TGF- β , Bone Morphogenetic Protein, and Activin Signaling and the Tumor Microenvironment

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The cellular and noncellular components surrounding the tumor cells influence many aspects of tumor progression. Transforming growth factor β (TGF- β), bone morphogenetic proteins (BMPs), and activins have been shown to regulate the phenotype and functions of the microenvironment and are attractive targets to attenuate protumorigenic microenvironmental changes. Given the pleiotropic nature of the cytokines involved, a full understanding of their effects on numerous cell types in many contexts is necessary for proper clinical intervention. In this review, we will explore the various effects of TGF- β , BMP, and activin signaling on stromal phenotypes known to associate with cancer progression. We will summarize these findings in the context of their tumor suppressive or promoting effects, as well as the molecular changes that these cytokines induce to influence stromal phenotypes.

As neoplasias progress to invasion and metastasis, specific microenvironmental changes are intricately involved in driving the process. In breast cancer, alterations in stromal gene expression are associated with progression from ductal carcinoma in situ (DCIS) to invasive ductal carcinoma (IDC) stages of cancer and correlate with poor patient prognosis (Ma et al. 2009). These changes are determined by alterations in infiltration and functions of cells that are found in the tumor microenvironment and ultimately result in phenotypic variations, which can either impede or promote epithelial cell malignancy. Phenotypic microenvironmental changes associated with enhanced tumor progression include desmoplasia, angiogenesis, inflammation, and suppression of antitumorigenic adap-

tive immune cell responses (Hanahan and Coussens 2012). Each of these processes can promote continued tumor growth. Mediated by the overactivation of resident and infiltrating fibroblasts, desmoplasia is the excessive deposition of extracellular matrix (ECM) proteins, which involves not only the production of ECM proteins but also the posttranslational modifications of these proteins necessary for stable ECM deposition (Kalluri and Zeisberg 2006). Such changes provide a scaffold for the infiltration of other cells and a substrate for tumor cell migration. When a tumor lacks necessary nutrient availability to maintain homeostasis, angiogenesis is induced to promote increased nutrient availability and facilitate continued tumor growth (Bergers and Benja-

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Editors: Rik Derynck and Kohei Miyazono

Additional Perspectives on The Biology of the TGF- β Family available at www.cshperspectives.org

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Cite this article as *Cold Spring Harb Perspect Biol* 2017;9:a022285

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min 2003). This process involves the stimulation of endothelial cell proliferation and cell-cell junction formation to adequately disseminate nutrients to tumor cells (Hida et al. 2008). Inflammation is a physiologic response to repair stressed or wounded tissue and is characterized by the influx of innate immune cells and subsequent release of growth factors and other proteins that promote tumor cell growth and migration and facilitate further alterations in the tumor microenvironment (Vesely et al. 2011). Of particular note is that tumors shift the phenotype of inflammatory cells toward a protumorigenic state rather than acting in an antitumorigenic fashion. The antitumoral immune responses attempt to clear the aberrant tissue, and tumors therefore must overcome the innate immune system to progress. To do this, tumors inhibit functions of the cellular mediators of the adaptive immune responses to allow continued malignant growth (Hanahan and Weinberg 2000).

Although the above processes have been identified and explored in the context of tumor progression, there remains a relatively poor understanding of the factors that drive these characteristics. Transforming growth factor β (TGF- β) signaling is an essential component of epithelial induction of stromal activation, and provides the molecular signals that induce the stromal cells to elicit these phenotypic responses during tumor progression (Pickup et al. 2013a). Overexpression of TGF- β ligands in tumor tissue is associated with poor patient prognosis in prostate cancer (Wikström et al. 1998). TGF- β signaling has a complex role in directing tumor progression with the prevailing view that active TGF- β signaling in the tumor epithelium suppresses tumorigenesis early, through inhibition of cellular proliferation, but promotes tumor progression through induction of an epithelial-to-mesenchymal transition (EMT) and increased tumor cell migration and invasion (Massagué 2008). However, it is interesting to note that decreased expression of TGF- β signaling mediators is associated with poor prognosis in numerous cancer types (Woodford-Richens et al. 2001; Pinto et al. 2003; Paiva et al. 2012; Owens et al. 2014). This could be caused by

increased availability of ligands in tumor tissue to induce changes not only in the tumor epithelium but also in the stromal cells of the tumor microenvironment. In support of this hypothesis, a TGF- β -induced gene expression profile in numerous stromal cell populations predicts poor patient prognosis in colorectal cancer (Calon et al. 2012). This review will focus on the roles of the TGF- β family in the alterations to the tumor microenvironment associated with tumor progression, with a focus on how different signaling ligands and receptors elicit similar and different phenotypic responses.

EPITHELIAL-STROMAL INTERACTIONS

The induction of stromal alterations during tumor progression is driven by numerous cell types, particularly by the tumor cells themselves. Distinct oncogenic mutations and signaling changes in response to extracellular cues from the microenvironment produce variable phenotypic changes as tumors progress. Along with its many tumor cell-intrinsic functions, TGF- β signaling in tumor cells also induces significant changes in the tumor microenvironment through alterations in the expression of various proteins, including secreted factors (Table 1) (Pickup et al. 2013a). In transgenic mice with constitutively active TGF- β signaling because of ligand overexpression or mutation of the type I TGF- β receptor T β RI/ALK-5 receptor, epithelial cells direct the phenotypic outcome of significant stromal changes, such as enhanced collagen deposition and angiogenesis (Muraoka-Cook et al. 2006; Safina et al. 2007). These stromal changes are associated with increased expression of cytokines and chemokines by the epithelial cells. These secreted factors influence stromal activation through differentiation of resident cells, alteration of noncellular tissue components, as well as recruitment of various mesenchymal and lymphocytic cells. For example, signaling by T β RI/ALK-5 increases matrix metalloproteinase (MMP) expression, which is associated with increased angiogenesis in vivo (Safina et al. 2007). Active TGF- β signaling in epithelial cells promotes an angiogenic phenotype through the

Table 1. Transforming growth factor β (TGF- β)-induced genes with known roles in microenvironmental modulation

Stromal phenotype	Genes	Regulation	References
Desmoplasia	<i>ADAM19</i>	Increased	Wei et al. 2001
	<i>COL1A1</i>	Increased	Lijnen and Petrov 2002
	<i>COL5A1</i>	Increased	Roepman et al. 2005
	<i>LOXL2</i>	Increased	Barry-Hamilton et al. 2010
	<i>TAGLN</i>	Increased	Yu et al. 2013
	<i>THBS1</i>	Increased	Bein and Simons 2000
	<i>ANGPTL4</i>	Increased	Okochi-Takada et al. 2014
Angiogenesis	<i>JAG1</i>	Increased	Steg et al. 2011
	<i>SEMA3C</i>	Increased	Ellis 2006
	<i>THBS1</i>	Increased	Simantov and Silverstein 2003
	<i>VEGFA</i>	Increased	Nakagawa et al. 2004
	<i>CEBPD</i>	Decreased	Duitman et al. 2014
Inflammation	<i>CXCL1</i>	Decreased	Bierie et al. 2009
	<i>CXCL5</i>	Decreased	Bierie et al. 2009
	<i>IL11</i>	Increased	Yashiro et al. 2006
	<i>CD73</i>	Increased	Beavis et al. 2012
	<i>SDF1A</i>	Increased	Karin 2010

TGF- β signaling drives tumor cell gene changes to influence stromal phenotypes. Significantly altered gene expression changes among three publicly available data sets obtained from TGF- β -treated carcinoma cells, and gene expression profiles from *TGFBR2*^{-/-} cancer cells (GSE23952, GSE17708, GSE10393; Bierie et al. 2009) were compared to derive a consensus of genes similarly altered in cancer cells on TGF- β 1 stimulation. These significantly altered genes were segregated into groups associated with the promotion of various stromal phenotypes.

increased expression and secretion of angiogenic factors, such as vascular endothelial growth factor (VEGF) and thrombospondin 1 (Wang et al. 2008a; Sartor et al. 2010). TGF- β signaling is also associated with a desmoplastic response through induction of ECM remodeling genes, such as MMPs and lysyl oxidase-like 4 (LOXL4), as well as ECM components themselves (Wang et al. 2008a). There are also emerging mechanisms by which TGF- β signaling in the tumor epithelium can alter immune responses through suppression of microRNA (miRNA) expression. Such data represent an exciting aspect of TGF- β signaling directing tumor-stromal interactions and tumor progression through altered miRNA biosynthesis. TGF- β upregulates the expression of CCL22, a chemokine shown to promote trafficking and activation of T lymphocytes to sites of inflammation, in hepatocellular carcinoma cells through the suppression of miR-34a expression, which correlates with increased regulatory T-cell (Treg) presence in the tissue (Yang et al. 2012). These activating effects are counteracted through inhibition of chemokine expres-

sion resulting in a decreased inflammatory response. Studies involving either activation of the TGF- β signaling pathway or targeted inactivation of TGF- β signaling mediators have shown that TGF- β signaling inhibits the secretion of immunomodulatory factors. Gene expression and functional analyses have established CXCL1 and CXCL5 as critical chemokines, whose expression is inhibited by TGF- β signaling (Bierie et al. 2009). Deletion of TGF- β signaling in mouse mammary epithelial cells by conditional inactivation of the *Tgfb2* gene, which encodes the type II TGF- β receptor (T β R2), results in increased infiltration of myeloid cells, in particular CD11b⁺ Gr1⁺ myeloid-derived suppressor cells (MDSCs) (Yang et al. 2008). Ultimately, increased MDSC infiltration suppresses T-cell activity allowing for enhanced tumor progression through immune evasion. Interestingly, conditional inactivation of *Tgfb2* as well as expression of a dominant-negative T β R2 in mammary epithelial cells both result in stromal changes similar to those arising from activation of TGF- β signaling and leading

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to more angiogenesis and collagen deposition (Amendt et al. 1998; Muraoka-Cook et al. 2006; Bierie et al. 2008). However, these stromal changes could be because of interactions between stromal cells and an altered cytokine environment elicited by the influx of myeloid cells.

Similar to TGF- β , bone morphogenetic protein (BMP) signaling in the tumor cells elicits epithelial as well as stromal changes. Clinical evidence in support of the stromal effects of BMP is apparent in juvenile polyposis, a disease characterized by germline mutations in the BMP type I receptor BMPRIA/ALK-3. Juvenile polyposis presents with dense stroma and increased infiltration of immune cells (Hardwick et al. 2008). However, the expression of BMP ligands is increased in patients with breast cancer and the expression of the BMP receptors BMPRIA and BMPRII correlates with a poor prognosis, indicating that BMP signaling in breast cancer cells remains intact and active during metastatic progression (Owens et al. 2014). With these data indicating that BMP signaling can act as a tumor promoter in breast cancer, it is interesting to note that, similar to TGF- β , the attenuation of BMPRII signaling in mammary carcinoma cells enhances metastasis (Owens et al. 2012). Such data underlie a key distinction between the cell autonomous and microenvironmental effects of the TGF- β family signaling to elicit different results during tumor progression. As with TGF- β signaling, a major change in the primary tumor with impaired BMP signaling and increased metastasis is a large influx of immune cells, notably F4/80⁺ macrophages and Gr-1⁺ MDSCs. This increase in immune infiltration is the result of a significantly altered chemokine expression profile of the mammary tumor cells with impaired BMP signaling (Owens et al. 2012). The role of activin signaling may be similar to that of TGF- β in that activin signaling mediators are largely inactivated in breast cancer, and downregulation of *ACVR2A* and *ACVR2B* encoding activin type II receptors correlates with a worse relapse-free survival (Owens et al. 2014). Further, studies of activin signaling in breast, liver, prostate, and pancreatic cancer cells show a growth inhibitory effect (Sozzani and Musso 2011). Unlike TGF- β and

BMP signaling there is little evidence that down-regulation of activin signaling results in significant stromal changes. However, further investigations are needed to reveal the role of activin signaling in the tumor epithelium in regulating the tumor microenvironment.

DESMOPLASIA

The excessive deposition of ECM is commonly referred to as desmoplasia and is associated with many disease states. In the context of tumor initiation, desmoplasia is associated with an increased tumor occurrence, as well as increased progression of the disease. In cancer, a noted feature in the progression of the disease is the accumulation of stromal cells including fibroblasts expressing α -smooth muscle actin (α -SMA), a microfilament protein associated with a mesenchymal morphology (Kalluri and Zeisberg 2006). These fibroblasts result from activation of resident or infiltrating fibroblasts, which then perform numerous functions in the tumor microenvironment. One of the most prominent features of fibroblasts in normal physiological function, as well as in cancer progression, is the deposition and remodeling of ECM proteins (Place et al. 2011). Increased mammographic density predicts increased development of breast cancer and promotes progression of the disease (Boyd et al. 2007). This mammographic density results from an increased deposition of collagen in the mammary tissue. As mentioned, microarray analysis of cancer-associated stroma has significantly impacted our understanding of the molecular changes that occur in the microenvironment during tumor progression. Interestingly, induction of ECM genes, most notably of those encoding collagens, in the stroma is associated with poor patient prognosis (Bergamaschi et al. 2008; Finak et al. 2008; Roman-Perez et al. 2012). Mouse models expressing mutations in the MMP targeting region of collagen corroborate these clinical findings. These mice present with tumors that have increased collagen deposition and ultimately lead to enhanced tumor cell metastasis (Provenzano et al. 2008). Not only the deposition of collagen influences tu-

mor progression but also the posttranslational modifications are necessary for proper incorporation of ECM proteins into the ECM (Lu et al. 2012). Expression of collagen-cross-linking enzymes has been shown to promote tumor progression, specifically tumor cell metastasis, in mouse models of cancer (Paszek et al. 2005; Erler et al. 2006; Levental et al. 2009). The concept of collagen architecture and remodeling playing a role in tumor progression has been taken one step further through the use of second harmonic imaging, which has allowed collagen structure to be segregated into three phenotypic signatures based on collagen alignment and orientation, known as the tumor-associated collagen signature (TACS) (Provenzano et al. 2006). Specific changes outlined in these signatures are associated with invasion of mammary carcinoma cells. Given the importance of TGF- β signaling in activating stromal fibroblasts, this pathway acts as a primary mediator of the various desmoplastic changes promoting tumor progression (Bierie and Moses 2006).

A TGF- β -induced expression profile in fibroblasts is the definition of an activated fibroblast and is critical in eliciting a desmoplastic response in the tumor microenvironment (Border and Noble 1994). Treatment of fibroblasts with TGF- β induces the expression of α -SMA as well as numerous ECM proteins, including type I collagen and fibronectin (Desmouliere et al. 1993). Consistent with these results, loss of TGF- β responsiveness in fibroblasts impairs the ability of the cell to produce ECM proteins and remodel the ECM (Martinez-Ferrer et al. 2010). Comparing these roles of TGF- β signaling in fibroblasts to tumor progression, we observe expected results. Increased expression of α -SMA is associated with high levels of TGF- β in squamous cell and other carcinomas (Kojc et al. 2005). Overexpression of TGF- β 1 in orthotopically injected human stroma results in the progression of normal breast epithelial cells to a hyperplastic phenotype (Kuperwasser et al. 2004). TGF- β expression is in part controlled by the transcription factor heat shock factor 1 (HSF1), and this induction promotes the progression of breast cancer in a TGF- β -dependent manner (Scherz-Shouval et al. 2014). Addition-

ally, the presence of TGF- β ligand is associated with increased deposition of multiple ECM proteins, including fibronectin and tenascin, in invasive breast and cervical carcinomas (Walker et al. 1994; Hazelbag et al. 2002). The culmination of these TGF- β actions results in a gene signature that drives specific gene expression changes in stromal fibroblasts (Calon et al. 2012). These changes have been identified in carcinomas and used to generate an ECM gene signature that is associated with poor patient prognosis (Bergamaschi et al. 2008). Interestingly, clinical and experimental data show that TGF- β signaling in fibroblasts is also tumor suppressive and that loss of TGF- β signaling components is associated with tumor progression and can even promote spontaneous tumor formation, particularly through the creation of an inflammatory microenvironment (Bhowmick et al. 2004; Achyut et al. 2013; Busch et al. 2015). Immune regulation represents another means by which fibroblasts can influence tumor progression in addition to their ability to cause desmoplastic stromal changes. Fibroblasts not only play a role in orchestrating the final stages of wound healing but act as professional secretory cells directing the appropriate cellular chemokines and cytokines for immune cell recruitment and elimination.

Other TGF- β family members have similar roles in fibroblast function; however, the substantial complexity and limited amount of data make their role in the desmoplastic phenotype of cancer poorly defined. BMPs are a prime example of complexity with various ligands associated with differing functional responses from fibroblasts. For example, BMP-7 has been shown to inhibit fibroblast activation in corneal fibroblasts, whereas BMP-4 induces the expression of the ECM proteins collagen and laminin in mammary-derived fibroblasts (Izumi et al. 2006; Owens et al. 2013). Additionally, BMP functions to suppress chemokine expression. Loss of BMP signaling in fibroblasts increases expression of CCL5/RANTES and granulocyte-colony-stimulating factor (G-CSF/CSF3) to promote infiltration of myeloid cells into the tumor microenvironment (Pickup et al. 2015). Activin signaling appears to have

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activities similar to TGF- β , with treatment of fibroblasts with TGF- β inducing higher α -SMA and collagen expression, and increased α -SMA immunostaining being associated with increased collagen deposition (Fumagalli et al. 2007; Mukhopadhyay et al. 2007). Tumor initiation and progression from DCIS is associated with activin A-induced fibroblast activation and ECM synthesis. Additionally, high levels of activin A associate with increased desmoplasia surrounding the foci of epithelial neoplasia (Fordyce et al. 2012; Dumont et al. 2013). The phenotypic changes induced by TGF- β family stimulation of stromal fibroblasts also promotes tumor progression through inhibition of tumor cell apoptosis and induction of tumor cell migration (Jinka et al. 2012).

Although production of ECM proteins is a critical hallmark of a desmoplastic tumor microenvironment, posttranslational remodeling is also an essential component in the development of a tumor-promoting desmoplastic microenvironment. Identified as an important part of the wound healing process, an essential function of fibroblasts is their ability to remodel and contract a collagen matrix (Kalluri and Zeisberg 2006). The aforementioned tumor-associated collagen signature depends on active production of enzymes involved in the modification of ECM components and the cells' ability to interact with the modified collagen (Provenzano et al. 2006). Processes such as maturation or degradation of collagen fibers play essential roles in determining tumor progression. Secreted protein, acidic, cysteine-rich (SPARC) and lysyl oxidase (LOX) are examples of matrix modifying enzymes that stabilize ECM components with well-established roles in the progression of the disease (Reed et al. 1994; Payne et al. 2007). TGF- β treatment of fibroblasts is associated with an increase in the expression of both of these enzymes (Boak et al. 1994; Reed et al. 1994). For example, SPARC is found in the ECM gene signature associated with TGF- β signaling and poor patient prognosis (Calon et al. 2012). Additionally, TGF- β induces LOX expression in both lung and mammary fibroblasts to promote tumor metastasis (Boak et al. 1994; Pickup et al. 2013b). The ultimate culmination

of the induced expression of these genes is the stiffening of the ECM in the tumors, which results in enhanced EMT and increased invasion and metastasis of tumor cells (Fig. 1) (Leight et al. 2012; Baker et al. 2013).

Other TGF- β family ligands have not been associated with this type of ECM remodeling, but are associated with increased expression of genes involved in ECM degradation. The most prominent protein families associated with ECM degradation are the MMPs and the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motif) proteins. TGF- β itself is an essential driver of the expression of both of these families of proteins, most notably MMP2 and MMP9 (Chambers et al. 2003; Dang et al. 2004). The evidence for involvement of BMPs as well as activins in the induction of ECM degradation enzymes is similar to that with TGF- β inducing the expression of numerous MMPs as well as ADAMTS proteins (Yamashita et al. 2004; Fessing et al. 2010; Owens et al. 2013). It is interesting to note that increased expression of either ECM stabilization or degradation enzymes is associated with enhanced tumor progression and poor patient prognosis (Murray et al. 1996; Erler et al. 2006). However, the effects of these stabilizing and degrading factors in the context of TGF- β family signaling, particularly BMP and activin signaling, on the composition and architecture of the tumor ECM remain to be elucidated.

ANGIOGENESIS

As carcinomas grow in size, more nutrients are required to maintain an environment that will sustain cell survival and growth. At first tumors are small enough to survive off the nutrients provided by the existing vasculature. However, as they reach a size of approximately one centimeter, the limited nutrient availability is unable to sustain continued tumor growth, and a hypoxic environment develops (Bergers and Benjamin 2003). Hypoxia, a lack of oxygen availability, influences tumor progression through induction of EMT and tumor cell invasion (Zhou et al. 2006). Hypoxic cues also drive the expression of angiogenic factors to promote

Figure 1. Transforming growth factor- β (TGF- β) family signaling promotes fibroblast activation and function to drive a desmoplastic stromal response. TGF- β , bone morphogenetic protein (BMP), and activin signaling drive the activation of fibroblasts to promote stromal changes, including increased extracellular matrix (ECM) production and remodeling. Overall, these changes are associated with highly invasive tumors and promote epithelial changes including increased proliferation, epithelial-to-mesenchymal transition (EMT), and invasion as well as angiogenesis leading to increased metastasis.

the sprouting and spreading of existing vasculature, known as the angiogenic switch. The angiogenic process involves two essential steps, induction of endothelial cell proliferation and migration and maturation of vessels through the establishment of endothelial cell–cell contacts and pericyte coverage. A primary driver in the expansion of the vascular network is the secreted VEGF, which promotes endothelial cell proliferation and maturation of expanded blood vessels (Franses et al. 2011). The central role of VEGF in angiogenesis provides the basis for therapeutic intervention in tumor patients using the drug bevacizumab (Ferrara et al. 2004). This humanized anti-VEGF antibody is used as an adjuvant therapeutic for colorectal and lung cancer as well as glioblastoma.

Targeted inactivation of the expression of Smad4, a central mediator in Smad signaling, specifically in endothelial cells results in a failure to form tube structures in vitro and in embryonic lethality in vivo (Lan et al. 2007). Given the

pleiotropic nature of these cytokines, numerous members of the TGF- β family play an important role in the angiogenic process in tumor progression. Breaking down the TGF- β family into specific signaling ligands, TGF- β significantly controls tumor angiogenesis. In a physiological context, activation of signaling and depletion of signaling components in the TGF- β pathway both result in significantly altered vascular phenotypes (Oshima et al. 1996; Lebrin et al. 2005; Allinson et al. 2012). Additionally, under hypoxic conditions, TGF- β and BMP signaling are increased and help resolve the hypoxic environment (Falanga et al. 1991; Akman et al. 2001; Maegdefrau et al. 2009).

In the context of tumor development, experimental models as well as human cancers that express high levels of TGF- β are associated with increased expression of proangiogenic factors and an overall increase in blood vessel formation (Pardali and ten Dijke 2009). TGF- β inhibition reverses this angiogenic phenotype

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(Mazzocca et al. 2009; Zhang et al. 2011). This induction occurs through both direct and indirect effects of TGF- β on endothelial cells. Directly, TGF- β has been shown to stimulate endothelial cell migration and maturation (Yang and Moses 1990). These phenotypic responses are attributed to effects of TGF- β signaling on miRNA processing and production. Treatment of endothelial cells as well as vascular smooth muscle cells with TGF- β induces a contractile phenotype. This contractile phenotype is in part caused by effects of TGF- β signaling on the expression and processing of miR-21 and miR-29a, resulting in an increase in their activities (Davis et al. 2008). The overall result of TGF- β signaling in endothelial cells leads to enhanced expression of miRNAs and induction of a contractile phenotype, and an enhancement of angiogenesis. Indirectly, TGF- β has been shown to induce the expression of other angiogenic factors, particularly VEGF, in tumor and stromal cells (Pepper 1997). However, TGF- β signaling has also been shown to inhibit these proangiogenic phenotypes in endothelial cells. These differential effects are controlled by the TGF- β coreceptors betaglycan (also known as TGF- β type III receptor) and endoglin, which modulate an endothelial cell's response to TGF- β between inducing either Smad2 and Smad3 or Smad1 and Smad5 activation and nuclear translocation, resulting in differential transcriptional activity (Pardali et al. 2010). In response to TGF- β stimulation, the coreceptor betaglycan promotes Smad2 and Smad3 phosphorylation by the type I TGF- β receptor T β RI/ALK-5 to inhibit endothelial cell proliferation and migration. However, the coreceptor endoglin alters this response by inducing phosphorylation by another type I receptor, ALK-1, which in turn phosphorylates Smad1 and Smad5 to induce endothelial cell proliferation and migration. Although induction of Smad2 and Smad3 phosphorylation in response to TGF- β inhibits endothelial cell proliferation and migration, induction of Smad1 and Smad5 phosphorylation, in response to TGF- β in the presence of endoglin, promotes the proangiogenic phenotypes. BMPs, which activate Smad1 and Smad5, have also been shown to promote angiogenesis dur-

ing tumor progression (Raida et al. 2005; Liu et al. 2007).

Acting in a similar fashion as TGF- β , BMPs, specifically BMP-4, -7, and -9, stimulate endothelial cell proliferation and tube formation in cell culture (Suzuki et al. 2008). This endothelial cell response to BMPs may require cooperation with VEGF signaling (David et al. 2007; Bieniasz et al. 2009). Evidence for a role of activin signaling in endothelial cell function suggests an inhibitory role of this ligand in angiogenesis. Several studies show that stimulation of endothelial cells with activin inhibits cell proliferation (McCarthy and Bicknell 1993; Panopoulou et al. 2005). As with TGF- β stimulation, activin acts through Smad2 and Smad3 supporting the notion that the differential response of endothelial cells to this family of ligands is primarily defined by the internal signaling components.

Proliferation and migration are only the first steps in the angiogenic process. The formation of a complete and functional blood vessel involves the inhibition of proliferation and migration, and the formation of cell-cell junctions and induction of pericyte coverage. As mentioned, the effects of TGF- β family signaling control this resolution of angiogenesis, and correlate with the overall effects of active TGF- β signaling on tumor vessel formation. The large majority of experimental models of tumor progression and human patients correlate high levels of TGF- β expression with increased vasculogenesis, when compared with control tumors that have lower TGF- β levels (Tuxhorn et al. 2002; Mazzocca et al. 2009; Hawinkels et al. 2010). However, as TGF- β has dual effects on endothelial cell function, it is not surprising that there are reports of TGF- β inhibiting tumor angiogenesis (Geng et al. 2013). It has been shown that inhibition of TGF- β signaling causes normalization of tumor vasculature with improved pericyte coverage of blood vessels and perfusion (Fig. 2) (Min et al. 2012).

BMP and activin signaling have also been implicated in angiogenesis. Implantation of tumor cells expressing high levels of BMPs was shown to promote angiogenesis in the resulting tumors, and increased expression of the BMP inhibitor, noggin, suppresses angiogenesis

Figure 2. Inhibition of transforming growth factor β (TGF- β) proangiogenic functions normalizes aberrant vessel formation associated with tumor progression. As tumors progress, endothelial cell proliferation and migration are required to provide nutrients to the increased demand in a growing tumor. Signaling by members of the TGF- β family has been shown to drive this process, thus introducing the potential for inhibition of this signaling axis to stunt blood vessel formation and thus slow tumor growth, spread, and potentially response to chemotherapeutics.

(Langenfeld and Langenfeld 2004). In addition, BMP expression positively correlates with VEGF expression in lung cancer patients (Bieniasz et al. 2009). Furthermore, neuroblastomas overexpressing activin A show decreased tumor growth that correlates with fewer capillaries (Panopoulou et al. 2005). Research on BMP and activin signaling in tumors has not yet established whether their angiogenic effects promote pericyte coverage and proper vessel perfusion. However, genetic ablation of Smad5, a cell-signaling mediator of the BMP response, results in numerous developmental abnormalities including improper formation of an intact vascular system (Chang et al. 1999). Additional developmental biology research shows that inhibition of BMP signaling and other TGF- β family signaling reduces angiogenesis in developing embryos, and suggests that this may also be true in tumors (Guillot et al. 2012). Further studies are needed to strengthen these correlations. Further evidence suggests that coreceptors mediate the angiogenic response by induc-

ing differential Smad phosphorylation. Thus, an additional layer of context is important to understand the dual response of endothelial cells to TGF- β signaling. Such data would be imperative to understand the role TGF- β , BMP and activin signaling in angiogenesis, and may provide new avenues for intervention into this tumor promoting process (Pardali et al. 2010). Although TGF- β is associated with enhanced angiogenesis in numerous studies, it would be interesting to determine whether this was through direct induction of an angiogenic response or through altering the signaling response of the cells to other TGF- β family ligands such as BMPs (Wiley et al. 2011).

INFLAMMATION AND THE IMMUNE RESPONSE

The TGF- β family is a critical regulator of immune responses in normal homeostasis as well as disease states. These effects are induced by alterations of immune cell recruitment, differ-

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entiation, and function. Targeted inactivation of Smad4 expression, resulting in impaired Smad signaling by the TGF- β family, in bone marrow cells results in a failure to develop normally functioning cells differentiated from hematopoietic stem cells (Karlsson et al. 2007). Mice with germline deficiency in the expression of TGF- β 1 show a marked inflammatory response mediated by altered lymphocyte cell function to promote innate cell activation (Kulkarni et al. 1993; Diebold et al. 1995). Conversely, systemic inactivation of T β RII expression leads to overactivation of the adaptive immune system associated with autoimmune disease (Oshima et al. 1996). Expression of a dominant-negative type II TGF- β receptor, T β RII, in CD8⁺ T cells results in a significant expansion of this cell population caused by a lack of the antiproliferative effects of TGF- β on the T cells (Lucas et al. 2000). Genetic ablation of Smad4 in CD4⁺ cell populations in vivo promotes the development of spontaneous gastrointestinal carcinomas (Kim et al. 2006).

In tumor progression, various cell populations of the immune system can have either pro- or antitumorigenic effects, depending on the context of cell maturation and function. These functions can largely be broken down by the adaptive and innate immune cell populations of the immune system. The innate immune system recognizes foreign antigen and promotes a general inflammatory microenvironment, whereas the adaptive immune system recognizes specific foreign antigens and kills off infected cells to prevent spread of foreign entities (Vesely et al. 2011). The TGF- β family significantly alters both innate and adaptive immune cell phenotypic responses with the outcome being largely protumorigenic through numerous mechanisms, including differentiation of T cells into Th17 and Treg cells, as well as macrophages into M2 identified cells.

ADAPTIVE IMMUNE RESPONSE

Despite being derived from “self-tissue,” tumor development can be associated with mutations that result in the production of abnormal proteins. Most cells can display antigens at their cell

surface, but dendritic cells in particular process proteins and display antigenic peptides in the context of presentation of major histocompatibility complex (MHC) proteins at their cell surface for potential recognition by T cells (Palucka and Banchereau 2012). This process represents a significant hurdle that the tumors must overcome lest they elicit an antitumorigenic adaptive immune response (Hanahan and Weinberg 2000). Although numerous factors can be influenced by a tumor to alter this adaptive immune response, and could induce T-cell-mediated cytotoxic action, this section will focus only on the adaptive immune system. TGF- β is an essential factor used by the tumors to suppress tumor-directed T-cell activation (Li and Flavell 2008). This function is achieved through action at several steps, with one of the first being the suppression of antigen presentation by MHC proteins on antigen presenting cells.

Acting to prevent the potential presentation of tumor antigens to the adaptive immune system, TGF- β suppresses not only the infiltration of antigen presenting dendritic cells into tumors, but also the ability of these cells to present antigens to T cells (Byrne et al. 2008; Novitskiy et al. 2012). Thus, as expected, alleviating this suppressive effect, through targeted inactivation of T β RII expression in dendritic cells, or suppression of TGF- β signaling by administration of the TGF- β inhibitory molecule SB-431542, induces antigen presentation as well as T-cell activation (Tanaka et al. 2010). Given that TGF- β promotes an immune suppressed microenvironment, targeted inhibition of TGF- β signaling in immune cells could lead to enhanced function of antigen presenting cells (APCs) and cytotoxic T lymphocytes (CTLs). It should be noted that the SB-431542 compound acts broadly to repress several TGF- β family type I receptors (i.e., ActRIB/ALK-4, T β RI/ALK-5, and ALK-7) (Inman et al. 2002), which may suggest similar actions of activin and TGF- β signaling in dendritic cell function.

BMP stimulation of dendritic cells promotes the activities of the cellular components of the adaptive immune system by inducing the expression of interleukin 8 (IL-8) and tumor necrosis factor α (TNF- α) (Martinez et al. 2011).

Additionally, BMP stimulation of dendritic cells increases the expression of the maturation markers programmed cell death 1 ligand 1 (PD-L1) and PD-L2, which promote T-cell and natural killer (NK) cell stimulation (Martinez et al. 2014). This once again points to similar effects of TGF- β and activin in the suppression of APC cell function, whereas BMP opposes this with stimulation of dendritic cell activity. Although these data are still preliminary and need further validation, it will be interesting to see if this could be because of differences in signaling pathways to mediate different phenotypic effects or cooperation with other signaling pathways unique to certain cell types.

An additional level of regulation of adaptive immune cell function by the TGF- β family is the expansion of a T-cell population in response to “non-self” antigens. As mentioned, systemic inactivation of the *Tgfb1* gene leads to myeloproliferative disorders (Yaswen et al. 1996). This results in the development of autoimmune disease, implying that TGF- β in the context of T-cell development during tumorigenesis suppresses the proliferation of activated T cells. Indeed, TGF- β inhibits T-cell proliferation, as is apparent from experiments with enhanced TGF- β signaling or suppression using a dominant-negative T β RII in T cells (Gorelik and Flavell 2000; Lucas et al. 2000). However, the number of T cells in the tumor microenvironment is not the only determinant of the protumorigenic function of TGF- β on T cells. TGF- β also inhibits the priming of T cells to an activated state that enables the cells to react to foreign antigens and elicit cytotoxic effects (Xu et al. 2003; Filippi et al. 2008; Oleinika et al. 2013). On identification of a “non-self” antigen, naïve T cells are primed into a Th1 cell phenotype to induce phagocytic-cell-mediated inflammation, or a Th2 cell phenotype to promote antibody production to potentiate a further immune response against the recognized antigen (Germain 2002). TGF- β suppresses the phenotypic development of both these cell types at a second level, stunting the function of T cells and its potential action against tumor cells (Gunturi et al. 2005; Bommireddy et al. 2006; Li et al. 2007; Li and Flavell 2008).

A third level of TGF- β -mediated T-cell suppression occurs in the induction of a differentiation program in T cells, which suppresses the activity of T cells. TGF- β plays an essential role in the development of Treg cells as well as Th17 cells in a Smad-dependent manner (Chen et al. 2003; Takimoto et al. 2010). Exposure of naïve T cells to TGF- β induces the expression of the Foxp3 transcription factor to prompt a cellular phenotype associated with suppression of CTL function. The primary mechanism by which CTL function is suppressed is through increased expression of TGF- β 1 (Whiteside et al. 2012). Alternatively, in an environment that is enriched in IL-6, the addition of TGF- β causes a phenotypic switch toward a Th17 cell phenotype. The inducing factors that drive Th17 cell differentiation determine whether they invoke pro- or antitumorigenic responses. TGF- β has been shown to induce a Th17 cell type, which is largely suppressive of antitumor immunity. Through the main effector, IL-17, this differentiation state results in a phenotypic outcome of suppressed T-cell activity (Mangan et al. 2006; Chalmin et al. 2012). The overall protumorigenic function of TGF- β in shifting T-cell differentiation has led to significant strides in research and clinical trials aimed at inhibiting these functions for the benefit of the patient (Akhurst and Hata 2012).

Preclinical trials with various TGF- β inhibitory small molecules and antibodies show that their therapeutic usage results in an antitumorigenic response that is generally associated with a more active adaptive immune system (Akhurst and Hata 2012). Interestingly, activin signaling cooperates with TGF- β to promote an immune suppressive environment. Specifically, the TGF- β -induced conversion of naïve T cells into Foxp3-expressing Treg cells is significantly enhanced when activin A is added (Huber et al. 2009). However, expression of a dominant-negative T β RII abrogated this response underlining the importance of TGF- β signaling in this process. Activin signaling also shows similarities to TGF- β signaling in the suppression of T-cell proliferation and differentiation (Hedger et al. 1989; Semitekolou et al. 2009; Ogawa and Funaba 2011). Treatment of naïve T cells with ac-

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tivin A decreases the induction of proliferation and activation in response to signaling by APCs (Semitekolou et al. 2009). Given the profound synergy between the induction of activin signaling and TGF- β signaling in eliciting Treg cell differentiation in T cells, it would be interesting to test this cooperative effect of TGF- β and activin stimulation in the antiproliferative and suppressed activation of T cells. Treatment of CD4⁺ T cells with dorsomorphin, an antagonist of BMP type I receptors, inhibits proliferation and Th1 differentiation (Yoshioka et al. 2012). Additionally, treatment of CD4⁺ cells with dorsomorphin prevents the induction of Th17 and Treg cell differentiation (Yoshioka et al. 2012). The significant evidence for a protumorigenic function of TGF- β family signaling in T-cell function remains one of the best appreciated aspects of TGF- β signaling with a relatively well-defined phenotypic response. Despite this, more studies are needed to provide the correct contextual evidence for disruption of these protumorigenic functions toward efficacious treatments for patients. Given the impact of the adaptive immune system on tumor progression, the TGF- β family is an attractive target for therapeutic intervention to enhance antitumor immune responses.

INNATE IMMUNE-CELL-MEDIATED INFLAMMATION

In tissue homeostasis, the innate immune response mediates removal of foreign antigens through activation of adaptive immune cell populations. Although complex in function, these cell types act in concert to induce cytotoxic cell death in foreign or abnormal tissue and clear the resulting cellular debris. The cytotoxic cell death induced by CTLs is mediated through numerous secreted factors and direct cell–cell interactions. In the cellular response, there is a notable shift in the activation of innate immune cells from a proimmunogenic phenotype (classical activation) toward a more anti-immunogenic response (alternative activation) (Biswas and Mantovani 2010). This change in activation is indicative of the system as a whole attempting to slow the adaptive immune system

and prevent tissue damage and autoimmune disease.

The natural interaction and progression of the immune response can be co-opted by tumors to prevent an antitumorigenic innate immune activation, or classical response, in favor of a protumorigenic, or alternative response. An important signaling system in determining this response is TGF- β signaling, which in many cell types has been shown to be critical in alternative activation of innate cellular components (Fig. 3) (Flavell et al. 2010). A well-established example of this is the induction of an M2 phenotype in tumor-associated macrophages. Classical activation of macrophages, induced by interferon γ (IFN- γ) treatment, is associated with promotion of adaptive immune response, particularly the conversion of T cells toward Th1 activation (Mills 2012). Overall, this type of macrophage activation would act to slow tumor progression through antigen presentation and promotion of cytotoxic T-cell function. The alternative activation of macrophages is associated with a gene expression signature that resembles promotion of tissue remodeling and suppression of the adaptive immune system (Murray and Wynn 2011). M2 macrophages secrete TGF- β and platelet-derived growth factor (PDGF) to promote stromal fibroblast activation, which in a wound-healing response would correlate with resolution of the wounding response (Qualls and Murray 2011). As mentioned earlier, abrogation of epithelial TGF- β signaling leads to recruitment of MDSCs, which inhibit antitumorigenic adaptive immune functions. TGF- β signaling in this cell type promotes tumor progression through one of the emerging functional roles of TGF- β signaling, the processing of miRNAs. Active TGF- β signaling in MDSCs results in increased expression of miR-494 to ultimately enhance the protumorigenic secretion of arginase-1 and MMPs (Liu et al. 2012).

BMP signaling has also been shown to promote macrophages into an alternative activation path through increased expression of IL-6, TNF- α , and monocyte chemoattractant protein 1 (MCP-1/CCL2), all of which are associated with an M2 phenotype in macrophages (Rocher et al. 2012). Recent studies into the use

Figure 3. Pro- or antitumorigenic immune functions are driven by transforming growth factor β (TGF- β) family signaling modulating immune cell differentiation and function. Numerous cell types in the innate immune system differentiate to a protumorigenic phenotype in response to TGF- β , bone morphogenetic protein (BMP) or activin signaling. One process through which these protumorigenic cells promote tumor progression is by shifting the adaptive immune system to a protumorigenic phenotype. The protumorigenic differentiation of adaptive immune cells is also driven in part by TGF- β family signaling. The end result is the inhibition of immune surveillance and tumor cell directed cytotoxicity and the promotion of Th2 T-cell responses and angiogenesis to promote tumor progression.

of BMP inhibitory drugs has shown that systemic inhibition of BMP signaling leads to an overall increase in immune cells, in particular F4/80 macrophages that are not M2-like but have a tumor suppressive M1-like phenotype (Owens et al. 2014). The role of activin signaling in macrophage activation is not as clear. Studies in mouse macrophages show that activin treatment results in suppression of iNOS and induction of arginase-1 expression, both of which are associated with an M2 macrophage polarization (Zhang et al. 2005; Ogawa et al. 2006). However, additional evidence indicates high-level activin signaling in association with M1 macrophage differentiation (Sierra-Filardi et al. 2011). Although these results seem contradictory, this

discrepancy may be explained by the models used in these studies. Activin signaling could promote resting macrophages toward an M1 activation phenotype, while maintaining an M2 activation state in macrophages that were already activated (Ogawa et al. 2006; Wang et al. 2008b; Zhou et al. 2009; Antsiferova and Werner 2012). As in other signaling contexts discussed, there is also the potential for these differential responses to be mediated by cooperative or competitive signaling between different ligands of the TGF- β family. Thus, it would be interesting to determine whether these different responses of macrophages to activin stimulation depend on the context of the other TGF- β family members.

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Although macrophages are the most abundant cell population of the innate immune system in the tumor microenvironment, other cell types still have a significant effect on tumor progression. Neutrophils can also have differential effects through a similar activation phenotype as the one associated with macrophages. In addition to promoting neutrophil migration, TGF- β promotes a protumorigenic differentiation program in neutrophils. In tumor-associated neutrophils (TANs) present in lung cancer, TGF- β signaling induces a gene expression program that suppresses T-cell activity and promotes myeloid cell infiltration through increased arginase and CCL5 expression (Fridlender et al. 2009). Similar studies on the functional effects of BMP and activin signaling in neutrophils have not been reported; however, neutrophils may serve as a primary source of activin A in inflammatory responses (Sideras et al. 2013). The effect of the release of this cytokine on tumor progression has yet to be established as pro- or antitumorigenic.

Dendritic cells represent another key cell population in the tumor microenvironment. TGF- β suppresses the migration and maturation of dendritic cells, thus slowing tumor-directed cytotoxic T-cell activity (Perrot et al. 2007). At an additional level, TGF- β has been shown to act on previously matured dendritic cells to promote T-cell differentiation toward a Treg cell path (Kobie et al. 2003), once again stunting T-cell activity directed toward tumor cells.

Other members of the TGF- β family do not share this inhibitory effect on dendritic cells. In fact, activin and BMP signaling both oppose TGF- β in that they promote dendritic cell migration and maturation and enhance T-cell stimulation (Scutera et al. 2008; Salogni et al. 2009). TGF- β family members direct an antitumorigenic immune response. Once the adaptive immune system has been activated, it can remove non-self cells through the activation of phagocytic NK cells. Acting through specific NK cell group 2 receptors (NKG2), NK cells direct targeted killing (Vivier et al. 2012). TGF- β inhibits the cytotoxicity of these cells to promote tumorigenesis through decreased

expression of NKG2 receptors as well as IFN- γ (Allan et al. 2010; Crane et al. 2010). Additionally, the immune suppressive effects of TGF- β in NK cells have been associated with a *Foxp3* gene expression program, which, much like in Treg cells, inhibits T-cell function, thus stunting potentially antitumorigenic adaptive immune responses (Monteiro et al. 2010). Activin signaling performs similar functions in NK cells, suppressing the expression of inflammatory cytokines such as IFN- γ , but does not have any significant effect on direct cytotoxicity of these cells. Important to the direct cytotoxic effect of these cells, activin A signaling does not inhibit the expression of NK cell lectin-like receptor subfamily K member 1 (NKG2D), an activating receptor that promotes NK-cell-mediated cell lysis (Robson et al. 2009). Studies into the effects of BMP signaling on NK cells are limited; however, BMP receptors are expressed by NK cells, and BMP signaling is associated with NK cell differentiation in the thymus (Hidalgo et al. 2012). BMP stimulation of NK cells promotes activation and activity through enhanced Th1-associated cytokine response (Robson et al. 2014). These limited studies point to an antitumorigenic response of NK cells to BMP stimulation, and further mechanistic studies should reveal whether this pathway is a potential target for therapeutic intervention to enhance chemotherapeutic response.

Although TGF- β has an inhibitory effect at all levels of immune regulation, the data for BMPs are less clear. BMP signaling in T cells and macrophages promote a protumorigenic effect, yet BMP signaling in dendritic cells and NK cells is associated with an antitumorigenic profile. Similarly, activin signaling appears to have diverse immunogenic effects dependent on the cell type to suppress immune function in T cells, macrophages, and NK cells while promoting immune function in dendritic cells. These many contradictory effects by BMP and activin make the systemic results of their signaling in the context of the immune system difficult to discern and confounds the implementation of therapeutic interventions without further investigation of the specific roles of these pathways in the broad scope of cancer-directed immune

functions. These studies, again, underscore the importance of further work identifying how to efficaciously target these pathways to provide significant patient benefit while avoiding complications from alleviating the antitumorigenic functions of activating these signaling pathways.

CONCLUDING REMARKS

The TGF- β pathways are highly studied and important because of their numerous and essential roles in embryonic development, tissue homeostasis, and disease initiation and progression. However, the successful implementation of therapeutics directed toward the TGF- β pathways remains elusive because of the multiple effects of TGF- β signaling on numerous cellular compartments. The role of TGF- β signaling in the epithelium is already confusing, showing both pro- and antitumorigenic roles in cancer progression, but add in the tumor microenvironment and there are several more factors and outcomes for consideration. The inhibition of the protumorigenic effects of TGF- β in one compartment, such as the immune system, may be met with inhibition of antitumorigenic effects in another, such as endothelial cells resulting in no net change in disease progression. Thus, research on the numerous effects of TGF- β signaling on the diverse cell populations in the microenvironment is critical. As discussed, activation of TGF- β signaling as well as BMP and activin signaling results in a variety of stromal changes, which have significant impacts on tumor progression.

For the most part, TGF- β signaling is associated with stromal phenotypes that promote cancer growth and dissemination. The stromal phenotypes resulting from activation of BMP and activin signaling are not as clear, with both pro- and antitumorigenic effects not only in different stromal compartments but also in individual drivers of stromal changes. Although the inhibition of BMP signaling has also been associated with disease progression, this is an example of multiple mechanisms resulting in a similar phenotype. For example, in T cells, active TGF- β , BMP, and activin signaling

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suppresses the activation of the adaptive immune system through numerous mechanisms. However, targeted inactivation of Smad4 expression, which partially inhibits canonical TGF- β family signaling, specifically in CD4⁺ T cells, results in gastrointestinal carcinoma formation, even without introduction of genetic alterations in the epithelial cells (Kim et al. 2006; Cejalvo et al. 2007; Li and Flavell 2008; Aleman-Muench and Soldevila 2012). Thus, although activation signaling by single TGF- β family ligands has been associated with an inhibitory response, the shared activity of Smad4 in TGF- β family signaling may indicate that these outcomes depend on interactions with other signaling pathways. Such findings underlie the importance of further investigation into these pathways in cancer to determine whether the TGF- β family can be therapeutically targeted in a way to benefit patient outcome. It is also important to determine whether these pathways cooperate with or antagonize other signaling pathways that may be identified for therapeutic targeting, such as the NF- κ B pathway (Bitzer et al. 2000; Freudlsperger et al. 2013).

As highlighted in this review, the cooperation among the TGF- β family pathways is an important determinant of the phenotypic outcome resulting from individual pathway activation. Although often acting in very distinct ways, TGF- β , BMP, and activin signaling share many similarities in their intracellular mediators. These overlapping interactions may explain why varying and often opposing phenotypes are obtained from similar experiments investigating phenotypic results of TGF- β family activation and could be an important contextual determinant for anticipating consequences of intervention. For example, activin signaling enhances TGF- β -mediated conversion of Foxp3⁺ Treg cells (Huber et al. 2009). Additional studies, particularly in fibroblasts, show pathway antagonism rather than cooperation between the TGF- β and BMP pathways. Specifically, disruption of TGF- β signaling enhances BMP signaling, whereas BMP signaling suppresses TGF- β signaling (Koli et al. 2004).

Individually, activation of each of these signaling pathways in fibroblasts is associated with

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an increase in fibroblast function, such as α -SMA expression and collagen deposition. However, preclinical studies with inhibitors of TGF- β signaling have not shown a specific effect on fibroblast function in mouse models of cancer (Akhurst and Hata 2012). Such data underlie the importance of establishing better preclinical models that allow for a more mechanistic understanding of the signaling complexities within these relevant systems. This includes establishing better markers for cell specification, interactions between clinically relevant oncogenes and microenvironmental components, and a large-scale breakdown of signaling within these cells and the context in which they are studied. It is possible that this is because of compensation by BMP signaling continuing to promote fibroblast activation and function. It would be interesting to determine whether TGF- β signaling inhibitors are more or less effective in diseases that are characterized by TGF- β signaling but also have high levels of BMP and activin signaling. These studies could also reveal additional targets for therapeutic intervention by modulating numerous TGF- β family pathways. An example of this could be betaglycan/T β RIII, which much like T β RII has a complex role in cancer progression showing both tumor promoting and suppressing activities (Dong et al. 2007; Criswell et al. 2008). Although betaglycan/T β RIII is known for its role in presenting TGF- β 2 to T β RII to activate TGF- β signaling, this coreceptor has also been shown to mediate activation of BMP and activin signaling (Cheifetz et al. 1990; Harrison et al. 2004). Thus, targeting of this receptor could antagonize both the TGF- β and BMP pathways together to prevent any compensation to obtain a positive therapeutic effect. However, the conceptual framework for the interactions between the TGF- β , BMP, and activin pathways is a very interesting field of research, in which new findings could finally elucidate effective targeting contexts for the TGF- β family as a whole.

Adding to the complexity of the interactions between the TGF- β family pathways is the fact that this is all occurring within the context of a heterogeneous cellular tumor microenvironment. Given the significant effects of each sig-

naling pathway on the development of stromal phenotypes associated with tumor progression, an understanding of how these pathways mediate stromal changes will be essential to furthering our understanding of the TGF- β family's mediation of stromal changes to influence tumor progression.

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