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REVIEW ARTICLE

Role of TGF-β signaling in tumorigenesis

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Abstract

Transforming growth factor β (TGF β) is one of the crucial cytokine playing an important role in developmental and pathological conditions. TGF β is primarily Smad mediated signal transducers but it can also cross talk with c-Jun N-terminal kinases (JNK), Mitogen-activated protein kinases (MAP), Phosphoinositide 3 (PI-3) kinase, Akt/protein kinase B signaling pathways to enhance cancer development. During early stages of cancer the TGF β induces a protective cytostatic effect. In advanced stages, TGF β fails to down regulate Myc, a transcription factor which represses expression of p21Cip1 and p15Ink4b involved proliferation and differentiation of normal epithelial cells and thus losses its cytostatic property. TGF β also involves in various metastatic processes including angiogenesis, extracellular modification, epithelial-mesenchymal transition, cell migration, immune suppression. Several preclinical animal model studies have encouraging outcomes, suggesting TGF β as a potential therapeutic target in advanced cancers.

Keywords: Transforming growth factor β; Smad; c-Jun N-terminal kinase; Mitogen-activated protein kinase; Phosphoinositide 3 kinase

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Introduction

TGF-β is a multipotent cytokine, involved in various cellular processes including cellular proliferation, apoptosis, angiogenesis, extracellular matrix production, immune response and it also involves in cancer progression. It is secreted by different cell types depending up on the cellular demand. Many studies revealed its involvement in inducing cellular differentiation, vasculogenesis and angiogenesis during embryogenesis. In normal and early stages of cancer epithelial cells, TGF-β acts as a tumor suppressor but its anti proliferation effect is lost in advanced cancers [1]. It has been shown to over express due to accumulation of somatic mutations and thus promotes tumor growth and metastasis [2, 3].

TGF-β signaling pathway

TGF-β belongs to a large cytokine family composed

of six subfamilies, TGF-β, nodal, growth and differentiation factor (GDF), bone morphogenetic protein (BMP), activin and mullerian inhibiting substance (MIS). TGF-β possesses nine conserved cysteine residues which are signatory residues for TGF-β family. A characteristic cysteine knot structure is formed by eight cysteine residues forming disulfide bonds within the molecule while the ninth cysteine forms a bond with the ninth cysteine of another TGF-β molecule to produce the dimer [4]. The fifth and sixth conversed residues are implicated in receptor specificity and sensitivity of TGF- β.

TGF -β is secreted as a precursor that has N-terminal propeptide latency- associated peptide (LAP) and C terminal active form of TGF- β [5, 6]. LAP is in turn bound covalently to latent TGF- β binding proteins (LTBPS) [7]. The LAP inhibits the active TGF -β from binding to its receptor while LTBP interacts with ECM. The activation of TGF -β ligand involves the proteolytic cleavage of LTBP by serine proteases. In cancer matrix metalloproteinases MMP2 and MMP9 are shown to over express and cleave the LTBPs to activate the TGF-β [8, 9]. Other molecules such as proteinase plasmin, elastase, calpain, cathepsin D are also involved in TGF-β activation. Thrombospondin a plasma protein and α v -β6 integrin interacts with LTBP and changes its conformation to convert into active form. It has been shown that pH and reactive oxygen species also induce TGF –β activation [10, 11].

TGF -β transduces signals by interacting with two classes of transmembrane serine/threonine kinases receptors, the TGF-β type-I receptors (TβRI) and the TGF-β type-II receptors (TβRII) and along with accessory receptors sometimes designated as TGF-β type-III receptors (TβRIII), endoglin and β glycan [12, 13]. Accessory receptors bind TGF-β with lower affinity and present it to TGF-β receptors [14]. TGF-β interacts with the extracellular domain of the TβRII which recruits and phosphorylates distinctive GS domain (SGSGSG sequence) of TβRI which inturn phosphorylates the Smads with its catalytic domain [15]. ALK5 and ALK1 are the two type I receptors, TGFβ1 signals through ALK5 in most cells and ALK1 in endothelial cells. Activated ALK1 phosphorylates Smad1/5 in the cytosol and ALK5 phosphorylate different classes of Smads 2/3. These Smads form complexes with Smad4 and translocates to the nucleus to regulate expression of a cascade of genes [16].

TGF -β transduces signal through Smad proteins where in eight types of Smads are identified in humans. Smad1, Smad2, Smad3, Smad5 and Smad8 which are phosphorylated by TGF -β family receptors and are called receptor phosphorylated Smads (RSmads). Smad4 is called co-Smad as it interacts and gets phosphorylated by RSmads. Smad 6 and Smad 7 are inhibitory Smads (Ismads) which are not phosphorylated by TGF receptors and interfere with Smad –receptor and Smad –Smad interaction [17]. Smads have two globular domains attached with a linker peptide. The N-terminal Mad homology 1 (MH1) domain binds with DNA and Mad homology 2(MH2) domain of C terminal possess serine residues which get phosphorylated by type-I receptors in case of RSmads and the linker region is a ubiquitin ligase binding site [15, 18]. The C-terminal phosphorylated RSmads complexes with MH2 domain of Smad4 and translocates into nucleus where this complex binds with other transcriptional cofactors to a form transcriptional complex which induces transcription. Depending upon the interaction transcriptional cofactor, the Smads form heterodimeres (Rsmad -Smad4) or heterotrimeres (2Rsmad-smad4) [19]. Most of the Smad partners identified are FoxH1, Fast1, Jun/Fos, Runx, ATF3, and E2F4/5.

TGF-β Smads independent signaling

TGF-β not only activates Smad pathway but also induces Smad independent signaling pathways like JUN NH2-terminal kinase (JNK), mitogen activated protein kinase (MAP kinases), PI-3 kinase, Akt (protein kinase B) signaling pathways [20, 21, 22]. TGF-β1 not only induces apoptosis but also simultaneously induces the EMT (epithelial-mesenchymal transition) in mouse hepatocyte AML-12 cell line [23]. Akt /PKB is a serine / threonine kinase which is also involved in regulation of TGF -β induced apoptosis and EMT. Akt inhibit apoptosis by inactivation of caspase-3 but recently it is found that Akt interacts with unphosphorylated Smad3 but not with Smad2 and prevent phosphorylation & translocation of Smad3 to the nucleus. As only Smad3 is involved in TGF -β induced apoptotic signaling Akt-Smad3 complex prevents the cell cycle arrest [1].

Akt also repress Smad activity through phosphorylation of FoxO transcription factors which regulate DNA repair and cell cycle progression. In glioblastoma brain tumor cells FoxO proteins form

a complex with Smad3 and Smad4 to activate p21 expression in response to TGF-β. AKT phosphorylates FoxO proteins preventing complex formation and inhibits apoptosis induced by TGF –β [24].

Regulation of TGF -β signaling

TGF -β is an important regulator of cell homeostasis by maintaining the fine balance between cell proliferation and cell death. TGF signaling is modulated by different mechanisms and at various levels. TGF -β transduces signal through Smad proteins in the expression of TGF -β responsive gene by interaction with transcriptional co activators and co repressors to regulate the signal. Expression of TGF-β as a proprotein, latency –associated protein is the primary TGF -β signal regulatory mechanism at ligand level.

Regulation of smads

Smad6 and Smad7 are the Inhibitory smads (Ismads) of TGF -β induced Smad mediated pathway. I Smads bind to TGF -β type-1 receptors and prevent R Smads to interact with type-1 receptors [25]. BMP and TGF -β induces feedback loop inhibition through expression of Smad6 and Smad7 respectively [26, 27]. Smad ubiquitination regulatory factor-1 (smurf-1) and WW domain-containing protein 1 (WWPl) interacts with Smad7 bound to type-I receptor and translocate type-I receptor into cytoplasm and degrade by proteosome mediated degradation [28]. Arkadia is a positive regulator of TGF -β signal induced ubiquitin-dependent degradation of Smad7 [29].

An invitro study on human arotic endothelial cells has shown another way of down regulation of TGFβ1 signaling pathways. TGFβ1 type II receptor and endoglin are internalized by thrombin a serine protease which activates PAR1 pathway which is critical for angiogenesis [30]. Additionally, Smad2 and Smad3 phosphorylated through extra cellular signal regulated kinase and Ca2+/ calmodulin dependent protein kinase II pathway resulting in impairment of their nuclear translocation. Smad3 is also prevented from translocation to the nucleus by phosphorylation via Akt/PKB pathway (protein kinase B) and protein kinase C (PKC) [30].

Regulation of transcription

Ski, SnoN are the oncoproteins that antagonize TGF -β signaling pathway at various levels [31]. v-Ski is an oncogene of the avian sloan– kettering virus homologus to human called c-ski [32]. These three homologs have a Zinc binding conserved cysteine and histidine amino acid residues and SAND domain which interact with Smad4 to block the signaling pathway [33, 34].

SnoN is also closely related homolog and has the two splice variant SnoA and SnoI isoforms found in human. SnoN and SnoA are expressed ubiquitously but SnoI is confined to malignant skeletal muscles [35]. SnoN and Ski interacts with RSmads, Co-Smads to block the transcriptional activity of TGF- β [36]. Ski or SnoN also interact with the active heteromeric Smad complex and disrupt them to break the signal cascade. In another mechanism these molecules interact with transcriptional corepressors like N-CoR to repress the transcription of TGF -β responsive genes [37]. Expression of Ski and SnoN is upregulated during certain phases of embryonic development and in cancers [38]. SnoN is over expressed in breast, esophageal and lung cancers. This may be due to its gene location i.e 3q26 which is a locus for gene amplification in certain lung cancer [39]. Ski expression is correlated with esophageal and melanoma cancer progression but in contrast some melanoma cell lines have reduced expression and in some cancers the gene is deleted very often [40]. Human ski gene located on chromosome 1p36 frequently undergoes deletion in neuroblastoma and melanoma [41, 42].

Arkadia induces degradation of SnoN and c-Ski in addition to Smad7. Arkadia interacts with SnoN and c-Ski in their free forms as well as in the forms bound to Smad proteins, and constitutively down-regulates of their expression. Arkadia thus up-regulates the TGF-β signaling through simultaneous down-regulation of two distinct types of negative regulators, Smad7 and SnoN/c-Ski, and may play an important role in determination of intensity of TGF-β family signaling in target cells [43].

Role of TGF-β in cancers

TGF -β (except TGF -β 2) targeted gene expression is mediated by SP1, FoxO, AP1 transcriptional cofactors [44]. TGF -β induces its cytostatic effect by inducing cyclin–dependent kinase inhibitors p21Cip1 and p15Ink4b and down regulates transcriptional factors Myc, Id1 and Id2, which represses expression

of p21Cip1 and p15Ink4b which is involved in proliferation and differentiation in normal epithelial cells. The Smad3-smad4 complex interacts with FoxO or Sp1 transcriptional factor and binds to the p21Cip1 promoter and expresses P21Cip1. Similarly Smads interact with a transcriptional cofactor and then binds to upstream promoter of p15Ink4b gene and activate transcription where AP1 mediates TGF-β auto-induction [45].

During tumorigenesis, TGF-β signaling components accumulate somatic mutations and losses it's potential to induce cytostatic effect through p21Cip1, p15Ink4b, Myc, Id1 and Id2. In gastrointestinal cancers TGF-β type II receptors is inactivated by micro satellite mutation accumulation due to DNA mismatch repair [1]. TGF-β type I receptor mutations are also seen in ovarian, breast and pancreatic cancers. Mutations are also detected in various Smads in human cancers. Studies on TGF-β receptor polymorphism showed that approximately 14% of general population carry TGFBR1*6A variant, which is increases the cancer risk up to 70% and 19% among TGFBR1*6A homozygote and heterozygote respectively [46].

Other then mutations, TGF-β signaling can be evaded to tumorigensis by Miz-1, a protein that specifically binds to the promoters of P21Cip1 and P15Ink4b and recruits Myc that suppresses the expression of P21Cip1 and P15Ink4b. This implies the important role of TGF-β which downregulates the Myc, involved P21Cip1 and P15Ink4b gene activation. In normal epithelial cells TGFβ counter the EGF and Ras-activated mitogen proliferating effect. In another mechanism, TGIF a Smad co-repressor is phosphorylated by hyperactive Ras thus preventing ubiquitination. The activated TGIF competes with p300 a transcriptional cofactor that binds to Smads and inhibits p15Ink4b expression by binding to promoter. In cancers, constitutive expression of Ras activates ERK MAP kinases to inhibit TGF beta signaling by phosphorylating Smad2 and Smad3 at specific sites other then TβRI target site [47].

Role of TGF-β in metastasis

During metastasis epithelial cell transdifferentiate to mesenchymal - like cell which increases the migration capacity. This phenomenon of transdifferentiation is known as epithelial-mesenchymal transistion (EMT). TGF-β induces EMT and promotes cancer development. de Graauw et al. (2010) hypothesise that Annexin A1 (AnxA1), an actin regulatory protein, is functionally involved in breast cancer progression. Annexin A1 (AnxA1), is consistently expressed in basal-like breast cancers (BLBC) a highly aggressive and progressive cancer compared to luminal-like, breast cancer cell lines. When expression of AnxA1 is inhibited by AnxA1 small interfering RNA (siRNA), BLBC-like cells from a mesenchymal is transformed to an epithelial morphology, an effect that was reversed by ectopic AnxA1 expression. AnxA1 siRNA also reduced TGF-β-induced Smad2 phosphorylation and nuclear translocation of Smad4, indicating that AnxA1 can to regulate TGF-β signaling [48].

Many studies have been shown that TGF-β induces angiogenesis, a crucial mechanism in tumour growth. TGF -β induces vascular endothelial growth factor (VEGF) and platelete derived growth factor (PDGF) expression by Smads [49, 50, 51]. During tumor angiogensis, TGF -β induces monocyte chemoattractant protein-1(MCP-1) that is necessary for migration of vascular smooth muscle cells towards endothelial cells during angiogenic sprouting [52].

Recent *in vivo* study revealed that constitutive signaling of TGF –β induces single cell motility in breast cancer. Single cell motility is capable of blood borne invasion and metastasis to distant tissues where as cohesive cell migration restricts the metastasis only to lymphnodes. This switching of cohesive cells migration to single cell motility is through Smad4, EGFR, Nedd9, M-RIP, FARP and RhoC transcriptional programming [53]. TGF –β induced IL-11, PTHrP acts on osteoblast to release RANK ligand(RANKL) which enhances the osteolysis and facilitates accumulation of migratory malignant cells [54, 55].

TGFβ a therapeutic target

In advanced stages of cancer, TGFβ loses its cytostatic property and promotes various processes like angiogenesis, extracellular modification, EMT, cell migration, immune suppression involved in disease progression. There are several evidences showing increased blood TGFβ level in various cancers [56, 57]. Drugs and antibodies that target TGFβ pathway in combination with conventional cancer therapeutics may contribute to lower the risk of cancer advancement or may inhibit the progression

of cancers detected in advanced stages improving the survival.

Animal models of breast cancer have shown that upon neutralization of TGFβ with anti- TGFβ antibodies slower the tumour progression rate [58, 59]. When additional doses of TGFβ were injected sub cutanaeously, induced cancer advancing processes like myofibroblast phenotype, angiogenesis in prostate cancer models [60]. These results suggest TGFβ can be a therapeutic target in cancer detected in advance stages. The possibility of TGFβ antagonists disturbing homeostasis cannot be ruled out. Thus, TGF –β signaling pathway is an emerging attractive therapeutic target in various cancers due to its multifunctional role in the regulation of cell proliferation, differentiation and survival or apoptosis. It can be predicted that inhibitors of this pathway will pave their way to cancer clinical trials leading to delay in tumour progression and enhance progression of the condition.

Conflict of Interest

The authors wish to express that they have no conflict of interest.

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