Review

Signaling interplay between transforming growth factor-β receptor and PI3K/AKT pathways in cancer

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The transforming growth factor (TGF)-β and phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) signaling pathways are used in cells to control numerous responses, including proliferation, apoptosis, and migration. TGF-β is known for its cytostatic effects in premalignant states and its pro-oncogenic activity in advanced cancers. The pro-cell survival response exerted by growth-factor-mediated activation of PI3K/AKT has been linked to stimulation of tumor formation. Both TGF-β receptor and PI3K/AKT pathways were initially modeled as linear signaling conduits. Although early studies suggested that these two pathways might counteract each other in balancing cell survival, emerging evidence has uncovered multiple modes of intricate signal integration and obligate collaboration in driving cancer progression. These new insights provide the rationale for exploring their dual targeting in cancer.

TGF-β and PI3K/AKT signal transduction: two pivotal pathways that control cell function

TGF-β was initially discovered due to its ability to induce fibroblasts to grow in soft agar; a feature associated with oncogenes. In contrast to oncogenic transformation, however, the effect of TGF-β was found to be reversible. Subsequently, TGF-β was found to have cytostatic effects on normal nonmalignant cells. These multifunctional effects of TGF-β that depend on cellular context are now well established. In normal cells and in early phases of tumor development, TGF-β frequently acts as a tumor suppressor, whereas in the late phases of tumor development, tumor cells become resistant to its antimitogenic effects and TGF-β can switch into a tumor promoter [1–3].

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TGF-β binds and activates specific, cell surface TGF-β type I and type II receptors (TβRI and TβRII, respectively) that are endowed with serine/threonine kinase activity. Stimulation of these receptors activates canonical intracellular signaling SMA- and MAD-related protein (SMAD) via transcription factor complexes. TGF-β receptor activation induces phosphorylation of receptor-regulated (R) SMADs (i.e., R-SMAD2 and R-SMAD3), which form heteromeric complexes with the common mediator, SMAD4. An inhibitory SMAD (i.e., SMAD7) competes with R-SMADs for binding to TβRI, and recruits SMAD ubiquitin regulatory factor (SMURF) E3 ubiquitin ligases to the activated receptor, which targets the receptor complex for degradation (Figure 1A) [1–3].

The TGF-β-induced growth inhibition in many cell types is mediated by a SMAD-dependent downregulation of *c-MYC* oncogene expression, and by inhibition of cyclin-dependent kinases (CDKs) via upregulation of *p15INK4B* and *p21CIP1* and downregulation of *CDC25A* expression [1–3]. In addition to causing cell cycle arrest, TGF-β/SMAD signaling can also induce apoptosis and differentiation of epithelial cells. Moreover, in a premalignant stage, TGF-β may exert tumor suppression by suppressing inflammation and expression of stromal-derived mitogens (Figure 1B). The tumor suppressing role of the TGF-β/SMAD pathway is exemplified in large subsets of colorectal, pancreatic, and ovarian cancers. These cancer cells have either a mutation or deletion in the TGF-β receptor gene or the SMAD gene. Thus, these cancer cells fail to respond to the tumor-antagonizing signals from TGF-β. However, in those conditions, late stage cancer cells may produce TGF-β to stimulate tumor progression by stimulating stromal cells to express growth factors [4] (Figure 1C).

Malignant clones that have lost TGF-β receptors or SMADs have a survival advantage, because these cancer cells can grow unconstrained; nevertheless, advanced tumors, including breast cancer, gliomas, and melanomas, draw benefit from the TGF-β/SMAD pathway. The most aggressive tumors preferentially acquire oncogenic mutations, such as in RAS/MAPK, PI3K/AKT or p53 signaling molecules, that are not core components of the TGF-β/SMAD pathway, but rather act in parallel or interacting pathways [5]. These oncogenic mutations in cancer cells

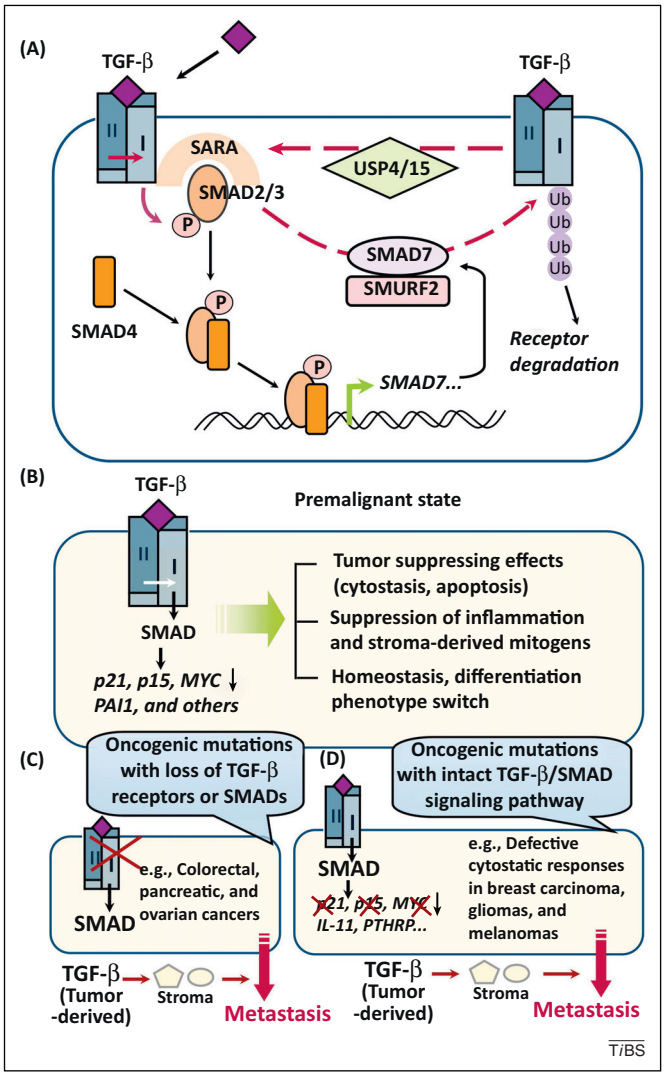

Figure 1. Transforming growth factor (TGF)-β/SMAD (SMA- and MAD-related protein) signaling in tumor suppression and tumor progression. **(A)** TGF-β signals via type II and type I serine/threonine kinase receptors (TβRII and TβRI, respectively). TGF-β binds first to the constitutively active TβRII. The binding event is recognized by TβRI, which mobilizes to form a receptor complex. Upon heteromeric receptor complex formation, TβRII kinase trans-phosphorylates TβRI on serine and threonine residues in a glycine/serine-rich domain. The activated TβRI phosphorylates R-SMAD2 and R-SMAD3. SMAD2 and SMAD3 are recruited to the activated TGF-β receptor complex via the SMAD anchor for receptor activation (SARA), which contains a FYVE domain that interacts with phosphatidylinositol 3-phosphate (PIP3). The R-SMADs form heteromeric complexes with SMAD4. Heteromeric SMAD complexes accumulate in the nucleus. There, in concert with other DNA-binding transcription factors, coactivators, and co-repressors, they bind to target gene promoters to regulate transcription. Inhibitory SMAD7 antagonizes TGF-β/SMAD signaling by competing with R-SMADs for receptor binding and by recruiting E3 ubiquitin ligases (SMURF2 and SMURF1) to the activated TGF-β receptor complex. These SMURFs target the receptor for ubiquitin-mediated proteasomal degradation. The covalent attachment of ubiquitin chains to TβRI is reversible; ubiquitin specific protease (USP)4/15 deubiquitinases can remove ubiquitin chains from TβRI, and thus stabilize the TGF-β receptor. The *SMAD7* gene is directly targeted by the TGF-β/SMAD signaling pathway; thus, it participates in a negative feedback loop to regulate the amplitude and duration of TGF-β/SMAD signaling. **(B)** In normal and premalignant cells, TGF-β enforces homeostasis by inducing tumor-suppressive effects, including cytostasis, differentiation, and apoptosis. TGF-β-induced growth arrest is mediated (in part) through the upregulation of cyclin-dependent kinase inhibitors, *p21CIP* and *p15INK4B*, and downregulation of the proto-oncogene, *c-Myc*. *Plasminogen activator-1* (*PAI1*, encoding an extracellular matrix protein, is a prototypic upregulated target gene of TGF-β. TGF-β exerts tumor suppressive effects by inhibiting expression of inflammation and stroma-derived mitogens. **(C)** In colorectal and pancreatic carcinomas or in ovarian cancers, frequent mutations occur in TGF-β receptors (*TGFβRI/TGFβRII*) or in *SMAD4*, which render cancer cells insensitive to the cytostatic effects of TGF-β. However, cancer cells benefit from elevated tumor production of TGF-β, which induces stromal production of cytokines that promote cell survival. **(D)** In breast cancer, gliomas, and melanomas, oncogenic mutations frequently do not occur in the central TGF-β receptor/SMAD components; instead, genetic or epigenetic changes selectively impair TGF-β-induced tumor-suppressive responses, such as upregulation of *p21CIP1* and *p15INK4B* and downregulation of *c-MYC*. Those mutations do not disturb pro-oncogenic responses such as EMT. In this context, cancer cells benefit from a TGF-β-rich environment. They respond to TGF-β activation of both SMAD-mediated and non-SMAD-mediated inductions of target genes. In the case of bone metastasis of breast cancer, TGF-β (which is highly abundant in bone) induces the expression of *interleukin* (*IL*)*-11* and *parathyroid hormone related protein* (*PTHRP*), which participate in a vicious cycle promoting bone resorption and osteolytic metastasis.

confer selective insensitivity to, for example, *p15INK4B*/*p21CIP1* induction or *c-MYC* reductions through the TGF-β/SMAD pathway; thus, the tumor-suppressive effects are abrogated. The SMAD pathway remains intact, therefore, SMAD-dependent gene responses in this context are advantageous for cancer cell migration and metastasis. The subverted TGF-β/SMAD signaling pathway then actively drives tumor cell progression. Tumor cells with this signature fail to execute TGF-β/SMAD-mediated growth arrest; rather, they undergo the epithelial-to-mesenchymal transition (EMT) in which cells lose cell polarity and cell–cell contacts, become more motile, and acquire fibroblast-like properties [4] (Figure 1D). In the latter process, the non-SMAD signaling pathways (and collaboration with oncogenic signals) are thought to contribute to the pro-oncogenic effects of TGF-β. In particular, the PI3K/AKT pathway is emerging as an important pathway and is the focus of this review. For discussion on other non-SMAD signaling pathways, we refer to excellent reviews [6,7].

The PI3K/AKT signaling pathway is also prominently activated by many growth factors that signal through receptor tyrosine kinases (RTKs). This pathway is well established and strongly linked to pro-survival. PI3Ks that are recruited to RTKs consist of two subunits: catalytic subunit p110 and regulatory subunit p85. Binding and activation of PI3K to membrane-bound RTKs stimulates the conversion of phosphatidylinositol- 4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-triphosphate (PIP3) that provides a docking site for pleckstrin homology domain containing AKT, which thereby becomes activated. Activated AKT mediates the phosphorylation of a multitude of effectors that mediate its antiapoptotic/pro-survival function and induces cell growth and protein translation (Box 1). Many cancer cells have mutations in components in the PI3K/AKT pathway that lead to hyperactivation of this pathway. These cells also exhibit a blunting of the TGF-β-induced growth inhibitory and proapoptotic effects at specific points in TGF-β/SMAD signaling cascade. This desensitization to cytostatic effects may contribute to switching TGF-β from a tumor suppressor into a tumor promoter.

In the following sections, we discuss recent advances in our understanding of the interplay between the TGF-β/SMAD and oncogenic signal-induced PI3K/AKT pathways in cancer progression. We end our review with an outlook toward an emerging rationale for combinatorial targeting of TGF-β and PI3K/AKT pathways in the treatment of cancer patients.

Direct and indirect activation of PI3K/AKT pathway by TGF-β

In addition to the canonical SMAD pathway (Figure 1A), there are other intracellular pathways that TGF-β receptors can initiate via either phosphorylation or direct interaction