Transforming Growth Factor-β, Smads, and Cancer

□□*Commentary on Baez et al., p. 3191*

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The Smad proteins are components of the transforming growth factor β (TGF-β) signaling pathway, which is a pathway that is deregulated in a variety of cancer types. Previous studies from the Munoz-Antonia lab and Reiss labs have shown the protein expression of members of the TGF-β signaling pathway is deregulated in head and neck squamous cell carcinomas (HNSCC; refs. 1, 2). In this issue of Clinical Cancer Research, Baez et al. have extended our understanding of the role of TGF-β signaling deregulation in HNSCCs by showing Smad4 expression is decreased more commonly in HNSCC that are human papillomavirus (HPV)-16 positive than in those that are HPV-16 negative. To appreciate the significance of these results, it is helpful to understand the molecular and cell biology of TGF-β. TGF-β is a multifunctional cytokine that controls a diverse set of cellular processes, including cell proliferation, apoptosis, and differentiation (3). TGF-β is one member of a superfamily of over 40 secreted signaling molecules that include the bone morphogenetic proteins, activin, and inhibin among others (4, 5). These ligands all function by binding to specific cell surface receptor complexes to activate post-receptor signaling pathways that mediate the biological responses of the cells.

Transforming Growth Factor-β Signaling and Smads

TGF-β mediates its effects on cells through a heteromeric receptor complex that consists of type I (TGFBR1) and type II (TGFBR2) components. TGFBR1 and TGFBR2 are serine-threonine kinases that phosphorylate downstream signaling proteins on activation (6). The receptor complex is activated by TGF-β binding to the TGFBR2 component of the receptor complex causing formation of the heteromeric TGFBR1/TGFBR2 receptor complex. The activated TGFBR2 component then phosphorylates the TGFBR1 component in the GS box of TGFBR1, a glycine-serine–rich region of the receptor. TGFBR1 then propagates the signal from the receptor through the activation of a variety of downstream signaling pathways (ref. 7; Fig. 1).

The best characterized of these signaling pathways is the Smad pathway, which consists of members of the Smad family of proteins, including Smad1, Smad2, Smad3, Smad5, and Smad4 (8). Different members of the Smad pathways have different roles in signal propagation and seem to be pathway restricted. Thus, TGF-β and activin activate Smad2 and Smad3, but bone morphogenetic proteins activate Smad1 and Smad5. Both the Smad2/3 and Smad1/5 complexes, however, form a hetero-oligomeric complex that often includes Smad4, which translocates to the nucleus to participate in the formation of transcription factor complexes (9). In the nucleus the Smad protein complexes can modulate transcription of specific genes through *cis*-regulatory Smad-binding sequences and through binding with other transcription factors such as p300/CBP, TFE3, Ski, and c-jun (10). The composition of these transcription factor complexes seems to be an important mechanism through which the specificity of the TGF-β mediated biological response is determined (10).

The Smad Family of Proteins

Thus, the Smad proteins are a family of proteins that serve as intracellular mediators to regulate TGF-β superfamily signaling. The Smad proteins compose an evolutionarily conserved signaling pathway that has been shown in *C. elegans*, *Drosophila melanogaster*, *Xenopus*, as well as humans. These proteins are characterized by two regions that are homologous to the *Drosophila* orthologue, Mad, and that are located at the NH_2 and COOH termini of the protein. These regions are termed the Mad-homology domains MH1 and MH2, respectively, and are connected by a less well-conserved, proline-rich linker domain. Numerous studies have identified three major classes of Smad proteins: (*a*) the receptor-regulated Smads (R-Smads), which are direct targets of the TGF-β receptor family type I kinases and include Smads 1, 2, 3, and 5; (*b*) the common Smads (Co-Smads: Smad4), which form heteromeric complexes with the R-Smads and propagate the TGF-β mediated signal; and (*c*) the inhibitory Smads (I-Smads: Smad6 and Smad7), which antagonize TGF-β signaling through the Smad pathway. Ligand binding to the TGF-β receptor complex results in TGFBR1-mediated phosphorylation of Smad2 and Smad3 on two serine residues in a conserved -SS(M/V)S motif located at the COOH terminus of the R-Smads (11). Phosphorylation of these serine residues is required for downstream signaling pathway activation (12). The specificity of Smad2 and Smad3 for the TGF-β receptor is dictated by a matching set of two residues within the COOH-terminal MH2 domain of these proteins (13). In addition, Smad2 and Smad3 are recruited to the TGFBR1 through an interaction with a membrane-associated, lipid-binding FYVE domain protein, Smad-anchor for receptor activation (SARA). Activation of Smad2 and Smad3 induces their partnering with Smad4. This complex then translocates to the nucleus and activates transcriptional responses as described above. In a similar fashion, the bone morphogenetic protein receptors activate Smad1 and Smad5, which then partner with

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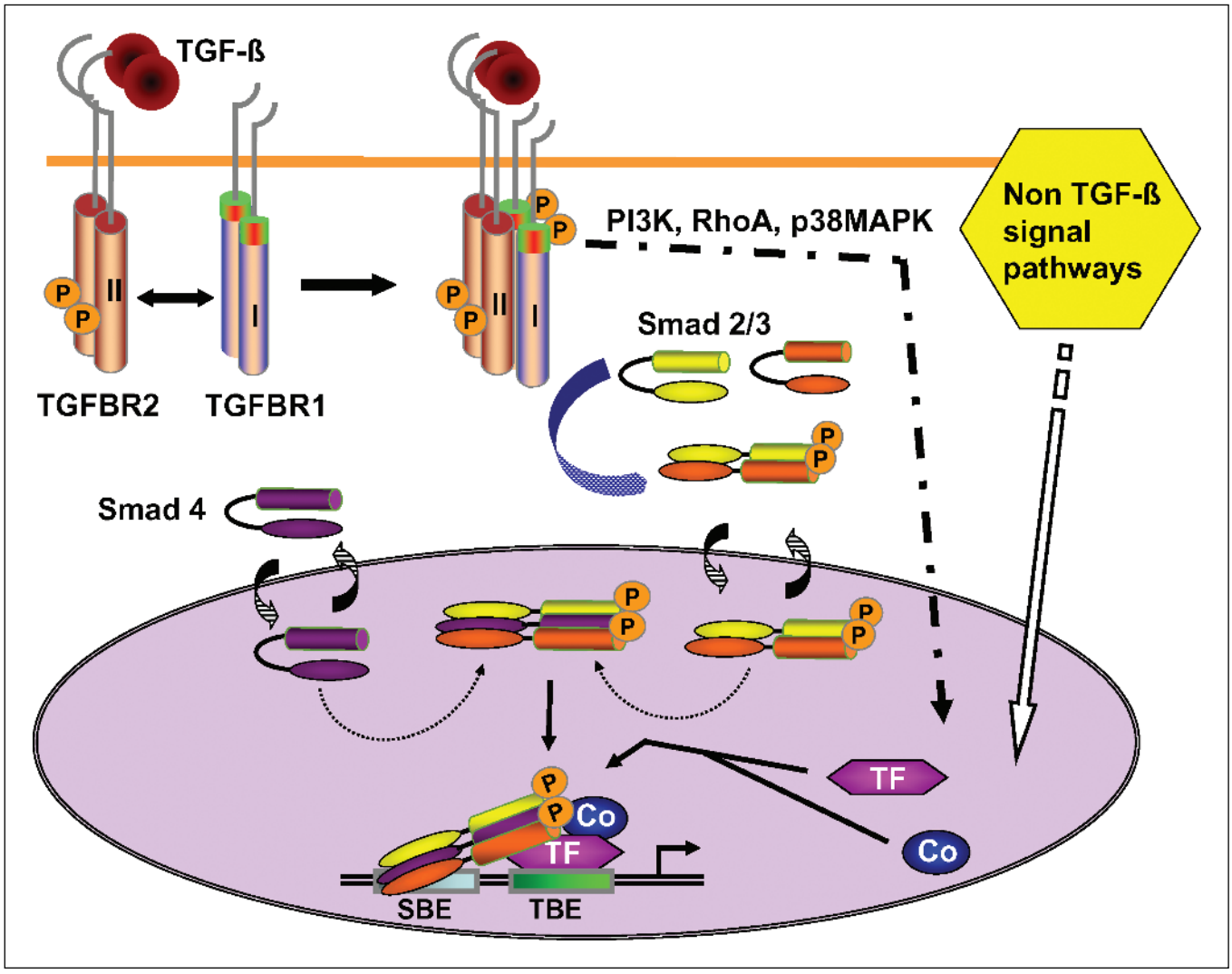

Fig. 1. Schematic diagram of TGF-β signaling from the cell membrane to the nucleus. Arrows, direction of the signal pathway flow. In addition to the Smad signaling pathway, which is shown in most detail, the non-Smad pathways are represented by the dashed line. The non – TGF-β pathways are also shown. One predominant area where these pathways interact with the Smads is at the level of the transcription factor complex formation. *Orange circles,* phosphate groups. *SBE,* Smad binding elements; *TBE,* transcription factor binding elements; *TF,* transcription factor; *Co,* coactivator or corepressor.

Smad4 and translocate to the nucleus to induce transcription of a different genes than those induced by the Smad2/Smad3/Smad4 complex.

Smad4 and Transforming Growth Factor-β Signaling

Thus, Smad4 acts as a convergent node in the Smad pathways that are downstream of the TGF-β superfamily receptors and can interact with all the R-Smad proteins. Smad4 seems to regulate the transcriptional activity of the Smad complexes by controlling nuclear trafficking or nuclear localization of the Smad protein complexes (14). Although initially felt to be an essential component of the TGF-β signaling pathway, Smad4 has since been shown to be dispensable for some TGF-β–mediated responses, such as fibronectin induction and TGF-β–induced cell cycle arrest (15, 16). In light of this data that TGF-β signaling can still occur despite the lack of Smad4, it is intriguing to speculate about what effect the loss of Smad4 has on the HNSCC tumors that Baez et al. have studied. It is possible that the loss of Smad4 changes the complement of genes that TGF-β and the TGF-β family members can transcriptionally regulate in a way that promotes tumorigenesis. Indeed, it is not known how Smad4 loss affects the transcription of a number of targets of the Smad signaling pathway that may play a role in carcinogenesis, including genes such as Smad7, c-Jun, Jun-B, p21, and TGF-β1 (5).

Transforming Growth Factor-β Signaling and Cancer: More than the Smad Pathway

The identification of inactivating mutations in *TGFBR2*, *TGFBR1*, *SMAD2*, and *SMAD4* in human cancers and the loss of expression of the TGF-β receptors and Smads in many cancers have suggested that these events are all selected for during tumor formation because of the common feature that they disrupt the TGF-β signaling pathway. This raises the question of how disruption of specific elements in the TGF-β/

Smad signaling pathway would promote tumor formation. It is possible that simple inactivation of the TGF-β activated Smad signaling pathway is sufficient to change gene expression that favors tumor formation. Indeed, the downstream transcriptional targets of the TGF-β signaling pathway are involved in the regulation of the diverse biological behaviors regulated by TGF-β. These functions not only are an integral part of organ development and tissue homeostasis but also are logical targets for deregulation in carcinogenesis. Elements involved in cell proliferation regulation, which have been clearly shown to be controlled in part by TGF-β, include the cyclin-associated proteins cyclin D1, cyclin-dependent kinase 4, p21, p27, p15, c-myc, Rb, p130, and p107 (17). In addition to the cyclin associated proteins, the extracellular matrix proteins and regulators of extracellular matrix proteins, fibronectin, tenascin, and plasminogen activator inhibitor 1, as well as a variety of other genes that affect apoptosis, differentiation, and other cell behaviors, seem to be transcriptionally regulated by TGF-β (5, 18).

However, it is also important to appreciate that TGF-β signal pathway activation not only regulates SMAD proteins but also other cytoplasmic signaling cascades (phosphatidylinositol 3-kinase/Akt, p38MAPK, Rho proteins, extracellular signal–regulated kinase, and stress-activated kinases) to produce the full spectrum of TGF-β responses (5). Smad2,3/Smad4 complexes interact with a variety of other transcription factors to regulate TGF-β mediated transcription. These interactions determine how a cell responds to TGF-β and may explain why the cell state and cell type, referred to as the "cellular context," can have a profound effect on the way in which TGF-β affects a cell (19) Current evidence suggests that the Smad pathway plays a central role in TGF-β–mediated growth inhibition and apoptosis whereas Rho proteins, stress-activated kinases (c-jun-NH_2-kinase and p38), and phosphatidylinositol 3-kinase predominantly regulate transcription, cell shape changes, loss of adherens junctions, and motility (20). There is also evidence that the activation of the Smad-dependent and Smad-independent pathways depends on the degree of TGFBR2 expression and activation (4).

Furthermore, the TGF-β signaling pathway(s) interacts with many other signaling pathways, and this interaction modulates the TGF-β responses. As previously mentioned, the biological effects of TGF-β are complex and dependent on cell type and state. This complexity seems to be governed not only by the TGF-β–mediated activation of Smad-dependent and Smad-independent signaling pathways but also by the integration of TGF-β–activated pathways with pathways activated by other factors, such as epidermal growth factor, or by mutations in genes such as *KRAS2* or *APC* (5, 21). These pathways interact at different levels of the signaling pathways to modulate the response of a cell to TGF-β. For example, overexpression of oncogenic v-Ha-Ras in the murine mammary epithelial cells EpH4 represses TGF-β/Smad signaling by inhibiting the nuclear translocation of Smad2 and Smad3 (22). In addition, other investigators have shown interaction of the Smad pathway with the Wnt signaling pathway, the IFN-γ/STAT pathway, and pathways that activate activator protein complexes (5). These pathways have been shown to have a role in altering the effect of TGF-β on the cell lines studied, especially with regard to apoptosis (17, 21). The integration of these TGF-β and non–TGF-β signaling pathways, especially in the setting of the known alterations in signaling pathways observed in cancer cells, has been proposed as one possible mechanism through which TGF-β can even have paradoxical effects on cancer cells (4).

Transforming Growth Factor-β/Smad Signaling, Human Papillomavirus, and Cancer

One of the particularly interesting observations by Baez et al. is that Smad4 expression is preferentially suppressed in the HPV-16–positive HNSCC tumors. HPV-16 is believed to promote tumor formation through the actions of the viral proteins E6 and E7. E6 and E7 drive the oncogenic process by deregulating cell cycle control by inactivating p53 and Rb, respectively (23). So, it is not immediately obvious why Smad4 expression would be preferentially selected in this context, but it is feasible that deregulation of the TGF-β signaling pathway could cooperate with other pathways that are preferentially deregulated in the setting of HPV to promote tumorigenesis. Another possible explanation is that Smad4, which has been shown to undergo proteosomal degradation in tumor cells that have activated oncoproteins such as Ras, may be undergoing a similar process in HPV-16–positive HNSCC tumors (9). It is also possible, as Baez et al. speculate, that the loss of Smad4 creates a susceptibility to HPV infection rather than HPV inducing deregulation of the TGF-β signaling pathway.

Although the specific mechanism linking decreased Smad4 expression to HPV remains unknown, there is considerable evidence that TGF-β and elements in the TGF-β signaling pathway, which include Smad2 and Smad4, act as tumor suppressors for many cancer types, including squamous cell cancers and adenocarcinomas. Much of this evidence is derived from studies in human cancers that show inactivating mutations in genes encoding proteins involved in TGF-β signal transduction, including *DPC4/SMAD4/MADH4* (24, 25), *SMAD2/MADH2* (26), and *TGFBR2* (27). Furthermore, invasive colon tumors develop in $Smad3^{-/-}$ and $Apc^{716\Delta}$ $Smad4^{+/-}$ mice, and mice that lack TGFBR2 in the colon, $Fabp^{4xat\text{-}132}$ Cre $Tgfbr2^{flx/flx}$ mice, are more susceptible to azoxymethane-induced colon neoplasms than are mice with intact TGFBR2 in the colonic epithelium (28–30). Perhaps the most intriguing animal model in relation to the studies by Baez et al. is a *Smad4* conditional knockout mouse that lacks Smad4 expression in the mammary epithelium and that develops squamous cell carcinomas in the mammary glands (31). Thus, it will be exciting to see how future studies in this area of cancer research shed more insight into the role of Smads and TGF-β signaling deregulation in HNSCC tumors.

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