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Crosstalk mechanisms between the mitogen-activated protein kinase (MAPK) pathways and Smad signaling downstream of TGF-β: implications for carcinogenesis

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Abstract

Transforming growth factor-β (TGF-β) superfamily members signal via membrane-bound heteromeric serine-threonine kinase receptor complexes. Upon ligand binding, receptor activation leads to phosphorylation of cytoplasmic protein substrates of the Smad family. Following phosphorylation and oligomerization, the latter move into the nucleus to act as transcription factors to regulate target gene expression. TGF-β responses are not solely the result of the activation Smad cascade, but are highly cell-type specific and dependent upon interactions of Smad signaling with a variety of other intracellular signaling mechanisms, initiated or not by TGF-β itself, that may either potentiate, synergize, or antagonize, the rather linear TGF-β/Smad pathway. These include, (a), regulation of Smad activity by mitogen-activated protein kinases (MAPKs), (b), nuclear interaction of activated Smads with transcriptional cofactors, whether co-activators or co-repressors, that may be themselves be regulated by diverse signaling mechanisms, and (c), negative feedback loops exerted by inhibitory Smads, transcriptional targets of the Smad cascade. This review focuses on how MAPKs modulate the outcome of Smad activation by TGF-β, and how cross signaling mechanisms between the Smad and MAPK pathways may take place and affect cell fate in the context of carcinogenesis.

Introduction

Members of the TGF-β superfamily (TGF-βs, activins, bone morphogenic proteins/BMPs) signal via heteromeric serine/threonine kinase transmembrane receptor complexes (Derynck & Feng, 1997; Javelaud & Mauviel, 2004; ten Dijke & Hill, 2004). Binding of the ligand to its primary (type II) receptor, a constitutively active kinase, allows the recruitment, transphosphorylation and activation of the signaling (type I) receptor. The latter is then able to exert its phosphorylation-dependent serine-threonine kinase activity to phosphorylate cytoplasmic protein mediators of the Smad family (Derynck et al., 1998; Miyazono et al., 2000; Shi & Massague, 2003). The ligand-specific, receptor-activated Smads (R-Smads), Smad1, Smad2, Smad3, Smad5 and Smad8, interact directly with, and are phosphorylated by, activated TGF-β receptors type I (Zimmerman & Padgett, 2000). Smads 1,5,8 are specific substrates of the BMP receptors, whereas Smad2 and Smad3 are activated by both TGF-β and activin receptors. R-Smads consist of two conserved Mad-homology (MH) domains that form globular structures separated by a linker region (Hill, 1999; Miyazono et al., 2000; Shi & Massague, 2003). The N-terminal MH1 domain has DNA-binding activity whereas the C-terminal MH2 domain has protein-binding properties. Phosphorylation of R-Smads by type I receptors occurs principally on two serine residues within a conserved -SS(M/V)S- motif at their C-terminus. Upon phosphorylation, they form heteromeric complexes with Smad4, a common mediator for all Smad pathways. The resulting Smad heterocomplexes are then translocated into the nucleus where they activate target genes, binding DNA either directly or in association with other transcription factors (Derynck & Feng, 1997; Hill, 1999; Javelaud & Mauviel, 2004; Miyazono et al., 2000; Shi & Massague, 2003; ten Dijke & Hill, 2004). Members of the third group of Smads, known as inhibitory Smads Smad6 and Smad7, control Smad signaling by preventing phosphorylation and/or nuclear translocation of receptor-

associated Smads and by inducing receptor complex degradation through the recruitment of ubiquitin-ligases that induce proteasomal degradation (Ebisawa et al., 2001; Hayashi et al., 1997; Kavsak et al., 2000; Zhang et al., 2001). More recently, Smad7 was also shown to recruit and stabilize the protein phosphatase PP1/GADD34 complex to the activated TGF-β receptors, thereby inducing receptor dephosphorylation and de-activation (Shi et al., 2004). Following target gene transcription, Smad complexes are released from the chromatin and may undergo ubiquitination followed by proteasomal degradation (reviewed in (Wang, 2003)), or may shuttle out of the nucleus for *de novo* phosphorylation by activated TGF-β receptors and re-entry into the nucleus for further gene regulatory functions (Di Guglielmo et al., 2003; Nicolas et al., 2004; Penheiter et al., 2002; Xiao et al., 2001).

The Smad pathway may not be viewed at a unique mean for TGF-βs to regulate cellular functions, as other signaling pathways including the mitogen-activated protein kinase (MAPK), the NF-κB or PI3 kinase/AKT pathways, can either be induced by TGF-β, or can modulate the outcome of TGF-β-induced Smad signaling (Derynck & Zhang, 2003; Lutz & Knaus, 2002; Massagué & Chen, 2000). Indeed, broad evidence exists for a tight integration of Smad signaling within a complex network of crosstalks with other signaling pathways that largely contribute to modify the initial Smad signals and allow the pleiotropic activities of TGF-β. Also, there may be instances whereby Smad signaling may even be dispensable for some of TGF-β responses, as exemplified by Smad-independent activation of the cyclin kinase inhibitors p15 and p21 in HaCaT keratinocytes (Hu et al., 1999), or transcriptional activation of the fibronectin promoter via MAPK-dependent mechanisms (Hocevar et al., 1999).

Interestingly, it appears now clearly that Smad proteins are not only the primary substrates for the TGF-β receptor kinases, but may also be phosphorylated by MAPKs, in response to either TGF-β itself or to various cytokines. Such R-Smad phosphorylation by MAPKs may serve to

regulate either Smad transcriptional activity or capacity to translocate into the cell nucleus (Derynck & Zhang, 2003; Lutz & Knaus, 2002; Massagué & Chen, 2000). Also, Smad proteins are also capable of physically interacting with transcription factors, themselves substrates of MAPKs, adding more complexity to the intricate relationship between MAPKs and the Smad pathway.

This review will summarize some of the latest data from the literature regarding MAPK and Smad interactions, with a main focus on the cellular events contributing to the various stages of carcinogenesis.

The MAPK signaling cascades and substrates

MAP kinases are a large group of proteins that allow numerous extracellular signals reaching the cell surface to rapidly activate nuclear transcription factors (Whitmarsh & Davis, 1999). They mainly consist in three subfamilies: the extracellular signal-regulated kinases (ERK1 and ERK2), the stress-activated protein (SAP) kinases known as c-Jun N-terminal kinase (JNK1, JNK2 and JNK3), and the p38/MAPKs (α, β, γ and δ) (Chang & Karin, 2001). ERK5, described as a mediator of Src activation (Zhou et al., 1995), is another member of the MAP kinase superfamily but thus far, unlike the first three groups of MAKs, it has not been shown to be activated by TGF-β, or to interfere with Smad signaling.

Signaling initiated by each MAPK pathway occurs through sequential activation of a MAPK kinase kinase (MAPKKK) by membrane-associated kinases such as cytokine or growth factor receptors, a MAPK kinase (MAPKK), leading to phospho-activation of an MAPK (Lowes et al., 2002). MAPK activation leads to downstream phosphorylation of nuclear kinases or, most commonly, transcription factors. A simplified view of the various MAPK pathways is

provided in Fig. 1, which refers to most MAPK members and substrates cited in the text below.

ERKs are phosphorylated by the MAPKKs MEK1 and MEK2, themselves substrates of the MAPKKK Raf-1, the latter being activated by the membrane-bound small G-protein Ras, for example following induction by mitogenic stimuli such as epidermal growth factor (EGF) upon binding and activation of their receptors. JNK family members are the substrates of MKK4 (also known as SEK1) and MKK7. p38/MAPK is phosphorylated by MKK3 and MKK6, themselves the substrates of several MAPKKKs, including, but not restricted to, apoptosis signal-regulating kinase-1 (ASK1), mixed lineage kinases (MLKs), and TGF-β-activated kinase-1 (TAK1) (Davis, 2000; Ip & Davis, 1998). ERK-mediated pathways are mostly involved in proliferation and differentiation and generally considered anti-apoptotic. JNK and p38 signaling pathways are activated by stress stimuli, many of which induce apoptosis, but in some cellular systems they have been implicated in proliferation and differentiation as well (Eferl & Wagner, 2003).

A variety of transcription factors, and some downstream kinases, serve as substrates for activated MAPKs (Treisman, 1996). Among them is activating protein-1 (AP-1), a family of pleiotropic transcription factors comprised of homo- and heterodimers of Fos, Jun and ATF family members, involved in the control of cell proliferation, death and survival, as well as tumorigenesis (Eferl & Wagner, 2003; Karin et al., 1997; Shaulian & Karin, 2001; Shaulian & Karin, 2002). ERK1/2 phosphorylate TCF/Elk-1, and activate CREB and c-Fos through the MSK1 and RSK kinases, respectively. p38 MAPKs activate Elk-1, CHOP, ATF-2, CREB and MEF2C (Hazzalin & Mahadevan, 2002). JNK is the only MAPK to phosphorylate c-Jun, the main component of AP-1 complexes, and also has ATF-2 and Elk-1 as substrate (Chang & Karin, 2001). Phosphorylation of c-Jun results in the activation of this key member of the AP-1 family of transcription factors, which can then bind specific AP-1 recognition sites

TGAG/CTCA, to transactivate target genes (Angel & Karin, 1991). Upon activation, CREB and ATF-2 bind to CRE sites (TGACGTCA) on target gene promoters (Smeal et al., 1994). Heterodimers of c-Jun and ATF-2 have also been shown to bind to CRE sites (Hai & Curran, 1991).

Activation of MAPKs by TGF-β

Many TGF-β-regulated gene promoters, including the TGF-β1 promoter, contain either AP-1- or CRE binding sites (Chung et al., 1996; Mauviel et al., 1993; Roberts et al., 1991; Wong et al., 1999). Complex interactions between the Ras pathway and TGF-β signaling exist at various stages of carcinogenesis, suggesting interference of signaling pathways (Akhurst & Derynck, 2001). Also, a MAPKKK homolog activated by TGF-β, TAK1, was identified in the mid 90s, a direct indication that TGF-β may indeed activate the MAPK kinase pathways (Yamaguchi et al., 1995). Of note, MAPK activation is, not a specific feature of TGF-β signaling and may be the result of various extracellular stimuli, including cytokines, ultraviolet irradiation, cell-cell or cell-matrix contacts, to cite a few. The outcome of Smad-MAPK interactions should therefore not be viewed solely as the result of multi-facetted TGF-β signaling downstream of its receptors, but also as a consequence of cytokine networks acting in concert to modulate Smad/MAPK signals.

TGF-β has been shown in numerous cell types to activate all ERK, p38 and JNK MAPKs (reviewed in (Derynck & Zhang, 2003; Wakefield & Roberts, 2002)). Not only is MAPK activation by TGF-β cell-type specific, but the activation of a given MAPK combination by TGF-β is also cell-type dependent. For example, in mink lung epithelial cells, TGF-β-induced activation of JNK mediates Smad3 phosphorylation, which is required for the transcriptional activation of Smad3-dependent responses (Engel et al., 1999). On the other hand, in rat

articular chondrocytes, TGF-β induces a rapid activation of ERK1/2, but not that of either p38 or JNK MAPKs (Yonekura et al., 1999). Multiple other examples have been described in the literature.

Activation of MAPKs by TGF-β has been described to occur either with slow kinetics, possibly resulting from Smad-dependent transcription responses, but also with rapid kinetics similar to those observed downstream of cytokine receptors. In the latter case, the rapid activation (5–15 min) of MAPK phosphorylation strongly not only suggests independence from Smad-driven transcription but also direct activation of MAPKKKs.

Initial evidence for Smad-independent activation of MAPK by TGF-β was obtained in Smad4-deficient cells, or in cells overexpressing dominant-negative Smads, where activation of the JNK/MAPK pathway was still possible in response to TGF-β despite the deficient Smad cascade (Itoh et al., 2002). Also, it has been shown that mutated TGF-β type I receptors, that can not phosphorylates R-Smad still activate p38 MAPK signaling in response to TGF-β (Yu et al., 2002). The mechanisms of Erk, JNK or p38 MAPK activation by TGF-β and its biological consequences are not fully characterized. ERK activation by TGF-β in epithelial cells may implicate Ras signaling (Yue & Mulder, 2000), while JNK and p38 MAPK signaling could be activated by various MAPKKKs in response to many stimuli. The first known MAPKKK to be activated by TGF-β family members is TGF-β-activated kinase 1 (TAK1), originally identified as a MAPKKK activated by TAB1 (for TGF-β-activated kinase binding protein-1) downstream of TGF-β/BMP receptors, positively regulating the SAPK/JNK and p38 kinase pathways (Yamaguchi et al., 1995). TAB1 is able to associate with the inhibitory Smad, Smad7, a phenomenon that may lead to inhibition of TAK-1-dependent p38 activation (Edlund et al., 2003). Alternatively, it has also been suggested that Smad7 could act as a scaffolding protein to provide structural support for MKK3/p38

activation by TAK1. Of note, because TAK1 also activates NF-κB, TGF-β/BMP receptors, due to their ability to activate TAK1, may, under certain circumstances, also induce NF-κB signaling (Arsura et al., 2003). XIAP (X-linked inhibitor of apoptosis), was then identified as a bridging molecule between TGF-β/BMP receptors and TAK-1/TAB-1, serving as a co-factor for TAK-1-dependent signaling (Yamaguchi et al., 1999). Uncertainty remains however, as (a), some of the cooperative activities of XIAP and TGF-β are not mediated by TAK-1-dependent signals, and (b), XIAP may also be dispensable for TGF-β signaling. For example, activation of TGF-β responsive genes by XIAP has been shown to depend on Smad4, while the anti-apoptotic effects of XIAP are Smad4-independent (Birkey Reffey et al., 2001). Furthermore, XIAP-deficient mice respond to TGF-β (Harlin et al., 2001). This could be explained if a direct link between TAK1 (in association with the cofactor TAB1) and the receptors is established by another upstream kinase, such as the hematopoietic progenitor kinase-1 (HPK1), which allows JNK activation by bridging the TGF-β receptors to TAK1 independently from XIAP (Zhou et al., 1999).

MEKK1 may also function upstream of TGF-β-mediated activation of MAPKKs (Brown et al., 1999); thus, MEKK1 and TAK1 could activate JNK through MAPK kinase 4 (MKK4), and p38 MAPK through MKK3 or MKK6, in response to TGF-β.

In a yeast two-hybrid screen designed to identify proteins interacting with the cytoplasmic tail of the TGF-β type II receptor, Daxx, a protein capable of blocking TGF-β induced apoptosis in B-cell lymphomas, and involved in the activation of the JNK pathway by TGF-β, was isolated (Perlman et al., 2001). Daxx was previously known as an adaptor protein for the Fas receptor, that mediates Fas activation of Jun amino-terminal kinase (JNK) and programmed cell death (Yang et al., 1997). Daxx thus appears to also function as an adaptor protein linking the TGF-β receptor complex to the apoptotic machinery and the JNK pathway.

TGF-β1 may induce both a rapid and a late activation of p38 depending on the cell type. Rapid, transient, p38 activation has been described in certain cell types including human neutrophils, HEK293, and C2C12 cells, and may be mediated by the induction of TAK1 in a R-Smad-independent manner. On the other hand, the delayed and sustained p38 activation observed in pancreatic carcinoma cells, hepatocytes, or osteoblasts, requires Smad signaling: Smad activation results in the induction of the expression of Gadd45b, an upstream activator of MKK4, which thus promotes the delayed activation of p38 MAPK (Takekawa et al., 2002). Likewise, JNK activation may occur rapidly or in a delayed manner (Engel et al., 1998), and among potential candidates that could mediate a delayed JNK activation following TGF-β treatment, is the inhibitory Smad, Smad7, a TGF-β/Smad target whose overexpression has been shown to induce persistent JNK activation in HepG2 cells (Mazars et al., 2001). At present, the exact mechanism(s) by which Smad7 activates the JNK pathway remains elusive. Depending on the cell line, TGF-β can rapidly activate the Rho-like GTPases, RhoA, RhoB, Rac and Cdc. Ras activation in response to TGF-β may also lead to activation of Rho-like GTPases. (Atfi et al., 1997; Edlund et al., 2002; Kamaraju & Roberts, 2005; Mucsi et al., 1996). Rac and Cdc42 regulate JNK and p38 MAPK pathway activation, presumably by directly interacting with MAPKKKs upstream of JNK and p38 MAPK.

Modulation of Smad activity by MAPKs

MAP kinases can modify Smad signaling by phosphorylation-dependent modification of ligand-dependent R-Smad nuclear translocation. Thus, Ras signaling has been proposed to inhibit TGF-β signaling via the ERK pathway. Specifically, ERK has been shown to phosphorylate the linker region of Smad1, Smad2 and Smad3, which results in blocking the nuclear translocation of these TGF-β-activated Smads (Kretzschmar et al., 1999). It was

proposed that such mechanism might explain why some cells with hyperactive Ras signaling do not respond to TGF-β (Calonge & Massagué, 1999; Kretzschmar et al., 1999). However, other reports have demonstrated impaired nuclear translocation of Smads in Ras-transformed cells or in cells with activated MAP kinase signaling, despite efficient phosphorylation of R-Smad linker regions by ERK (Engel et al., 1999). Furthermore, cooperativity between Ras/MAP kinase signaling and TGF-β signaling has been observed during tumor cell differentiation and behavior (Lehmann et al., 2000; Oft et al., 1996), not compatible with the hypothesis of a blocade of TGF-β signaling by Ras.

The issue of whether MAPK phosphorylation of Smads impairs or not their nuclear translocation is very complex: ERK activation, while all potential ERK or JNK sites in the middle-linker regions of Smad2 are mutated, is associated with phosphorylation of the R-Smad outside the C-terminal –SSMS motif and leads to enhanced nuclear translocation of Smad2 (Brown et al., 1999; de Caestecker et al., 1998). Furthermore, TGF-β-dependent phosphorylation sites in Smad2 overlap with the Smad2 phosphopeptide maps seen following activation of the ERK1/2 MAP kinase pathway by HGF (de Caestecker et al., 1998), suggesting that common sites of MAP kinase-induced phosphorylation may also be required for TGF-β-dependent nuclear translocation of the R-Smad/Smad4 complex.

ERKs are not the only MAPKs capable of phosphorylating Smads. Work by Matsuzaki's group showed JNK-dependent phosphorylation of Smad2 and Smad3 in response to HGF, PDGF and TGF-β itself, with no loss of R-Smad capacity to translocate into the nucleus or to transactivate target genes (Mori et al., 2004; Yoshida et al., 2005).

Recently, it was shown that the human breast cancer cell line MCF10CA1h that the Rho/ROCK and p38 pathways cooperate to allow TGF-β-induced growth arrest (Kamaraju & Roberts, 2005). This effect is achieved by phosphorylation of the R-Smad linker regions by both kinases, resulting in an increased transactivation potential of R-Smads, ultimately

leading to cell cycle withdrawal.

MAPKs may indirectly affect Smad signaling by controlling Smad7 expression. Initial observations by Brodin et al. indicated that TGF-β-induced Smad7 expression depends on cooperative interactions between AP-1, Sp1 and Smad proteins (Brodin et al., 2000). More recently, both the JNK and TAK1/p38 pathways were shown to regulate Smad7 expression, in a cell-type specific manner (Dowdy et al., 2003; Uchida et al., 2001). Also, ultraviolet irradiation, a know activator of MAPKs, activates Smad7 transcription in a c-Jun-dependent manner (Quan et al., 2005).

Finally, MAPKs, and in particular the p38 pathway, is involved in the control of post-translational modification of Smads. Specifically, it has been demonstrated that p38 regulates the sumoylation of Smad4 by proteins of the PIAS family of E3 ligases, contributing to an enhancement of Smad4-dependent transcription (Ohshima & Shimotohno, 2003).

Together, these results indicate that activation of MAPK pathways may have positive or negative regulatory effects on R-Smads, depending on the nature of MAPK activation, which, in turn, may affect both the specificity and multiplicity of MAP kinase-dependent phosphorylation events.

Nuclear interactions between Smad complexes and MAPK-activated transcription factors

Activated R-Smad proteins have been shown to participate in a number of heterogenous transcription complexes bound to DNA, as they exhibit a broad capacity to interact with numerous transcription factors, such as Sp1. Downstream components of MAP kinase-signaling pathways, and especially transcription factors of the AP-1 family, may also interact with the R-Smad/Smad4 complex in the nucleus, providing an additional level of crosstalk

between these pathways (Zhang et al., 1998). c-Jun and JunB, both downstream substrates of JNK, are components of the AP-1 complex that are transcriptionally regulated by the TGF-β/Smad pathway (Jonk et al., 1998; Mauviel et al., 1996; Wong et al., 1999), and contribute to an autocrine regulatory loop of Smad activity (Verrecchia et al., 2001a). Interestingly, transcriptional cooperation depends on the structure of the target promoters, as Smad and AP-1 cooperate to activate AP-1-dependent promoters, while they tend to antagonize each other with regard to Smad-specific transcription dependent on Smad-binding site (Verrecchia et al., 2001b). While Fos/Jun-Smad3/4 physical interactions may participate in a hetero-tetrameric complex bound to AP-1 elements or their adjacent nucleotides on DNA (Zhang et al., 1998), data from our own laboratory indicate that both c-Jun and JunB are capable of interrupting Smad3-mediated transcription, as Jun/SMAD3 complexes may form off-DNA, preventing Smad3 binding to cognate DNA sequences (Verrecchia et al., 2000). We also showed that JNK activity promotes such off-DNA association of Jun proteins with Smad3 (Verrecchia et al., 2003). Accordingly, in $JNK_1^{-/-}$-$JNK_2^{-/-}$ ($JNK^{-/-}$) fibroblasts, TNF-α had no effect on TGF-β-driven, Smad-dependent, gene transactivation unless *jnk1* was introduced exogenously. Aside from preventing Smad3 from binding to its cognate DNA binding sites, the JNK pathway may also regulate Smad2/3-dependent transcription via alternate mechanisms, for example by facilitating c-Jun association with transcriptional co-partners, such as the co-activators p300/CBP, or the co-repressors c-Ski and TGIF: c-Jun association with CBP/p300 has been shown to interrupt Smad3-driven transcription by squelching of p300/CBP away from Smad complexes ; also c-Jun may physically associate with c-Ski and TGIF and allow the latter to exert their repressory activity by interfering with the assembly of Smad2/p300 complexes (Pessah et al., 2002; Pessah et al., 2001).

It is interesting to note that the JNK/Jun axis is instrumental to the HTLV-1 Tax oncoprotein in repressing TGF-β signaling, a mechanisms that may contribute to leukemogenesis (Arnulf

et al., 2002). Also, the JNK pathway may contribute to regulate autocrine TGF-β1 expression, as JNK-deficint fibroblasts constitutively express TGF-β1, expression that can be repressed by complementation of the cells with JNK (Ventura et al., 2004).

ATF-2, a downstream substrate of both JNK and p38 MAPKs participates in certain AP-1 complexes. Its expression is induced by TGF-β and transcriptionally regulated by both Smad- and TAK1-dependent mechanisms (Hanafusa et al., 1999; Sano et al., 1999). Furthermore, it is also possible that AFT-2 participates in transcription complexes in association with Smad proteins (Sano et al., 1999).

Although these are just a few examples of the intricacy of transcriptional control by AP-1 and Smad complexes, they suggest a very complex level of integration of the signaling pathways resulting in activation of AP-1 via a MAPKs with Smad-driven signals originating from the TGF-β receptors, leading to either amplification or negative feedback loops controlling TGF-β effects. Naturally, the outcome of these interactions is further diversified by the presence and nature of distinct regulatory sequences within target gene regulatory sequences.

Relevance of the MAPK-Smad interactions to carcinogenesis

TGF-β plays a dual role during tumorigenesis. In early stage of carcinogenesis this cytokine displays tumor suppressor activities characterized by its antiproliferative activity, its ability to induce apoptosis and to promote genomic stability, while in advanced stages of tumor development, TGF-β acts as a promoter of tumor and metastasis, stimulating the epithelial to mesenchymal transition (EMT), matrix metalloproteinase (MMP) expression and angiogenesis, and inhibiting immunosurveillance (reviewed in (Akhurst & Balmain, 1999; de Caestecker et al., 2000; Derynck et al., 2001; Gold, 1999; Siegel & Massague, 2003). There is a particularly complicated and intimate interrelationship between the TGF-β system and

Ras/MAPK pathways in tumorigenesis.

Responses that are directly proportional to the level of Smad activity in the nucleus may be attenuated by the opposing effects of Ras signaling, as is the case with the antiproliferative response to TGF-β in epithelial cells (Calonge & Massagué, 1999; Oft et al., 1998; Oft et al., 1996). Ras-Smad antagonism may for example occur at the level of Smad nuclear accumulation (Kretzschmar et al., 1999), but other mechanisms such as opposite regulation of cyclin-dependent kinases (Cdks) during the G1 phase of the division cycle may also contribute to attenutate TGF-β tumor suppressive activities (Hannon & Beach, 1994; Reynisdottir et al., 1995).

Nevertheless, recent data suggest that aberrant activation of MAPK pathways may play an important role in diverting the TGF-β response towards a pro-oncogenic outcome, and that TGF-β and activated Ras may cooperate to promote invasive, metastatic disease. For example, in the presence of oncogenic Ha-Ras or Ki-Ras, the growth-inhibitory response of human prostate and colon cancer cells to TGF-β is converted to a Smad-independent mitogenic response. In kidney epithelial cells, activation of Raf confers protection against TGF-β-induced apoptosis while enhancing its proinvasive effects (Lehmann et al., 2000), and induction of EMT in breast tumor cells is dependent on the presence both of activated Ras and of a functional TGF-β autocrine loop that is enhanced by Ras (Lehmann et al., 2000; Xie et al., 2004). Gene arrays data obtained from human keratinocytes induced by TGF-β to undergo EMT has provided the first insights into ERK-dependent gene targets with roles in cell–matrix interactions and cell motility (Xie et al., 2003).

The interaction between TGF-β and Ras signaling can also be cooperative, resulting in outcomes that neither pathway would achieve on its own. In fact, several studies indicate that aberrant activation of MAPKs may divert the “normal“ TGF-β cytostatic response toward a pro-oncogenic outcome. Oncogenic Ras in mammary epithelial cells not only attenuates

Smad-mediated antiproliferative responses but also endows these cells with the ability to respond to TGF-β with transdifferentiation into a highly invasive and metastatic phenotype (Oft et al., 1998). Breast cancer cells with a hyperactive Ras pathway (owing to EGF receptor gene amplification) respond to TGF-β with an increased ability to metastasize to bone ((Yin et al., 1999), see details below). Thus, the activity of oncogenic Ras with regard to TGF-β signaling does not simply consist in blocking Smad signaling, but to reprogram epithelial cell response to TGF-β.

Induction of epithelial-mesenchymal transition (EMT), which marks the acquisition of an aggressive phenotype in certain cancers, has been shown to require cooperation between Ras/MAPK and TGF-β/Smad cascades (Derynck et al., 2001). In studies designed to identify the mechanisms underlying EMT, it has been shown that treatment of Ha-Ras-transformed mouse mammary epithelial cells, EpRas, with TGF-β results in loss of expression of E-cadherin, and this effect of TGF-β is blocked by inhibition of Ras activity (Janda et al., 2002; Oft et al., 2002). Also, by means of mutations in TβRI that selectively disable Smad binding and activation but not signaling through the MAPK pathways, it has been shown that both Smad and MAPK signaling are required for EMT (Itoh et al., 2003; Yu et al., 2002).

TGF-β-regulated apoptosis is cell type and context dependent. Indeed TGF-β provides signals for both cell survival and apoptosis (Sanchez-Capelo, 2005; Siegel & Massague, 2003). Activation of p38 and JNK MAPK contributes in the TGF-β induced apoptosis in numerous cell types, such as in prostate cancer cells, murine myeloid cell line M1 and the human hepatoma cell line Hep3B (Edlund et al., 2003; Sanchez-Capelo, 2005). It has been proposed that delayed p38 activation by TGF-β, rather than rapid Smad-independent p38 activation, participates in the induction of apoptosis by TGF-β, as seen in AML12 murine hepatocytes (Yoo et al., 2003). Nevertheless, TGF-β can also rescue several cell type from serum

withdrawal –induced apoptosis. In the latter case, activation of c-Jun may contribute to this rescue (Sanchez-Capelo, 2005).

In numerous tumors, acquisition of matrix metalloproteinase (MMP) activity is associated with increased migration and invasiveness of cancer cells. Studies have shown an involvement of p38 kinase activity in TGF-β induced several MMP biosynthesis in fibroblasts, breast epithelial cells or in transformed keratinocytes (Johansson et al., 2000; Kim et al., 2004; Kim et al., 2005; Ravanti et al., 1999).

Recent studies suggest that TGF-β plays a specific role in directing metastatic cells to particular organ sites such as bone, which is a common site of metastatic foci of breast and prostate cancer, a phenomenon that may require cooperation of Smad and MAPKs. Elegant *in vivo* studies by Guise and collaborators, using a breast cancer cell line that metastasizes to the bone, demonstrated that expression of a constitutively active TGF-β type I receptor enhances the expression of PTH-related protein (PTHrP) by tumor cells. PTHrP in turn recruits bone-resorbing osteoclasts, thus increasing osteolytic bone metastases, and decreasing survival. On the other hand, expression of a dominant negative TGF-β type II receptor had the opposite effects, reducing tumor metastases (Yin et al., 1999). The authors further identified that both TGF-β/Smad and p38 signaling pathways cooperate to induce the expression of PTHrP and promote metastasis of breast cancer cells to bone (Kakonen et al., 2002). Together these data point to tumor cell autonomous oncogenic effects of TGF-β and its gene targets on metastases.

MAP kinase activation can also contribute to the TGF-β metastasis promotion by stimulating migration of tumor cells (Dumont et al., 2003). Furthermore, it has been shown that expression of a mutant TGF-β type I receptor unable to bind R-Smad but maintaining kinase activity enhances tumorigenesis but suppresses metastasis of MCF10A-derived cell lines

(Tian et al., 2004; Tian et al., 2003). Clearly emphasizing the bifunctional role of TGF-β in carcinogenesis.

Conclusions

Although experimental carcinogenesis has proven that targeting TGF-β is likely to be interesting to treat human malignant tumors, much remains to be understood before one can precisely unravel the bi-functional tumor suppressor/pro-oncogenic role of TGF-β, and propose adequate therapeutic options aimed at interfering with TGF-β signals. The fine line drawn between mechanisms involving TGF-β signaling that are either deleterious or beneficial in the context of tumor progression (cell-type specificity, organ context, degree of progression of tumor before treatment, etc) and the complexity of the interactions with other signaling cascades make it extremely difficult to identify the proper context in which inhibition of TGF-β signaling will be really advantageous to the patient, i.e. restore TGF-β tumor suppressive functions.

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Legends to figures

Figure 1

MAPK network

Transduction of the signal from the plasma membrane to the nucleus occurs through the sequential activation of MAPKKKs, MAPKKs, MAPKs and their direct nuclear targets, kinases (italics) and transcription factors. For further details, refer to the corresponding text within the manuscript.

Figure 2

Activation of Smad and MAP kinases by TGF-β and their interactions

Upon TGF-β ligation toTβRII, the latter phosphorylates and activates TβRI, which in turn phosphorylates the R-Smads, Smad2/3. Activated R-Smads bind Smad4 and translocate to the nucleus to act as transcription factors, controlled by a balance between transcriptional co-activators (co-A) or co-repressors (co-R). The three principal MAPKs can be activated by TGF-β in Smad-dependent or -independent ways (see text for further details). MAPKs, following activation by TGF-β or others stimuli such as growth factors or pro-inflammatory cytokines, can regulate Smad activation by a direct phosphorylation or through their downstream effector molecules. An example is shown, whereby activated Jun and ATF-2 modulate Smad transcriptional activity through direct physical contacts or by altering the balance between transcriptional co-activators or co-repressors. : phosphorylation.