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Review

Signaling lansdscape of prostate cancer

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Abstract: Research over the decades has gradually and sequentially shown that both intratumor heterogeneity and multifocality make prostate cancer difficult to target. Different challenges associated with generation of risk-stratification tools that correlate genomic landscape with clinical outcomes severely influence clinical efficacy of therapeutic strategies. Androgen receptor mediated signaling has gained great appreciation and rewiring of AR induced signaling cascade in absence of androgen, structural variants of AR have provided near complete resolution of genomic landscape and underlying mechanisms of prostate cancer. In this review we have attempted to provide an overview of most recent advancements in our knowledge related to different signaling cascades including TGF, SHH, Notch, JAK-STAT in prostate cancer progression and development.

Key words: Prostate cancer, molecular therapeutics, signaling, apoptosis.

Introduction

Prostate cancer is a multifaceted and genomically complex disease and rapidly developing resistance against mainstream therapeutics has added another layer of complexity to standardization of therapy (1,2). Overwhelmingly increasing high-impact research has significantly enhanced our understanding of the positive and negative regulators which modulate different steps of prostate cancer development, progression, castration resistant prostate cancer and metastasis (3,4). It is becoming progressively more understandable that deregulation of spatio-temporally controlled intracellular signaling cascades play key role in prostate carcinogenesis.

In this review, we systematically put pieces of published studies together to summarize advancements in our understanding related to how misrepresentation of signal transduction cascades contributes to development and progression of prostate cancer.

TGFβ mediated signaling

Transforming growth factor-β1 (TGF-β1) is functionally inactive initially and undergoes activation before its release as an active cytokine. Functionally Active TGF-β1 transduces the signals intracellularly through TGF receptor (5). Ligand-Receptor association induced an autophosphorylation of the receptor followed by phosphorylation of R-SAME (5). TGFR may undergo different fates context dependently through synchronized protein-protein interactions. TGFR may be proteasomally degraded upon binding with inhibitory SMADs and the SMAD ubiquitylation regulatory factor (SMURF), or remain intact and functional on surface of the cell to transduce the signals to downstream effectors (6). SMURF1, a C2-WW-HECT-domain E3 ubiquitin ligase has been studied to be involved in regulation of cancer cell metastasis (Figure 1). Phosphorylated R-SMADs have been noted to homomerically and heteromerically complex with co-mediator SMAD (Co-SMAD) (Figure 1). These protein complexes accumulate in nucleus and interact with coactivators and/or corepressors and DNA-binding cofactors to transcriptionally modify activity of target gene network. Dephosphorylated SMAD complexes are functionally inactive and rapidly exported to the cytoplasm (5,6).

Intratibial tumors generated from RUNX2-WT-expressing cells produced osteolytic disease, whereas tumors developed by mutant RUNX2 expressing cancer cells contained mixed osteolytic/osteoblastic lesions (7). Data clearly indicated that disruption of the protein-protein interaction between RUNX2 and SMAD significantly reduced incidence and size of lung tumors (7).

There is an exciting piece of evidence suggesting that mutant p53 enhances TGF-β/Smad signaling in prostate cancer cells. Mutant p53 expressing prostate cancer cells were more sensitive to TGF-β dose-dependently as evidenced by notably raised levels of phosphorylated levels of Smad2/3 (8).

Conditioned medium from TGF-β1 treated bone

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marrow stromal cells (BMSCs) markedly reduced apoptosis of cancer cells. Phosphorylated SMAD2 was functionally active in BMSC-triggered trans-differentiated prostate cancer cells, as evidenced by luciferase reporter and immunoblotting assays (9).

Hexamethylene bisacetamide-inducible protein 1 (Hexim1) is reportedly involved in regulation of TGFβ activity and SMADs turnover in a CDK9 dependent way (10). Upregulated Hexim1, mild expression of SMAD7 and higher expression of SMAD2 associated significantly with disease progression. Higher expression of SMAD2 was associated with shorter disease free survival (DFS) (10).

Proteolytic Cleavage of TGFR: Transcriptional Control of Target Genes

Tumor necrosis factor receptor-associated factor 6 (TRAF6) has been investigated to proteolytically cleave intramembrane region of the TβRI in cancer cells (11). APPL1 and APPL2 are Rab5 effector proteins and reportedly involved in facilitating the nuclear accumulation of Intracellular domain (ICD) of TβRI in TGFβ treated prostate cancer cells (11). However nuclear accumulation was not detected in TGFβ treated APPL2 and APPL1 silenced prostate cancer cells. TβRI polyubiquitination at lysine residue is triggered by TRAF6 and later proteolytically cleaved into TβRI- ICD in TGFβ treated cells (11). Mechanistically it has been revealed that TRAF6 modulated endosomal sorting of TβRI to APPL1-positive endosomes in TGFβ treated cells. Intriguingly these endosomally sorted TβRI were not noted in TRAF6 silenced cells. APPL proteins also efficiently modulate translocation of endosomally located TβRI-ICD to the nucleus via microtubules in a TRAF6-dependent manner (11).

TGFβ has been shown to efficiently enhance catalytic activity of γ-secretase core components, to modulate proteolytic processing of trans-membrane receptors (12). Mechanistically it has been demystified that different molecules work synchronously to recruit presenilin-1 (PS1) to the TβRI complex to polyubiquitylate and activate PS1 (12). Furthermore, PS1 proteolytically cleaved TβRI in the transmembrane domain to form ICD, which accumulated in the nucleus to transcriptionally activate expression of TβRI (Figure 1). PS1 and TRAF6 mediated cleavage of TβRI enhanced TGFβ-induced invasion of cancer cells (12). However, γ-secretase inhibitor remarkably reduced TβRI-ICD formation, its nuclear accumulation and tumor growth formation in mice xenografted with prostate cancer cells (12).

Interplay of Androgen Receptor and TGF induced signaling

Substantial fraction of information has been added into the existing pool of knowledge and it is now clear that individual cells within the tumor microenvironment, including endothelial cells, bone marrow mesenchymal stem cells (BM-MSCs) and macrophages are contributory in prostate cancer progression (13). Prostate cancer recruited more pre-adipocytes that consequently enhanced TGFβ1, MMP-9 and p-SMAD3 levels in prostate cancer (CWR22Rv1 and C4-2) cells. Suppression of Androgen Receptor induced signals not only promoted invasive potential of prostate cancer cells but also enhanced ability of these cells to attract and recruit more pre-adipocytes (13). Data clearly suggested that pre-adipocytes infiltration into tumor mass dramatically reduced AR expression in prostate cancer cells and simultaneously enhanced TGFβ1, MMP-9 and p-SMAD3 levels to increase invasive potential (13).

Androgens transcriptionally downregulate SMAD3

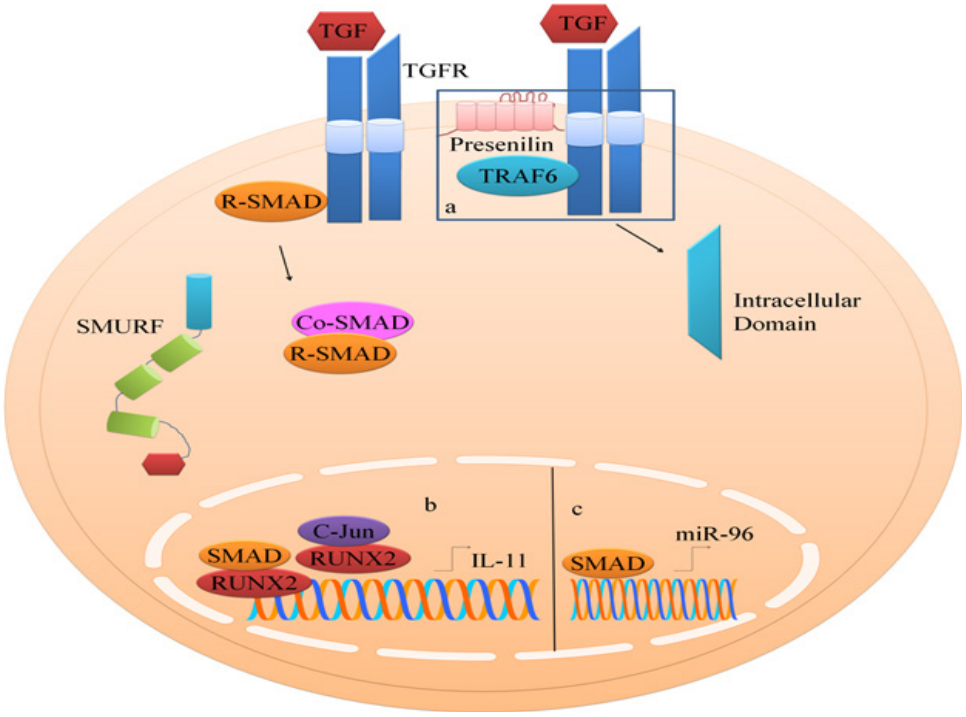

Figure 1. shows TGF mediated intracellular signaling. TGF transduced signals intracellularly through TGFR. R-SMADs after phosphorylation interacted with co-activator SMADs and accumulated in nucleus. (a) Presenilin, TRAF6 worked synchronously to modulate proteolytic cleavage of TGFR. Cleaved intracellular domain transcriptionally upregulated target gene expression. (b) IL-11 is controlled byRUNX2-SMAD and RUNX2-c-Jun. (c) SMAD also transcriptionally regulates miR-96.

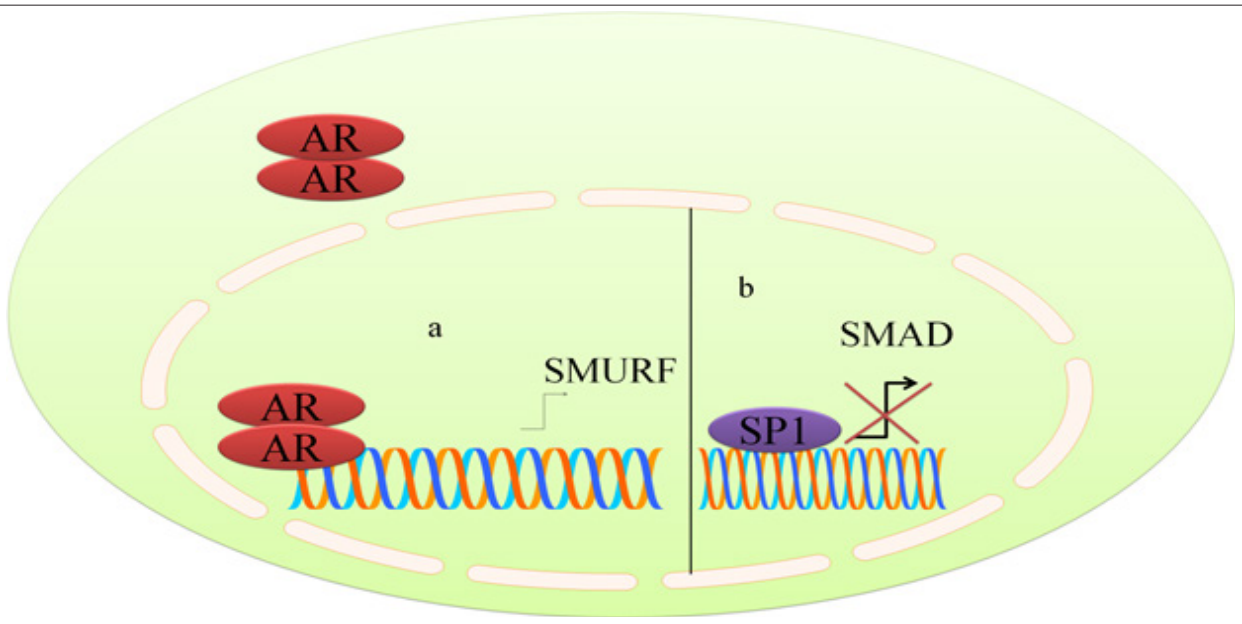

Figure 2. shows androgen receptor mediated control of SMURF. 2(b) Androgen treatment induced loading of Sp1 to promoter region of SMAD.

by facilitating the binding of Sp1 to the promoter region (14). (Figure 2). SMURF1 is transcriptionally controlled by AR as evidenced by enhanced loading of androgen bound AR to enhancer that contains a canonical half androgen responsive element (ARE) (15). (Figure 2).

Regulators of TGF signaling

Significantly enhanced expression levels and enzymatic activity of Matrix Metalloproteinases including MMP9 and MMP2 were noted in Dkk3 silenced prostate epithelial cells (16). Dkk3 exerted inhibitory effects on TGFβ mediated migration/invasion of prostate cancer cells. Structurally it has been confirmed that C-terminal cysteine rich domain of Dkk3 inhibited TGF-β-mediated upregulation of MMP13 and MMP9 (16).

ERG (ETS Related Gene), an ETS family of transcriptional factors has been extensively studied as DNA transcriptional activators. There is a direct piece of evidence suggesting that ERG binds to both inactive and phosphorylated-SMAD3 proteins (17). SB431542, a TGF-β type I receptor inhibitor effectively repressed transcriptional activity of TGF-β/SMAD signaling axis however, increasing expression of ERG counteracted SB431542 mediated inhibitory effects (17).

Transcriptional Regulation of Different Genes

RUNX2 gene encodes a Runt-related transcription factor, reportedly involved in promoting the expression of osteolytic and metastasis related genes (18). It has been experimentally verified that IL-11 gene promoter contained binding sites for AP-1, SMAD and RUNX2. Accordingly, different protein complexes consisting either of RUNX2-SMAD or RUNX2-c-Jun have been noted to transcriptionally stimulate expression of IL-11 (18). Significantly reduced level of IL-11 was detected in prostate cancer cells in the presence of the Runx2-HTY mutant protein. Treating RUNX2 expressing cancer cells with TGFβ1 induced 30-fold increase in expression of IL-11, accompanied by enhanced loading of c-Jun to promoter region of IL-11 (Figure 1) (18).

Smad2/3-binding elements (SBEs) have been noted within promoter region of miR-96 (19). TGFβ has been observed to transcriptionally upregulate miR-96 by facilitating association between promoter region of pri-miR-96 and Smad2/3/4 complex (Figure 1). Upregulated expression of miR-96 consequently promoted aggressive phenotypes of prostate cancer cells (19).

γ-Tocotrienol effectively downregulated precursor and the mature forms of TGFβ2 to inhibit growth of prostate cancer cells (20).

In the upcoming section we discuss how SHH signaling regulates prostate cancer progression.

Sonic Hedge Hog Signaling

In the absence of signal, smoothened (SMO) activity is inhibited by patched that allowed protein kinase A (PKA)-mediated phosphorylation and truncation of GLI2 and GLI3 (21). Nuclear accumulation of GLI2 and GLI3 repressors results in transcriptional inhibition of target genes. Structural association of SHH with patched (PTCH1) relieved patched mediated inhibitory effects on SMO (21). Activated SMO protected GLI proteins from PKA-mediated post-translational modification and activated them. GLI1–GLI3 activators translocated into the nucleus to trigger expression of the target genes (21).

Loss of olfactomedin 4 (OLFM4) has been reported to be associated with prostate cancer progression. Knock-out study revealed that knockout mice developed lesions in prostate epithelium and other organ tumors (22). OLFM4 protein directly interacted with SHH, to reduce SHH levels in the culture media of prostate cancer cells that consequently resulted in inhibition of GLI-reporter activity (22). mRNA levels of GLI and SHH were notably suppressed in OLFM4 overexpressing prostate cancer cells (22).

Prostate cancer DU145 and LNCaP cells reconstituted with SHH demonstrated markedly increased resistance to paclitaxel (23). Therefore, targeting of SHH is essential to overcome resistance against different drugs.

It is intriguing to note that ascorbic acid, acts as a cofactor in synthesis of collagen and mouse pre-osteo-

blasts cocultured with SHH overexpressing prostate cancer cells formed collagen matrix with characteristically distinct fibril ultrastructures (24). Ascorbic acid induced 2 fold increase in PTC1 and GLI1 expression in pre-osteoblasts cocultured with SHH overexpressing prostate cancer cells (24). The ability of Ascorbic acid induced upregulation of SHH signaling was blocked in dominant negative (DN) GLI1 expressing MC3T3 cells. Interestingly, ascorbic acid mediated activation of SHH signaling and differentiation of osteoblasts was also inhibited upon treatment with collagen synthesis inhibitor (24).

TAK-441, a smoothened antagonist notably reduced castration resistant prostate cancer (CRPC) in mice xenografted with LNCaP cells. Murine orthologs of Ptch1, Gli1 and Gli2 were notably inhibited in xenografted mice treated with TAK-441 (25). The data clearly suggested that TAK-441 treatment delayed CRPC by disruption of paracrine HH signaling with stroma of tumor (25). *Sutherlandia frutescens* , a medicinal plant dose and time dependently inhibited growth of prostate cancer LNCaP and PC3 cells. *Sutherlandia frutescens* efficiently inhibited signaling via blockade of Ptched1 and Gli1 gene expression. Development of poorly differentiated carcinoma was significantly reduced in TRAMP mice fed with diet supplemented with *S. frutescens (*26).

JAK STAT pathway

Cytokine induced intracellular signaling operates through activation of Janus Kinases (JAKs) that consequently leads to JAK mediated phosphorylation of Signal Transducers and Activators of Transcription (STATs). These STATs undergo dimerization and accumulate in the nucleus to trigger expression of target genes (27).

Lycorine, a phytochemical isolated from Amaryllidaceae plant, effectively inhibited migration of prostate cancer cells expressing high levels of STAT3/p-STAT3 (28). Lycorine considerably suppressed phosphorylated STAT3 levels in EGF treated prostate cancer cells. Lycorine efficiently inhibited nuclear accumulation of STAT3 in EGF treated prostate cancer cells (28).

IL-6 dose dependently and time- dependently enhanced phosphorylated levels of STAT3. S3I-201 is a compound reported to chemically inhibit STAT3 activity in prostate cancer cells as evidenced by markedly reduced levels of p-STAT3 (29). JAK2 tyrosine kinase inhibitor (AG490) also significantly downregulated MCL-1, STAT3 and VEGFA levels alongwith notably rise in CASP8 and CASP9 mRNAs (29).

MLS-2384, a new 6-bromoindirubin derivative dose-dependently inhibited Janus Kinase-2 and STAT3 levels in prostate cancer cells (30).

It has previously been convincingly revealed that inhibition of functionally active STAT3, by either pSTAT3 inhibitor (LLL12) or anti-IL-6 antibody (siltuximab) suppressed clonogenicity of stem-like cells in high-grade prostate cancer patients. LLL12 remarkably inhibited growth of a patient-derived castration resistant prostate cancer in tumor model (31).

Notch Signaling

It is now known that expression levels of Jagged1, 2, Hey1, Notch3 expression are notably enhanced in prostate tumor lesions. Notch3 was notably higher in prostatic tumors that co-expressed Hey1 and Jagged1 (32). Significantly higher levels of Notch1, Notch4, HES1 and Jagged1 were detected in tissues of prostatic intraepithelial neoplasia (33).

Jagged1 overexpression differentially enhanced prostate cancer cell proliferation in androgen receptor positive LNCaP and LAPC4 cells. However, Jagged1 overexpressing PC3 and DU145 cells did not show any notable increase in proliferation (34). Surprisingly, AR overexpression in Jagged1 overexpressing PC3 and DU145 cells induced cellular proliferation. There is evidence of higher phosphorylated Akt levels in AR and Jagged 1 overexpressing prostate cancer cells (34).

γ-Secretase Inhibitor (PF-03084014) has been shown to work effectively when used synergistically with docetaxel to markedly inhibit both docetaxel resistant and -sensitive CRPC growth of tumor in soft tissue and bones (35).

Conclusion

Data obtained through high-throughput technologies has deepened our knowledge of molecular subtypes of prostate cancer according to their genomic profiles. Keeping in view the heterogeneously natured prostate cancer, it is clear that multifaceted molecular mechanisms underpin resistance against clinically effective drugs in patients, and there may be time dependent changes in response to different therapeutics. Collection of biopsies of metastases before initiation of new treatments and from the patients who have developed resistance against different treatments, coupled with integrative genomic analysis, should be helpful in identification of these evolving resistance mechanisms, to get a step closer to personalized medicine.

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