate tumorigenesis, overexpression of TGF-β1 in prostate cancer cells enhances invasion and metastasis [27].

Biological activities of TGF-β other than those directed at cell growth (such as invasion and migration) and dictated by Smad-independent effectors could provide a selective advantage to the transformed prostatic epithelial cells towards a highly aggressive phenotype with metastastic ability, by promoting cell motility, extracellular matrix modulation and metastasis (**Figures 1 & 2**). Indeed, the phosphatase and tensin homolog (PTEN) in human cancers may contribute to a role for TGF-β1 as a tumor enhancer with specific effects on cell motility and migration [28]. Since the PTEN prostate-specific knockout mouse model mimics human prostate cancer initiation and progression, from high-grade prostatic intraepithelial neoplasia to metastasis, it might be a valuable tool for profiling TGF-β1 signaling during tumor progression. The significance of the tumor microenvironment in the context of loss of TGF-β1 responsiveness is evident from the profound impact of stroma on tumorigenesis of adjacent epithelial cells. *In vivo* studies have shown that conditional inactivation of the TβRII gene in fibroblasts resulted in prostate intraepithelial neoplasia [29]. The challenge remains to identify the key molecular events and characterize the cellular dynamics of the tumor microenvironment responsible for converting TGF-β1 action from tumor suppressive to oncogenic. The key aspects linked to the mechanistic exploration of the 'double-edged-sword' action of TGF-β1 are the following:

- Loss of TGF-β signaling (due to TβRII loss/or intracellular effector Smad4 loss) in prostate cancer cells is responsible for the loss of apoptotic control, despite the presence of an active ligand, in early stages of malignant development [7,8]. Additional changes can promote metastasis in the presence of active TGF-β1 (**Figure 2**).
- TGF-β1 signaling players responsible for 'functional swinging' could act in a Smad-independent fashion and concurrently with a mutant AR to promote prostate cancer cell invasion by reducing apoptosis (**Figure 2**).
- In addition to the effects directly targeting the tumor epithelial cells, the tumor microenvironment (reactive stroma) could indirectly mediate the tumor cellular response to the apoptotic effects of TGF-β1 (**Figure 2**).

The concept prevailing is that TGF-β1 has tumor-suppressor activity in the early stages of prostate tumorigenesis when the epithelial cell response is intact and, with progression to advanced disease, epithelial cell response is lost and the oncogenic activities dominate. Consistent with this concept, we documented that

Figure.1. TGF-β1 activation and cellular targets.



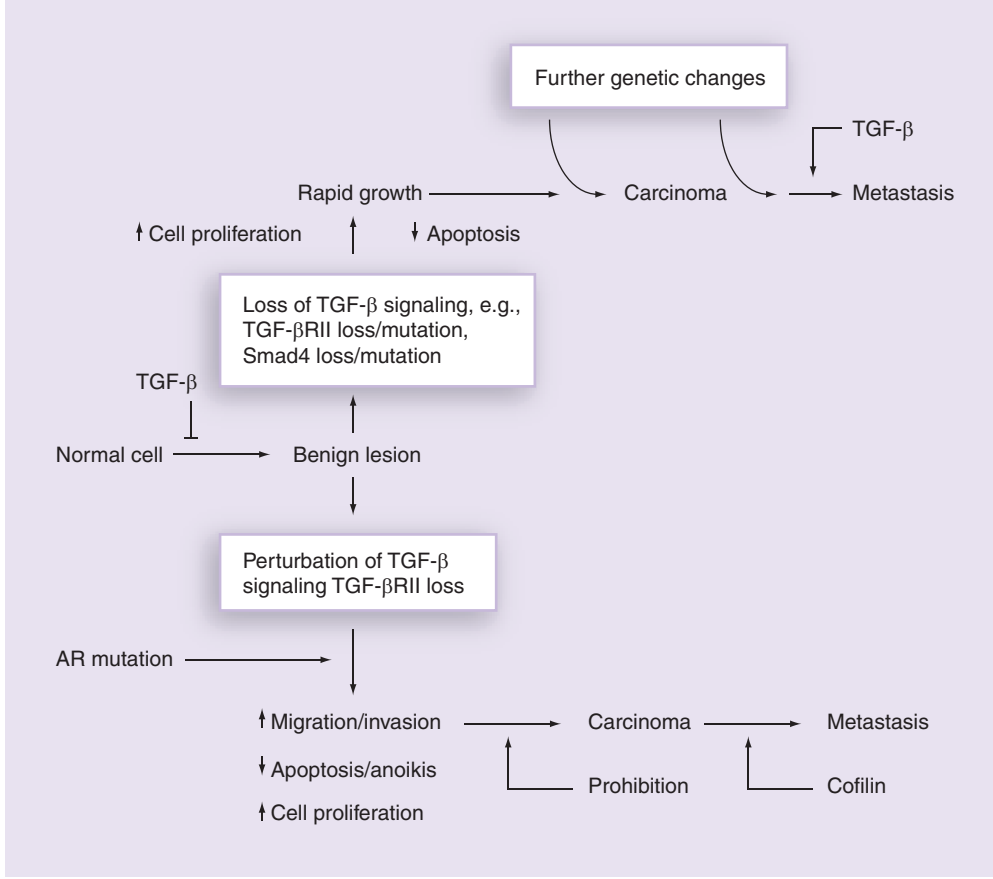
ECM: Extracellular matrix.

TGF-β1 functions as a tumor suppressor in the prostate [8,23], and disruption of TGF-β1 signaling contributes to early malignant changes and subsequent emergence to androgen independence and metastasis. More recent *in vivo* studies evaluated the effect of functional inactivation of TGFβ1 signaling in murine prostate epithelium by expressing a dominant-negative version of the human TGFβRII in transgenic mice under the metallothionein promoter MT1 [30]. Pathological examination of mouse prostates from human TGF-β receptor II dominant negative (hTβRII DN) mice revealed that loss of TGFβ signaling results in prostatic hyperplasia and low-grade prostatic intraepithelial neoplasia in hTβRII-DN transgenic mice, potentially via a significant increase in the proliferative index paralleled by reduced apoptosis. Inactivation of TGF-β1 signaling leads to significant changes in prostate tissue kinetics, increased proliferation and reduced apoptosis towards a malignant phenotype. The physiological consequences of a dysfunctional TGF-β1 signaling via loss of the TβRII receptor on the initiation and progression of prostate cancer, are currently being investigated in the dominant negative TGF-β receptor II (DNTRII) transgenic model (dysfunctional TGFβ1 signaling) crossed with the transgenic adenocarcinoma mouse prostate (TRAMP) transgenic mouse [31].

'While defects in the Smad intracellular signaling may contribute to deregulation of TGF-β1 signal transduction and TGF-β1 resistance in prostate tumor progression, the functional TbRII heterodimeric signaling complex can also regulate activation of downstream Smad-independent pathways'

The involvement of TGF-β1 signaling effectors in prostate cancer development and progression awaits full dissection. While defects in the Smad intracellular signaling may contribute to deregulation of TGF-β1 signal transduction and TGF-β1 resistance in prostate tumor progression, the functional TβRII heterodimeric signaling complex can also regulate activation of downstream Smad-independent pathways, potentially altered in human cancer. Our recent proteomic studies identified changes in intracellular trafficking of two proteins, prohibitin and cofilin, as novel signaling effectors of TGF-β-induced apoptotic and migration responses of human prostate cancer cells via Smad-dependent mechanisms [32].

Figure 2. TGF-β1 dysfunctional signaling promotes cell proliferation and migration and inhibits apoptosis during prostate tumorigensesis.



Future efforts focus on identifying the response of prostates to castration-induced androgen ablation in the absence of a functional TβRII signaling, and sensitizing prostate tumors to undergo TGF-β1-mediated apoptosis using androgens, with therapeutic implications in androgen-independent advanced prostate tumors. Finally, effective new imaging technologies will provide insights into the neurovascular network of prostate tumors, with targeting value.

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