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The TGF- β Paradox in Human Cancer: An Update

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Summary

Transforming growth factor- β (TGF- β) plays an essential role in maintaining tissue homeostasis through its ability to induce cell cycle arrest, differentiation, apoptosis, and to preserve genomic stability. Thus, TGF- β is a potent anticancer agent that prohibits the uncontrolled proliferation of epithelial, endothelial, and hematopoietic cells. Interestingly, tumorigenesis typically elicits aberrations in the TGF- β signaling pathway that engenders resistance to the cytostatic activities of TGF- β , thereby enhancing the development and progression of human malignancies. Moreover, these genetic and epigenetic events conspire to convert TGF- β from a suppressor of tumor formation to a promoter of their growth, invasion, and metastasis. The dichotomous nature of TGF- β during tumorigenesis is known as the “TGF- β Paradox,” which remains the most critical and mysterious question concerning the physiopathological role of this multifunctional cytokine. Here we review recent findings that directly impact our understanding of the “TGF- β Paradox” and discuss their importance to targeting the oncogenic activities of TGF- β in developing and progressing neoplasms.

Keywords

Angiogenesis; Cancer; Cell Invasion; Epithelial-mesenchymal Transition; Metastasis; Signal Transduction; Transforming growth factor- β

1. TGF- β and the Tumor Microenvironment

1.1. TGF- β and Fibroblasts

Tumor development in many respects mirrors that of an organ, albeit in a highly dysfunctional and disorganized manner. For instance, whereas normal tissue specification requires reciprocal signaling inputs from distinct cell types and matrix proteins, the phenotype of developing carcinomas is similarly dictated by the dynamic interplay between malignant cells and their accompanying stroma, which houses fibroblasts and endothelial cells (ECs), as well as a variety of infiltrating immune and progenitor cells [1,2]. Moreover, tumor reactive stroma not only plays an important role during cancer initiation and progression, but also in determining whether TGF- β suppresses or promotes tumor formation (Figure 1; [3–5]). Along these lines, TGF- β exerts its anti-tumor activities by regulating epithelial cell behavior, and by regulating that of adjacent fibroblasts, which synthesize and secrete a variety of cytokines, growth factors, and extracellular matrix (ECM) proteins that mediate tissue homeostasis and suppress cancer development. Thus, inactivating paracrine TGF- β signaling between adjacent epithelial and stromal compartments promotes cellular transformation, as well as induces the growth, survival, and motility of developing neoplasms [6,7]. For instance, rendering fibroblasts deficient in the expression of the TGF- β type II receptor (T β R-II), which manifests as

insensitivity to TGF- β , results in the formation of prostate intraepithelial neoplasia and invasive carcinoma of the forestomach [3]. Conditional deletion of T β R-II in mammary gland fibroblasts enhanced their proliferation and abundance within abnormally developed ductal units [8]. Interestingly, grafting a mixture of T β R-II-deficient mammary fibroblasts with mammary carcinoma cells under the subrenal capsule significantly enhanced the growth and invasion of breast cancer cells. The enhanced tumorigenicity of implanted mammary carcinoma cells was not recapitulated in grafts containing TGF- β -responsive fibroblasts, which failed to synthesize and secrete the high levels of TGF- α , MSP (macrophage-stimulating protein), and HGF (hepatocyte growth factor) produced by their T β R-II-deficient counterparts [3–5,8]. Thus, TGF- β signaling in fibroblasts suppresses their activation of cancer-promoting paracrine signaling axes that target adjacent epithelial cells. Somewhat surprisingly, T β R-II-deletion in mammary carcinoma cells resulted in the activation of two tumorigenic paracrine signaling axes comprised of SDF-1:CXCR4 and CXCL5:CXCR2, which collectively function in recruiting immature GR1⁺CD11b⁺ myeloid cells to developing mammary tumors [9]. Upon their arrival within mammary tumor microenvironments, GR1⁺CD11b⁺ cells promote breast cancer cell invasion and metastasis by attenuating host tumor immunosurveillance, and by stimulating MMP expression [9]. Recently, the ability of TGF- β to induce cell cycle progression in glioma cells required initiation of autocrine PDGF-B signaling. Importantly, the proliferation promoting properties of TGF- β and Smad2/3 only occurred in glioma lacking methylation of the PDGF-B gene, suggesting that the methylation status of PDGF-B determines the oncogenic activities of TGF- β in part *via* autocrine PDGF-B signaling within tumor microenvironments [10].

Tumorigenesis often is accompanied by intense desmoplastic and fibrotic reactions, which elicit the formation of rigid tumor microenvironments that enhance the selection and expansion of metastatic cells [11,12]. Lysyl oxidases (LOX) belong to a 5 gene family of copper-dependent amine oxidases (*i.e.*, LOX, LOXL, LOXL2, LOXL3, and LOXL4) that function in cross-linking collagens to elastin in the ECM [13,14]. Mechanistically, the activation of these cross-linking reactions by LOXs secreted by fibroblasts and epithelial cells serves to increase the tensile strength and structural integrity of tissues during embryonic development and organogenesis, as well as during the maintenance of normal tissue homeostasis [13,14]. Similar to TGF- β , members of the LOX family have been associated with tumor suppression and tumor promotion. Indeed, the transformation of fibroblasts by oncogenic Ras is suppressed by LOX and its ability to bind, oxidize, and inactivate growth factors housed in cell microenvironments, which presumably contributes the loss of cyclin D1 expression observed in LOX-expressing fibroblasts [15,16]. More recently, LOX was observed to interact physically with TGF- β 1 and alter its ability to stimulate Smad3 in cultured osteoblasts [17], while LOXL4 expression inhibited TGF- β stimulation of liver cancer cell invasion through synthetic basement membranes [18]. Thus, these findings implicate LOXs as potential suppressive agents within tumor microenvironments. In stark contrast, aberrant LOX activity also is associated with cancer progression, particularly the selection, expansion, and dissemination of metastatic cells [13–15,19–22]. Indeed, upregulated LOX expression is (*i*) essential for hypoxia-induced metastasis of human MDA-MB-231 breast cancer cells in mice [19]; and (*ii*) is observed most frequently in poorly differentiated, high grade mammary tumors and, consequently, predicts for increased disease recurrence and decreased patient survival [15,19]. Recently, we observed LOX expression to be induced strongly by TGF- β in normal and malignant MECs, and in mammary tumors produced in mice. Moreover, inhibiting LOX activity or degrading its metabolic byproduct, hydrogen peroxide, both antagonize the ability of TGF- β to induce the proliferation, EMT, and invasion in normal and malignant MECs. Furthermore, we find that LOX antagonism uncouples TGF- β from stimulating Src and p38 MAPK (M. Taylor and W.P. Schiemann, *unpublished findings*), whose activities are essential for mediating oncogenic signaling by TGF- β in breast cancer cells [23–25]. Along these lines, future studies need to enhance our understanding of (*i*) the role of tumor reactive fibroblasts and their production of

TGF- β in protecting carcinoma cells from tumoricidal radiotherapies, and (ii) the molecular and cellular mechanisms whereby anti-TGF- β therapies selectively sensitize carcinoma cells, not their adjacent normal counterparts, to ionizing radiation treatments [26–28].

Collectively, these findings highlight the important role TGF- β plays in governing autocrine and paracrine signaling networks, and more importantly, demonstrate how disrupting the delicate balance between these systems contributes to carcinoma development and progression.

1.2. TGF- β and Immunosurveillance

In addition to its regulation of stromal fibroblasts, TGF- β present in cell microenvironments also plays an essential role in governing the delicate balance between host immunosurveillance and inflammation, which collectively can determine whether tumor development and progression is induced or inhibited [29,30]. The importance of TGF- β in regulating immune system function and homeostasis is underscored by the finding that (i) TGF- β 1-deficient mice exhibit lethal multifocal inflammatory disease [31,32]; (ii) Smad3-deficient mice exhibit defects in the responsiveness and chemotaxis of their neutrophils, and their T and B cells [33]; and (iii) transgenic expression of truncated T β R-II specifically in T cells results in severe autoimmune reactions characterized by multifocal inflammation and autoantibody production [31]. Furthermore, T cell-specific deletion of Smad4 in mice drives T cell differentiation towards a Th2 phenotype and their elevated secretion of interleukins (ILs) 4, 5, 6, and 13 [34]. Similar to fibroblasts, the net effect of disrupting paracrine T cell signaling networks is the development of gastrointestinal carcinomas in these genetically engineered animals [34]. In addition, cancer cells typically increase their production and secretion of TGF- β into tumor microenvironments, as well as into the general circulation of cancer patients [35–37]. Abnormally elevated TGF- β concentrations also are detected within the tumor milieu in response to ECM degradation mediated by resident and recruited leukocytes – *i.e.*, monocytes/macrophages, dendritic cells, granulocytes, mast cells, T cells, and natural killer (NK) cells – that either promote or suppress tumor development in a context-specific manner [38].

1.2.1. TGF- β and Adaptive Immunity—TGF- β suppresses host immunosurveillance by inhibiting the proliferation and differentiation of NK and T cells, and by diminishing their synthesis and secretion of cytotoxic effector molecules, including interferon- γ , lymphotoxin- α , perforin/granzyme, and Fas ligand [30,39,40]. TGF- β also inhibits the tumor-targeting activities of T and NK cells through its stimulation of Tregs housed within tumor microenvironments [41]. Whereas TGF- β potently inhibits the proliferation of naïve CD8⁺ T cells, this cytokine elicits little-to-no activity in fully differentiated CD8⁺ T cells due to their downregulation of T β R-II. Administration of ILs 2 or 10 to differentiated CD8⁺ T cells restores their responsiveness to TGF- β , as does expression of the co-stimulatory molecule CD28, which promotes the survival of memory/effector phenotypes in thymic and peripheral T cell populations [30,39,42]. Mechanistically, the immunosuppressive effects of TGF- β transpire in part *via* Smad3, whose phosphorylation and activation prevents the mitogenesis of CD8⁺ T cells by (i) inhibiting their production of IL-2; (ii) repressing their expression of c-Myc, cyclin D2, and cyclin E; and (iii) stimulating the expression of the CDKs p15, p21, and p27 [30, 39,40]. In contrast to its stimulation of cytostasis in CD8⁺ T cells, TGF- β has no effect on the proliferation of CD4⁺ T cells, but does inhibit the differentiation of CD4⁺ T cells into Th1 and Th2 lineages by (i) downregulating T cell receptor expression; (ii) reducing intracellular Ca⁺⁺ signaling; and (iii) repressing the expression and activation of transcription factors [30, 39,40], all of which weaken host immunosurveillance. Collectively, these findings predict that inactivating TGF- β signaling in CD8⁺ or CD4⁺ T cells will inhibit tumor formation by elevating host immunosurveillance, a supposition shown to occur during T cell-mediated eradication of skin [43] and prostate [44] cancers in mice. More recently, TGF- β was observed to promote the development and progression of breast and colon cancers by inducing CD8⁺ T cells to

secrete IL-17, which exerts pro-survival signaling in carcinoma cells [45]. Thus, in addition to improving host immunosurveillance, neutralizing TGF- β function in T cells also will improve tumor resolution by suppressing the activation of carcinoma survival pathways.

1.2.2. TGF- β and Innate Immunity—In addition to its role in regulating adaptive immunity, TGF- β also plays an essential role in directing activities and behaviors of components of the innate immune system, including NK cells, dendritic cells, mast cells, monocytes, and macrophages. Indeed, we defined a novel TAB1:IKK β :NF- κ B signaling axis that forms aberrantly in breast cancer cells, and in normal MECs following their induction of EMT by TGF- β . Once formed, this signaling axis enables oncogenic signaling by TGF- β in part *via* activation of NF- κ B and its consequential production of proinflammatory cytokines, which promote breast cancer growth in mice in a manner consistent with regulation of innate immunity by TGF- β [46]. Along these lines, TGF- β receptors were observed to associate with those for IL-1 β , thereby enabling (i) TGF- β to activate NF- κ B; (ii) IL-1 β to activate Smad2; and (iii) both pathways to potentiate inflammatory cytokine production [47] their ability to promote inflammation and the enhanced the survival of tumor-associated monocytes [48,49]. In addition, transgenic expression of IL-1 β in the stomachs of mice promoted the activation of myeloid-derived suppressor cells (MDSCs) *via* an IL-1R/NF- κ B signaling axis, whose inappropriate and constitutive activation results in the formation of stomach neoplasias [50]. TGF- β is a potent inhibitor of the cytolytic activity of NK cells, presumably by attenuating the activation of their NKp30 and NKD2D receptors, and by inhibiting their production of interferon- γ . In addition, TGF- β also represses the activities of dendritic cells by inhibiting their expression of MHC class II, CD40, CD80, and CD86, and TNF- α , IL-12, and CCL5/Rantes [30,39,40,51]. Mast cells are actively recruited to tumor microenvironments by TGF- β where they synthesize and secrete numerous tumor promoting factors, including histamine, proteases, and cytokines (*e.g.*, VEGF and TGF- β) [40,52]. Lastly, TGF- β stimulates monocytes and macrophage chemotaxis to tumor microenvironments, leading to enhanced tumor invasion, angiogenesis, and metastasis, and to diminished antigen presentation and immunosurveillance towards developing neoplasms [53,54].

1.3. TGF- β and Endothelial Cells

Angiogenesis is the process whereby new blood vessels sprout and form from preexisting vessels; it also is an essential physiological process that transpires during embryonic development, wound healing, and the female reproductive cycle [55,56]. The initiation of pathological angiogenesis has been linked to numerous human diseases, including rheumatoid arthritis, diabetic retinopathy, and age-related macular degeneration [56,57]. Interestingly, all solid tumors larger than 1 cm³ suffer from hypoxia [58], and as such, initiate angiogenesis as a means of acquiring an efficient supply of nutrients and waste removal, as well as a route for their metastasis to distant locales. Two distinct phases are involved in angiogenesis, namely angiogenic activation and resolution. During the activation phase of angiogenesis, ECs initially exhibit increased vessel permeability and elevated rates of cell proliferation, migration, and invasion. In addition, new vessel sprouting is further enhanced by a reduction in EC adhesion, coupled to an alteration in basement membrane integrity. In contrast, angiogenic resolution essentially restores activated ECs to their resting, quiescent phenotypes, as well as promotes the recruitment of perivascular cells that maintain vessel stability and hemodynamics [55–57].

TGF- β plays critical roles in regulating both the activation and resolution phases of angiogenesis [59–62]. Indeed, homozygous deletion of various components of the TGF- β signaling system in mice routinely results in the appearance of vascular and EC defects, particularly in animals lacking TGF- β 1 [63], T β R-I [64], T β R-II [65,66], T β R-III [67,68], Smad1 [69], or Smad5 [70]. In humans, loss or inactivation of endoglin leads to hereditary

hemorrhagic telangiectasia type 1 (HHT1) [71,72], while that of ALK1 results in HHT2 [73, 74]. Moreover, the defects associated with HHT1 and HHT2 in humans are phenocopied in knockout mice lacking expression of either endoglin [75,76] or ALK1 [77–79], respectively. Thus, altered expression and/or activity of TGF- β in tumor microenvironments clearly will impact the ability of hypoxic tumors overcome this impediment to their growth and survival.

ECs have been reported to express two distinct T β R-Is, namely T β R-I/Alk5 and ALK1. The importance of these two receptors in mediating vessel development by TGF- β is evidenced by the embryonic lethality observed at day E11.5 and E10.5 in mice lacking ALK1 [79] or ALK5 [64], respectively. Recent evidence also suggests that these two type I receptors differentially regulate the coupling of TGF- β to angiogenic activation and resolution. For instance, T β R-I/ALK5 activation stimulates Smad2/3 and the subsequent expression of genes operant in mediating vessel maturation, including plasminogen activator inhibitor 1 (PAI-1) and fibronectin [78,80,81]. Moreover, microarray gene expression analyses of EC cells before and after their stimulation with TGF- β confirmed that the activation of a TGF- β :T β R-I/ALK5:Smad2/3 signaling axis does indeed promote angiogenic resolution [61,82]. In contrast, ALK1 activation stimulates Smad1/5 and the subsequent expression of genes operant in mediating angiogenesis activation, including Id1 and interleukin 1 receptor-like 1 [78,80–82]. Moreover, ALK-1 signaling stimulated by TGF- β requires this cytokine to initially activate T β R-II and ALK-5, which then recruit and activate ALK-1 following its association with T β R-II:ALK-5:TGF- β ternary complexes [78]. Thus, activation of ALK-1 and the induction of angiogenesis by TGF- β must first proceed through its assembly of angiostatic TGF- β receptor complexes (*i.e.*, T β R-II:ALK-5). At present, the molecular mechanisms that initially exclude and then recruit ALK-1 to angiostatic TGF- β receptor complexes remain unknown, but may reflect a delicate balance between TGF- β and other angiogenic factors located within tumor microenvironments. Indeed, low TGF- β concentrations enhance the ability of bFGF and VEGF to stimulate EC proliferation and angiogenic sprouting, while high TGF- β concentrations inhibit these angiogenic activities [62,83]. Along these lines, the pro-angiogenic functions of TGF- β also have been linked to its ability to regulate the expression and/or activities of other angiogenic factors, such as bFGF and VEGF [84]. It is interesting to note that inclusion of TGF- β to Matrigel plugs implanted into mice only promotes angiogenesis and vessel development in the presence of bFGF and its ability to create a pro-angiogenic microenvironment (M. Tian and W.P. Schiemann, *unpublished observation*). Thus, it is plausible that the recruitment of ALK-1 to angiostatic TGF- β receptor complexes may first require the stimulation of accessory angiogenic signals or proteins within activated EC microenvironments. Along these lines, the coupling of TGF- β to angiogenesis is controlled by the presence of endoglin, whose expression is induced by ALK1 and serves to promote EC proliferation, migration, and tubulogenesis by antagonizing the activities of T β R-I/ALK5 [60,82].

Collectively, these studies highlight the complexities associated with the ability of TGF- β to regulate EC activities coupled to angiogenesis. Future studies clearly need to (*i*) better define the precise mechanisms that enable TGF- β and its downstream effectors to govern the induction of angiogenic or angiostatic gene expression profiles; (*ii*) establish the impact of EC and perivascular cell differentiation states to influence the angiogenic response to TGF- β ; and (*iii*) identify the microenvironmental cues and signals that cooperate with TGF- β in mediating angiogenesis activation and resolution.

2. TGF- β , EMT, and Metastasis

The acquisition of invasive and metastatic phenotypes by carcinomas ushers in their transition from indolent to aggressive disease states, during which time immotile, polarized epithelial cells undergo EMT and transdifferentiate into highly motile, apolar fibroblastoid-like cells

[85–87]. In doing so, post-EMT carcinoma cells remodel their ECM and microenvironments in a manner that facilitates their intravasation into the vascular or lymphatic systems, as well as their extravasation at distant locales to form micrometastases that ultimately develop into secondary carcinomas [88]. Interestingly, a recent study identified a set of potential metastatic gene signature whose expression is highly associated with the acquisition of pulmonary metastasis by human breast cancers [89]. Included in this metastatic gene signatures are IDs (**I**nhibitor of **D**ifferentiation) 1 and 3, which mediate constitutive proliferative signals in newly established pulmonary micrometastases [89]. In addition, the ability of TGF- β to induce ANGPTL4 (angiopoietin-like 4) expression in breast cancer cells enables their retention, extravasation, and colonization specifically in the lungs, not the bone [90]. Pathological reactivation of EMT programs in differentiated cells and tissues not only promotes their invasion and metastasis, but also underlies the development of several human pathologies, such as chronic inflammation, rheumatoid arthritis, and chronic fibrotic degenerative disorders, all of which are characterized by dysregulated microenvironmental signaling [85–88,91,92]. In the following sections, we summarize recent developments linking TGF- β to the induction of EMT and metastasis, to the selection and expansion of cancer stem cells, and to the regulation of microRNA expression in developing and progressing neoplasms.

2.1. TGF- β Signaling and EMT

2.1.1. Canonical TGF- β Effectors and EMT—The ability of TGF- β to induce EMT and metastasis transpires through the activation of canonical (*i.e.*, Smad2/3-dependent) and noncanonical (*i.e.*, Smad2/3-independent) TGF- β signaling inputs. For instance, inactivating canonical TGF- β signaling in human MCF10ACA1a breast cancer cells by engineering their expression of a dominant-negative Smad3 construct [93] or a T β R-I mutant incapable of activating Smad2/3 (*i.e.*, L45 mutant) [94] significantly reduced their ability to colonize the lung. Along these lines, Smad4-deficiency not only diminished the expression of PTHrP, IL-11, and CTGF in human MDA-MB-231 breast cancer cells, but also abrogated their metastasis to bone in response to TGF- β [95–98]. Interestingly, whereas Smad4-deficiency cooperates with oncogenic K-Ras to induce the initiation and development of pancreatic cancer, the expression and activity of Smad4 is essential for TGF- β stimulation of pancreatic cancer EMT and growth [99]. Similar inactivation of canonical TGF- β signaling by overexpression of Smad7 [100, 101] prevents the invasion of breast [102] and head and neck cancers [103,104], as well as the pulmonary metastasis of melanomas [105]. Collectively, these findings highlight the importance of Smad2/3/4 signaling in mediating EMT and metastasis stimulated by TGF- β , and suggest the potential benefit of Smad2/3 antagonists to improve the clinical course of patients with metastatic disease.

2.1.2. Noncanonical TGF- β Effectors and EMT—Noncanonical TGF- β signaling also plays an essential role in mediating TGF- β stimulation of EMT, invasion, and metastasis [106]. Included in this growing list of noncanonical effectors targeted by TGF- β are Ras/MAP kinase [107–115], PI3K/AKT [116], Rho/ROCK [117], Jagged/Notch [118], mTOR [119], and Wnt/ β -catenin [120]. Collaborative signaling events occurring between NF- κ B and oncogenic Ras also mediate EMT and pulmonary extravasation of breast cancer cells in response to TGF- β [121]. Similarly, we identified TGF- β stimulation of NF- κ B as an essential pathway operant in coupling TGF- β to the expression of Cox-2, whose activity and production of PGE2 are critical for EMT induced by TGF- β in normal and malignant MECs [122]. We [23–25] and others [108] also established integrins as key players in mediating EMT, invasion, and p38 MAPK activation by TGF- β , as well as its ability to stimulate the growth and pulmonary metastasis of breast cancers in mice [25]. Essential effectors targeted by the formation of integrin:TGF- β receptor signaling complexes are (*i*) the protein proto-oncogene Src and its phosphorylation of T β R-II at Y284, which creates a docking site for Grb2 and ShcA [23–25]; (*ii*) the adapter molecule Dab2, which facilitates TGF- β stimulation of Smad2/3 and FAK

[123,124]; and (iii) the protein tyrosine kinase FAK, which coordinates the formation of $\alpha\beta3$ integrin:T β R-II complexes and, together with its effector p130Cas, is essential for TGF- β stimulation of breast cancer pulmonary metastasis in mice (M.K. Wendt and W.P. Schiemann, *unpublished observation*). In addition, $\alpha\beta3$ integrin also mediates TGF- β -dependent metastasis of breast cancer cells to bone [125,126]. Collectively, these findings implicate T β R-II as an essential mediator of oncogenic signaling by TGF- β , particularly its ability to promote the acquisition of invasive and metastatic phenotypes at the expense of significantly impacting primary tumor growth [127]. Along these lines, a missense mutation in T β R-II identified in human head and neck carcinomas was observed to promote their EMT and invasion in part *via* (i) hyperactive protein kinase activity in mutant T β R-II proteins, and (ii) inappropriate coupling of TGF- β receptors to Smad1/5 activation, as opposed to Smad2/3 [128]. Interestingly, following its phosphorylation by T β R-II, the tight-junction assembly protein, PAR-6, associates with T β R-I and coordinates the ubiquitination and degradation of RhoA by Smurf1 [129]. The net effect of these TGF- β -dependent events results in the dissolution of epithelial cell tight junctions and the disassembly of their actin cytoskeleton, leading to the induction of EMT.

2.2. TGF- β and Cancer Stem Cells

It is important to note that EMT is a normal and essential physiological process that directs tissue development and morphogenesis in the embryo, as well as promotes the healing, remodeling, and repair of injured tissues in adults [85–87]. Thus, tumorigenic EMT in many respects reflects the inappropriate reactivation of embryonic and morphologic gene expression programs, and as such, points towards a potential link between EMT and the maintenance of stem cell properties. Accordingly, aggressive and poorly differentiated breast cancer and glioma cells exhibit gene signatures characteristic of stem cells [130], while human and mouse MECs induced to undergo EMT acquire stem cell-like properties in part *via* activation of the TGF- β signaling system [131]. Because TGF- β is a master regulator of physiological and pathological EMT [91], these findings suggest that the conversion of TGF- β from a tumor suppressor to a tumor promoter mirrors its ability to induce the selection and expansion of stem cell-like progenitors in post-EMT cells. In fact, TGF- β treatment of malignant, but nonmetastatic human breast cancer cells suppressed their tumorigenicity by diminishing the size of the cancer stem cell pool, and by reducing ID1 expression that results in the differentiation of the progenitor pool [132]. Thus, uncoupling TGF- β from regulation of ID1 expression may dictate whether TGF- β either promotes or suppresses the maintenance and/or expansion of cancer stem cells. Indeed, pharmacological inhibition of TGF- β signaling in cancer stem cells induced a mesenchymal-epithelial transition that resulted in their acquisition of a more epithelial-like morphology [133]. Along these lines, Future studies clearly need to (i) identify the molecular mechanisms that link TGF- β and EMT to the generation of cancer stem cells, and (ii) establish the therapeutic impact of TGF- β in promoting chemoresistance *via* its stimulation of EMT and the expansion of cancer stem cells.

2.3. TGF- β and microRNAs

Finally, accumulating evidence now positions microRNAs as potentially important regulators of the “TGF- β Paradox.” Indeed, expression of miR-21 in breast cancers predicts for elevated TGF- β 1 expression and a poor clinical prognosis [134], while that in gliomas results in the suppression of multiple components of the TGF- β signaling system, including its ligands (*e.g.*, TGF- β s 1 and 3), its receptors (*e.g.*, T β R-II and T β R-III), and its effector molecules (*e.g.*, Smad3, Daxx, and PDCC4) [135,136]. Recently, TGF- β was shown to promote contractile phenotypes in vascular smooth muscle cells by stimulating the processing of primary miR-21 transcripts into their pre-miR-21 counterparts *via* the formation of Smad2/3:DROSHA complexes. In doing so, cellular levels of miR-21 accumulate rapidly, resulting in diminished expression of PDCC4 (**programmed cell death 4**) and its inability to

suppress contractile machinery expression in vascular smooth muscle cells [136]. Similar induction of miR-21 expression took place in Smad4-deficient carcinoma cells, suggesting that TGF- β -regulated miR processing also takes place in epithelial cells in a manner independent of Smad4 [136]. Moreover, miR-21 expression also functions to promote EMT stimulated by TGF- β [137], although the molecular mechanisms underlying this event remain to be determined definitively. In contrast to miR-21 and its role in promoting EMT by TGF- β , microRNA-200 family members and miR-205 function in maintaining epithelial cell polarity and, consequently, in suppressing EMT. Importantly, the ability of TGF- β to induce EMT first requires this cytokine to downregulate microRNA-200 family member and miR-205 expression, which promotes ZEB1 and ZEB2 expression and their initiation of EMT [138]. Thus, aberrant microRNA expression may play a significant role in determining whether epithelial cells sense and respond to the tumor suppressing functions of TGF- β , or rather to its oncogenic activities.

Conclusions and Future Perspectives

Despite considerable progress over the last decade in defining the molecular mechanisms that underlie the initiation and maintenance of the “TGF- β Paradox,” science and medicine still lack the necessary knowledge and wherewithal to explain and, more importantly, to manipulate the physiopathological actions of TGF- β to improve the clinical course of human malignancies. While it is abundantly clear that TGF- β plays a major role, both directly and indirectly, in regulating the ability of cancer cells to acquire each of the 6 hallmarks necessary for their malignant progression [139], it remains unclear as to how these events conspire in regulating the response of developing and progressing neoplasms to TGF- β . For instance, defects in TGF- β function rarely effect primary tumor growth, but more commonly play a significant role in enabling cancer cells to acquire EMT and invasive/metastatic phenotypes. Thus, while it is easy to rationalize why tumors require TGF- β to provide them with a selective EMT and metastatic advantage, teleologically it remains troublesome to assume that these phenotypic changes induced by TGF- β are permanently ingrained in aggressive carcinoma cells. Indeed, cancer cells perpetually locked into a “vagabond” mentality is counterintuitive to the processes underlying metastasis development and the formation of secondary carcinomas at distant locales. Instead, it appears that the exquisite balance between the functions and behaviors of TGF- β in distinct tissue types become unbalanced and incapable of suppressing disease development, particularly that of neoplastic transformation. Along these lines, the processes underlying the maintenance of normal tissue and cellular homeostasis have been likened to those necessary in facilitating the existence of a well-balanced and harmonious society [140]. The studies highlighted herein are consistent with a role for TGF- β in serving either as a benevolent or corrupt village manager, one whose ultimate agenda is dictated by the prevailing mood of the village’s stromal and microenvironmental constituents. Thus, these findings also underscore and reinforce the need to develop novel pharmacological agents designed to antagonize the oncogenic activities of TGF- β in cancer cells, as well as in their supporting stromal compartments.

Executive Summary

TGF- β and the Tumor Microenvironment Fibroblasts

- Tumor reactive stroma plays a critical role in determining whether TGF- β suppresses or promotes tumor formation.
- Loss and/or disruption of paracrine signaling systems between fibroblast and adjacent epithelial cells results in cellular transformation, and in the progression of developing neoplasms.

- Similar inactivation of TGF- β function in epithelial cells also elicits aberrant epithelial:fibroblast paracrine signaling networks that drives malignancy.
- TGF- β stimulation of desmoplastic and fibrotic reactions promotes the formation of stiff, noncompliant microenvironments that select for the expansion of metastatic cells.
- LOX family members are essential for desmoplasia induced by TGF- β , and for stimulating breast cancer metastasis in hypoxic tumor environments.
- Fibrotic reactions enhance TGF- β signaling and may facilitate tumor protection to radiotherapies.

TGF- β and Immunosurveillance

- TGF- β is a potent suppressor of inflammation and immune suppression.
- Similar to fibroblasts, altered paracrine signaling by immune cell contributes to tumor formation, particularly that in the gastrointestinal track.
- TGF- β is a potent inhibitor of adaptive immunity, which contributes to weaken host immunosurveillance.
- TGF- β also is a potent activator of innate immunity, which contributes to carcinoma progression and metastasis.

TGF- β and Endothelial Cells

- Angiogenesis is the process whereby new blood vessels develop from pre-existing vessels.
- Angiogenesis also provides cancer cells a route for their metastatic spread.
- Aberrant TGF- β signaling elicits developmental vascular defects that typically result in embryonic lethality.
- Human hereditary hemorrhagic telangiectasia (HTT) is phenocopied in mice lacking expression of either ALK1 or endoglin.
- TGF- β regulates both the activation and resolution of angiogenesis by differential activation of ALK1 (*i.e.*, pro-angiogenic *via* Smad1/5/8 activation) or T β R-I/ALK5 (*i.e.*, anti-angiogenic *via* Smad2/3 activation).
- Activation of ALK1 by TGF- β requires the presence of ALK5 and T β R-II.

TGF- β , EMT, and Metastasis

- EMT is a process whereby polarized, immotile epithelial cells transdifferentiate into apolar, highly motile fibroblastoid-like cells.
- TGF- β is a master regulator of normal and tumorigenic EMT.
- EMT is essential for the acquisition of invasive and metastatic phenotypes in carcinoma cells.
- TGF- β induces EMT *via* stimulation of canonical (*i.e.*, Smad2/3) and noncanonical (*i.e.*, Ras/MAP kinases, PI3K, AKT, and Rho/ROCK) pathways.
- β 3 integrin, Src, and p38 MAPK are essential in facilitating EMT stimulated by TGF- β .

- Aberrant expression of microRNAs in response to TGF- β may drive EMT and metastasis.
- EMT induced by TGF- β may play important roles in generating chemoresistant cancer stem cells.

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Figure 1. Cellular Targets of TGF- β During the Development and Progression of Human Cancers TGF- β is a multifunctional cytokine that normally suppresses cell proliferation, differentiation, and apoptosis, as well as regulates cell and tissue homeostasis. Under normal physiological conditions, TGF- β functions as a tumor suppressor by preventing the ability of cells to progress through the cell cycle, or by stimulating the ability of cells to undergo apoptosis or differentiation. However, genetic and epigenetic events that transpire during tumorigenesis can convert TGF- β from a tumor suppressor to a tumor promoter, particularly the ability of cancer cells to acquire invasive and metastatic phenotypes. The oncogenic activities of TGF- β also are coordinated by dysregulated autocrine and paracrine signaling networks that take place between epithelial, fibroblasts, endothelial, and immune cells, that collectively promote tumor angiogenesis, invasion, and metastasis, and inhibit host immunosurveillance within tumor microenvironments. See text for specific examples of how TGF- β signaling becomes dysregulated during tumorigenesis