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TGF-β Signaling in Health and Disease

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Abstract

The TGF-β regulatory system plays crucial roles in the preservation of organismal integrity. TGF-β signaling controls metazoan embryo development, tissue homeostasis, and injury repair through coordinated effects on cell proliferation, phenotypic plasticity, migration, metabolic adaptation, and immune surveillance of multiple cell types in shared ecosystems. Defects of TGF-β signaling, particularly in epithelial cells, tissue fibroblasts, and immune cells, disrupt immune tolerance, promote inflammation, underlie the pathogenesis of fibrosis and cancer, and contribute to the resistance of these diseases to treatment. Here we review how TGF-β coordinates multicellular response programs in health and disease, and how this knowledge can be leveraged to develop treatments for diseases of the TGF-β system.

Introduction

The development, homeostasis, and repair of metazoan tissues rely on the multipotency and proliferative capacity of rare progenitor cell populations and their progenies, the support of neighboring cells, the surveillance of the immune system, and the input of potent signals. The transforming growth factor β (TGF-β) family of cytokines stands out as the most pleiotropic of these signals and, frequently, also the most dominant. The discovery of TGF-β[1] and the elucidation of its signaling pathway from membrane receptors to target genes[2] enabled the delineation of the biology of these factors,[3-8] the structural basis for TGF-β signaling,[9-11] the context-dependent nature of the TGF-β effects,[12] and how congenital skeletal, connective and cardiovascular diseases, as well as chronic inflammation, fibrosis, and cancer arise from malfunctions in this pathway.[13-17] But as the basis for the different effects of TGF-β on myriad cell types became clear, questions of a higher order emerged: Do the many effects of TGF-β serve a common purpose? How are these effects coordinated? And how can this knowledge be leveraged to treat diseases of the TGF-β system?

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Declaration of Interests

Joan Massagué holds company stock of Scholar Rock, Inc. Dean Sheppard is a founder of Pliant Therapeutics, a member of the Genentech Scientific Review Board, a member of the Amgen Immunology Scientific Advisory Board, and a member of the Scientific Review Board for Lila Biologics.

Many effects – one overarching role

Among the plethora of TGF-β effects on different cells and tissue environments (Figure 1), some relate to growth control ranging from suppression of proliferation through cell cycle inhibitors in epithelial and hematopoietic cells to stimulation of fibroblast proliferation through the release of mitogens. Other effects of TGF-β relate to the regulation of phenotypic plasticity though genome-wide chromatin changes that modify the developmental state and transcriptional landscape of a cell. Examples include the regulation of pluripotency in stem cells, mesenchymal phenotypic transitions in epithelial and endothelial progenitors, migration and axon formation in neurons, and differentiation in mesenchymal, hematopoietic and epithelial lineages. TGF-β is also a potent fibrogenic signal for fibroblasts, connective tissue, and epithelial cells to produce and remodel the extracellular matrix (ECM). As a key enforcer of immune tolerance and a suppressor of inflammation, TGF-β restricts multiple functions of the adaptive and innate immune systems. The pleiotropic nature of TGF-β distinguishes it from WNT, Hedgehog, Notch, and tyrosine kinase effectors which primarily act to promote organized tissue growth.

Notably, TGF-β triggers these diverse effects through a common membrane receptor and a common set of SMAD transcription factors. Although the signaling activity of the TGF-β pathway determines the strength and duration of a response, the nature of this response depends on contextual determinants such as the type and developmental state of the target cell and the presence of response-modifying signals. As a result of these variables, TGF-β can have diverse, sometimes opposite effects. For example, TGF-β can function as an enforcer of homeostasis in a healthy epithelium, as an apoptotic signal in pre-malignant cells arising in this tissue, and as a tumor progression agonist in carcinoma cells that avert this tumor suppressive effect.

The opposite roles of TGF-β as guardian of homeostasis and instigator of pathogenesis have baffled biologists and the pharmaceutical industry, earning TGF-β epithets like “jack of all cytokine trades” and “Jekyll and Hyde growth factor”. However, when taken together, the disparate effects of TGF-β fulfill a common purpose of balancing homeostasis and injury repair. Three cell types – epithelial cells, immune cells, and tissue fibroblasts– are central targets of TGF-β in this overarching function as well as in the most common diseases of TGF-β signaling: chronic inflammation, fibrosis, and cancer (Figure 1). Connective tissue, skeletal, smooth muscle cells and endothelial are also highly responsive to TGF-β, as demonstrated by the consequences of TGF-β malfunctions in these tissues. Yet overall, the whole tissue, more than any of its constituent cell types, is the target of the TGF-β system, and preserving tissue integrity is the ultimate output of multicellular TGF-β responses.

Here, we review the current knowledge on TGF-β signaling, its effects on its principal target cell types, its involvement in common diseases of inflammation, fibrosis and cancer, and efforts to treat TGF-β dysfunctions. The emerging concepts are also relevant to all other members of the TGF-β family, their signaling functions, and their roles in development and homeostasis, as well as other disorders of TGF-β signaling including rare cardiovascular, connective tissue, and skeletal developmental diseases resulting from inherited mutations in

TGF-β system components. [4,16,17] Our aim is to distill the basic principles and essential knowledge that inform this vast field.

Active cytokines and latent forms

The TGF-β family of cytokines includes two subfamilies, based on structural and biological criteria (Table 1). In mammals, the TGF-β/Nodal subfamily comprises TGF-β1, TGF-β2 and TGF-β3 (jointly referred to as TGF-β), Nodal, four Activins, and five Growth and Differentiation Factors (GDFs). It also includes the antagonistic ligands Inhibin, which blocks activin receptors, and Lefty1 and Lefty2, which block Nodal co-receptors. The Bone Morphogenetic Protein (BMP) subfamily includes eleven BMPs, four GDFs, and the Anti-Muellerian Hormone (AMH). BMP3 is a BMP receptor antagonist. Members of both subfamilies have pleiotropic effects during development and in adult tissues, although Nodal and AMH play only a few critical roles mostly in development.

The three TGF-β isoforms are produced by many cell types. Each isoform is synthesized as a disulfide-linked dimeric precursor which is cleaved by the endoprotease furin in the Golgi. The cleaved N-terminal portion of the precursor is called the latency-associated peptide (LAP), and the C-terminal dimeric domain constitutes the mature TGF-β cytokine (Figure 2A). Three intrachain disulfide bonds within each TGF-β monomer form a structurally tight, highly stable “cystine knot” with protruding flexible loops that interact with receptors and ligand regulators. After cleavage, TGF-β remains noncovalently associated with LAP, and multiple contacts with LAP occlude the receptor-binding sites of TGF-β.[18] In most cells this complex, called the “small latent TGF-β complex”, is disulfide linked to one of three latent TGF-β binding proteins (LTBPs 1, 3 and 4) or, in certain cells, to transmembrane leucine-rich repeat containing proteins LRRC32, also known as GARP (glycoprotein A repetitions predominant protein)[19] or LRRC33.[20] After secretion, LTBPs bind to the ECM and can be covalently cross-linked to fibronectin by tissue transglutaminases. GARP/LRRC32 and LRRC33 tether latent TGF-β to the surface of the TGF-β synthesizing cell (Figure 2A).

All other TGF-β family members are also dimers, disulfide-linked in most cases, synthesized as the C-terminal portion of a precursor. Homodimers are the prevalent forms, but natural heterodimers such as TGF-β1.2,[21] activin AB[22] and BMP2.7[23,24] further diversify the family. Cells can sense and compute inputs from multiple TGF-β family members and receptors simultaneously.[25] Latent forms like those of TGF-β are known for only a few family members. However, several families of secretory molecules bind BMPs and Activins to withhold these ligands from membrane receptors.[11]

Critical steps in TGF-β activation

Because the association between native TGF-β and LAP is non-covalent, TGF-β can be activated *in vitro* by heat and extreme pH. However, no convincing data implicate changes in temperature or pH as activators of TGF-β *in vivo*. LAP presents potential cleavage sites for proteases to release active TGF-β,[18] and various serine proteases (e.g. plasmin and cathepsin D) and metalloproteinases (e.g. MMP9 and MMP14) can activate TGF-β *in vitro*.[26,27] However, the phenotypes of mice lacking these proteases do not phenocopy the

loss of TGF-β function.[28] Thus, the *in vivo* functional significance of proteolytic activation of TGF-β remains uncertain.

The ECM protein thrombospondin 1 (TSP1) contains an exposed peptide sequence (KRFK) that can bind a conserved sequence (LSKL) in the LAP of all three TGF-β isoforms. This interaction disrupts the association of LAP with the captive TGF-β.[29] Mice lacking TSP1 manifest some of the phenotypes of TGF-β1-deficient mice, including inflammation and epithelial hyperplasia in multiple organs.[30] TSP1 from infiltrating monocytes is an important mediator of TGF-β activation during vascular remodeling in a model of schistosomiasis-induced pulmonary arterial hypertension.[31] However, several allosteric and force-driven processes are now recognized as the main mechanisms for activation of latent TGF-β *in vivo*. Delineating these mechanisms is a focus of current research and manipulating them is a goal of the pharmaceutical industry and the clinic.

Role of integrins.

The possibility that members of the integrin family of cell adhesion receptors activate latent TGF-β was first suggested by the phenotype of mice lacking the β6 subunit of the integrin αvβ6. αvβ6 is highly induced on epithelial cells in several organs by tissue injury and inflammation.[32] *Integrin β6* (*Itgb6*) knockout mice develop exaggerated inflammatory responses in the lungs and skin but are protected from tissue fibrosis in multiple tissues.[33-35] Both phenotypic features are consistent with a deficit in TGF-β. Cells expressing αvβ6 can activate TGF-β1 and TGF-β3 by binding the sequence RGD that is present in an exposed loop of their LAP.[34,36] Integrin αvβ8, which is expressed in neuroepithelial cells, astrocytes, and in subsets of myeloid cells, T cells, epithelial cells, and fibroblasts, binds to the same RGD site and can also activate TGF-β1.[27] Mice lacking *Itgb8* die during embryonic development or immediately after birth from intracerebral hemorrhage caused by defective vascular development in the central nervous system.[37] Intracerebral hemorrhage in these mice is caused by the absence of αvβ8 on neuroepithelial cells which activates TGF-β for presentation to endothelial cells.[38]

The integrins αvβ6 and αvβ8 are essential for many of the developmental and homeostatic roles of TGF-β1, as shown by knock-in of a point mutation in TGF-β1 which prevents integrin binding.[39] Administration of αvβ6 blocking antibody to *Itgb8* knockout mice bred to bypass perinatal mortality, or crossing these mice to mice lacking *Itgb6* recapitulates most of the developmental phenotypes of mice lacking TGF-β1 and TGF-β3.[40] These phenotypes include severe multiorgan inflammation (a central feature of TGF-β1 knockout mice) and cleft palate (seen in TGF-β3 knockout mice). These observations indicate that integrins αvβ6 and αvβ8 are crucial for TGF-β activity during development and immune homeostasis. Equivalent protection from hepatic and pulmonary fibrosis by deletion of the integrin αv subunit from fibroblasts or treating mice with a small molecule inhibitor to the αvβ1 integrin support the idea that αvβ1 is the main TGF-β activating integrin in fibroblasts.[41] In contrast to the TGF-β1 and TGF-β3 LAPs, the TGF-β2 LAP lacks an RGD sequence but contains an alternate sequence in an exposed loop that can bind to αvβ6 for activation.[42] TGF-β2 might also be spontaneously active after secretion.[43]

Mechanisms of activation by integrins.

Integrin αvβ6 activates latent TGF-β1 and TGF-β3 by binding to the RGD sequence in the respective LAPs (Figure 2B). When αvβ6-expressing epithelial cells are induced to contract, physical force deforms the tethered latent complex either releasing free active TGF-β or changing the conformation of the captive cytokine to expose its receptor binding sites. Although expression of this integrin is restricted to epithelial cells, which are not generally considered to be highly contractile, evidence supports an important role for actin-myosin contraction and mechanical deformation of the latent complex in integrin αvβ6–mediated TGF-β activation.[34,44]

Deletion of LTBP1, required for tethering the latent complex to the extracellular matrix, also inhibits αvβ6-mediated TGF-β activation, and this defect can be rescued by a fusion protein composed of the LAP-tethering and fibronectin-binding domains of LTBP1.[45] The crystal structure of the LAP-TGF-β1 complex shows that the cysteine residue in LAP used for tethering to LTBP1, GARP and LRRC33 and the integrin binding loop of LAP are located on opposite poles of the latent complex.[18] These findings suggest that force applied across the tethered αvβ6–LAP complex unfolds the latency loop and releases the active cytokine.

Integrin αvβ8 does not seem to activate TGF-β through cell contraction.[27] TGF-β activation by integrin αvβ8 is retained even after the entire β8 cytoplasmic domain is deleted. Recent high resolution cryo-EM structural data, together with studies showing that αvβ8 can activate a mutant form of latent TGF-β that cannot release the active cytokine from LAP, suggest that αvβ8 binding to LAP induces a conformational change in the latent complex that allows the captive TGF-β to bind to its receptors without release from LAP.[46] The importance of this mechanism for activation by αvβ6 and/or αvβ1 remains to be determined.

Unlike LTBPs, which are widely expressed, GARP and LRRC33 are each expressed on distinct subsets of immune cells and other cell types. GARP is restricted to regulatory T cells, endothelial cells, platelets, and some fibroblasts, whereas LRRC33 is expressed in macrophages and microglia.[19,20] GARP and LRRC33 tightly tether latent TGF-β1 to the cell surface, and this tethering plays a critical role in activation of these latent complexes by αvβ6 and αvβ8 integrins (Figure 2B). Integrin-expressing cells can induce TGF-β signaling in adjacent cells. For example, αvβ8 expressed in one cell activates TGF-β signaling in the cell expressing GARP-tethered TGF-β. [46] This pattern fits with the observation that in vivo deletion of TGF-β ligands or TGF-β receptors from the same T cell[8] often share many phenotypic features. On the other hand, deletion of *Itgb8* from neuroepithelial cells results in a very similar phenotype as deletion of TGF-β receptors in microglia, which do not express *Itgb8*.[47]

TGF-β signal transduction

The TGF-β pathway epitomizes membrane-to-nucleus signaling by direct receptor-mediated activation of transcription factors (Figure 2C). The receptor subunit composition, ligand-driven activation mechanism, and signal propagation through SMAD proteins elucidated for TGF-β apply to the rest of the TGF-β family. The composition, function, structural

basis, and the many layers of regulators of this pathway have been comprehensively reviewed.[9,10,12] Here, we present the key features.

Receptors.

TGF-β ligands bind to pairs of transmembrane serine/threonine protein kinase subunits known as receptors type I and type II. Mammalian genomes include 7 type I receptors and 5 type II receptors[9] which are bound in various pairwise combinations by specific ligands (Table 1). In the case of TGF-β1, each monomer contacts one TGF-β type II receptor (TGFBR2) molecule forming a composite ligand-receptor protein surface that is then recognized by one type I receptor molecule (TGFBR1).[48] In the case of BMPs and Activins, each monomer contacts independent surfaces of the corresponding type I and type II receptors.[49,50] Thus, ligand binding results in the assembly of a hetero-tetrameric receptor complex bound by the dimeric ligand (Figure 2C). The TGFBR2 subunits then phosphorylate a Gly/Ser-rich region (GS region) situated near the kinase domain of the TGFBR1 subunits. Binding of the small protein FKBP12 to the GS region in the unliganded TGFBR1 locks the kinase activity in an inactive state.[51] Once phosphorylated by TGFBR2, the GS region is thought to release FKBP12 and serve as a docking site for SMAD proteins as substrates of the TGFBR1 kinase.[52] Numerous small-molecule kinase inhibitors have been developed against TGFBR1 and TGFBR2 that block all TGF-β responses.[14]

Co-receptors.

Co-receptors are crucial for binding of TGF-β and several other family members to the signaling receptors (Table 1). The core protein of the membrane-anchored proteoglycan betaglycan (also known as the type III TGF-β receptor) binds TGF-β for presentation to TGFBR2. [9] This step is particularly important for TGF-β2, which has low intrinsic affinity for the signaling receptors compared to TGF-β1 and TGF-β3.[53,54] The transmembrane protein endoglin is an essential co-receptor for BMP9 and BMP10. Other co-receptors are anchored to the cell surface via glycosylphosphatidylinositol tails, including the essential Nodal co-receptors Crypto and Cryptic, and Repulsion Guidance Molecules (RGM) as co-receptors for certain BMPs.

SMAD transcription factors.

SMAD transcription factors are direct substrates of type I receptor kinases (Figure 2C). SMAD proteins consist of N-terminal (or MH1) and C-terminal (or MH2) globular domains connected by a flexible linker region.[9,10] The N-terminal domain binds to DNA whereas the C-terminal domain includes sites for SMAD interaction with type I receptors, receptor adaptor proteins, other SMADs, nucleocytoplasmic shuttling factors, DNA binding cofactors, histone acetylases such as p300 and CBP, and chromatin remodeling proteins. The type I receptors for the TGF-β subfamily primarily phosphorylate SMAD2 and SMAD3, whereas those for the BMP subfamily phosphorylate SMADs 1, 5 and 8, with crossover SMAD signaling occurring in certain contexts. These five SMAD proteins are called “receptor-regulated SMADs (R-SMADs).

In the basal state, R-SMAD proteins shuttle between the cytoplasm and the nucleus. Receptor-mediated phosphorylation targets two serine residues at the C-terminus. The

resulting pSer-X-pSer-carboxyl group mediates SMAD-SMAD binding for the assembly of trimeric complexes with SMAD4 (R-SMAD–R-SMAD–SMAD4 complexes). SMAD4 is not a receptor substrate nor is it required for R-SMAD nuclear translocation, but it is an essential participant in most SMAD-mediated transcriptional responses. The specific function served by SMAD4 remains unknown. SMAD6 and SMAD7 are inhibitory SMADs which antagonize SMAD4 and the type I receptors, respectively. TGF-β, BMP, interferon-γ, and other signals induce the expression of SMAD7 for negative feedback and antagonistic crosstalk in the pathway.[55]

Receptor-activated SMAD complexes bind to hundreds of genomic loci in the nucleus, where SMADs undergo phosphorylation at the linker region by the RNA polymerase II (RNAPII) kinases CDK8 and CDK9.[56] This stimulates the transcriptional activity of SMAD complexes while leading to further phosphorylation of the linker by glycogen synthase kinase 3β. GSK3β creates binding sites for the HECT domain ubiquitin ligases SMURF1/2 (in SMAD1 and 5) and NEDD4L (in SMAD2 and 3) to mark the activated SMADs for degradation.[56-58] Alternatively, SMADs undergo dephosphorylation by SCP1/2 phosphatases[59] and dissociation by poly(ADP-ribose) polymerase-1 (PARP-1) mediated ribosylation, to be recycled for new rounds of signaling.[60] The regulation of SMADs and RNAPII by related kinases and phosphatases suggests a close coordination in SMAD-dependent activation of RNAPII transcription. The linker region of SMADs is also phosphorylated in the cytoplasm by mitogen-activated protein kinases (MAPKs) and cell cycle CDKs.[61,62] Additional regulators of the TGF-β pathway include decoy receptors, mediators of SMAD nucleocytoplasmic shuttling, transcriptional co-activators and co-repressors, non-coding RNAs, and ubiquitination-based receptor and SMAD turnover[12] (Figure 2C).

Pathway conservation and mutation.

X-ray crystal structures for ligands, receptors, and SMAD proteins have provided a wealth of insights into the function and specificity of the TGF-β pathway, including the steps of latent TGF-β activation, ligand interactions with traps and receptors, and the interaction of SMAD with receptors, regulators, DNA, and DNA-binding cofactors.[9,10,50,63]

TGF-β and BMP ligands, receptors, co-receptors, and SMAD proteins, as well as the dichotomy of these two subfamilies are highly conserved across metazoans. The functional complementarity between the two subfamilies is manifest in many contexts, for example, in primordial germ cell development,[64] hair follicle progenitor differentiation,[65] and epithelial-mesenchymal transitions (EMT).[66] Although TGF-β gave name to the entire gene family, TGF-β is restricted to deuterostomes (vertebrates, crinoids, and sea stars) whereas BMPs and Activins are present across all metazoan phyla.[63] TGF-β pathway agonists have also emerged by convergent evolution. The rodent intestinal parasitic helminth *Heligmosomoides polygyrus* encodes a structurally unrelated TGF-β mimic (Hp-TGM) which binds to TGF-β receptors in host T cells to suppress immune attack.[67]

The central components of the TGF-β system are essential for mammalian development, as shown by gene knockouts in mice. There is no gastrulation without Nodal, and deletion of TGF-β receptors, SMAD2, or SMAD4 is embryonic lethal. As we discuss

below, loss-of-function somatic mutations in *TGFBR1, TGFBR2, SMAD2, SMAD3* and *SMAD4* are frequent in certain types of cancer, and inherited *SMAD4* mutations cause a juvenile polyposis and hemorrhagic telangiectasia syndrome with propensity to intestinal cancer. Immune dysregulation, fibrosis, and cancer are the most common diseases involving somatically mutated or otherwise altered TGF-β signaling in the adult, and hence are the focus of this review. Notably, inherited mutations in the TGF-β system in human are the cause of rare if devastating diseases of skeletal, connective, and cardiovascular tissues in human (Table 2). Inherited mutations in *TGFB1* encoding a hyperactive TGF-β1 variant cause Camurati-Engelmann disease, a debilitating syndrome characterized by abnormally thick skull and limb bones, joint deformities, and spine curvature.[68] Inherited and occasionally spontaneous mutations in *TGFB2, TGFB3, TGFBR1, TGFBR2,* and *SMAD3* cause the five known types of Loeys-Dietz aortic aneurysm syndrome, which is characterized by multiple connective tissue alterations and is highly variable in penetrance, age of manifestation, and severity of the symptoms. These alterations include craniofacial anomalies (e.g. premature skull bone fusion, hypertelorism, bifid uvula), deformities in spine, chest and foot bones, osteoarthritis, a brittle skin prone to bruising, and, most ominously, an enlarged aorta prone to bulging (aneurysm) and rupture.[69] Paradoxically, while the mutant alleles causing Loeys-Dietz syndrome encode functionally weakened protein products, the affected tissues show heightened TGF-β signaling activity perhaps resulting from an imbalance in the regulation of other branches of the TGF-β family in these tissues.

Pathway variations.

Although the type I receptors are the main substrates for the type II receptor kinases, TGFBR2 also phosphorylates PAR6 (partition defective 6) to regulate intercellular tight junctions and cell migration in epithelial cells.[70,71] A long C-terminal extension in BMPR2 mediates the activation of LIM domain kinase 1 (LIMK1) which phosphorylates cofilin to inhibit actin polymerization. In neurons this phosphorylation regulates neurite outgrowth.[72-74]

As transducers of TGF-β signals, SMADs primarily mediate transcriptional activation responses with recruited co-activators.[12] However, SMADs can also recruit co-repressors. In the absence of TGF-β, SMAD4 forms a complex with SKI (Sloan Kettering Institute proto-oncogene) and the related SKIL (also known as SnoN) which recruit histone deacetylases (HDACs) to prevent the leaky expression of TGF-β target genes under basal conditions (Figure 2D). In $CD4^+$ T cells, the SMAD4-SKI-SKIL complex inhibits expression of RORγt (encoded by *RORC*) to prevent differentiation into T helper 17 (T_H17) cells.[75] A failure of SMAD4 with SKI to inhibit intestinal $CD8^+$ T cells triggers chronic inflammatory bowel disease.[76] The SMAD4-SKI-SKIL complex is dismantled when TGF-β-activated SMAD2 and SMAD3 join this complex and the E3 ligase Arkadia causes SKI and SKIL polyubiquitination and degradation.[77,78] TGF-β activated SMADs increase the expression of SKIL and SMAD7, creating negative feedback loops.[79] Other SMAD-binding repressors include TGIF1 and TGIF2, which also interact with retinoid acid receptors and are implicated in the intermodulation of these pathways.[80,81]

Gene activation by R-SMAD not always requires SMAD4. For example, induction of the transcription factor SOX4 by TGF-β in pancreatic epithelial progenitors requires SMAD2 and SMAD3 but not SMAD4.[82] This is in line with the requirement of SMAD2 and SMAD3, but not SMAD4, for pancreas development.[83,84] Beyond transcriptional regulation, TGF-β- and BMP-activated SMADs bind pri-miRNA microRNA precursors and the Drosha/DGCR8 microprocessor and enhance pri-miRNA processing into pre-miRNA.[85]

Early studies on TGF-β and BMP signaling implicated MAPK kinase kinase 7 (MAP3K7), renamed TGF-β activated kinase 1 (TAK1) .[86] MAP3K7/TAK1 is a central signal transducer of receptors for major pro-inflammatory signals including interleukin-1β (IL-1β), tumor necrosis factor (TNF), and Toll-like receptors.[87] The MAPKs ERK1 and 2, p38MAPK, and JNK, and the phosphatidylinositol 3-kinase (PI3K) can be activated by TGF-β in cell culture.[88] The receptor adaptor proteins TRAF4, TRAF6, and SHC have been implicated in TGF-β receptor coupling to MAPK pathways[89-91] (Figure 2D). However, genetic evidence and a structural basis for MAPKs and PI3K serving as direct mediators of TGF-β receptor signaling are lacking. TAK1, ERKs, p38, JNK, and PI3K have well-established agonists of their own, including inflammatory signals, receptor tyrosine kinases, cellular stresses, and metabolic sensors. These agonists typically abound in the microenvironment of TGF-β target cells *in vivo*, raising questions about the significance of TGF-β as an activator of these pathways. In contrast, there is strong genetic and functional evidence that RAS-MAPK activation by canonical agonists or oncogenic RAS mutations is a key collaborator of TGF-β-activated SMADs in the induction of EMTs (see below). The prevailing consensus is that most effects of TGF-β are mediated by the SMAD pathway and influenced by the activity of the MAPK, WNT, and other major pathways.

Basis for contextual responses

The DNA binding activity of SMADs is essential for their role as signal-activated regulators of gene expression. SMAD binding to DNA is mediated by a protruding β-hairpin in the MH1 domain of identical sequence among R-SMADs and SMAD4.[92] SMAD2 also binds to this motif but contains a unique flexible loop that occludes the β-hairpin when in the closed conformation.[93] Regardless, all R-SMADs and SMAD4 bind with similar affinity to 5-bp GC-rich motif variants including CAGAC, GGCGC, and others.[94] Although the DNA binding activity of SMADs is necessary for their function, it is insufficient to dictate pathway-specific and cell-type specific choice of TGF-β target genes.

TGF-β target gene selection depends on the ability of R-SMADs to differentially associate with context-specific transcription factors, forming complexes that combine the DNA binding specificity of the various components. By combining with different partners, TGF-β-activated SMADs and BMP-activated SMADs gain access to different target genes and generate pathway-specific responses. And, by combining with different partners in different cell types, TGF-β-activated SMADs give rise to cell-type specific responses. The paradigm is forkhead box H1 (FOXH1, previously known as Fast1), an essential maternal factor in Nodal-driven mesendoderm differentiation during gastrulation.[95] In epiblast cells, which have a relatively nucleosome-dense chromatin, FOXH1 functions as a pioneer transcription factor that binds to cis-regulatory elements in endoderm specification genes (*Gsc*, *Eomes*,

Mixl, *Foxa2*) for activation by Nodal-driven SMADs.[93,96] FOXH1 selectively binds to SMAD2 and SMAD3, directing these factors to FOXH1-loaded loci. Consistent with a role as a pioneer factor, FOXH1 binds to DNA with extensive interactions over the minor and major grooves and shows higher affinity for its cognate sequence in nucleosomal DNA than in a linear DNA fragment.[97]

Lineage-determining transcription factors (LDTFs) like FOXH1 act as determinants of cellular responses to TGF-β signaling in many other contexts.[12] TGF-β-activated SMADs co-bind the genome with the transcription factor MyoD1 in myoblasts to regulate myogenic differentiation, with PU.1 in pro-B cells to regulate B cell differentiation,[98] and with other partners to inhibit cell proliferation and regulate immune cell functions, as mentioned below. BMP-activated SMADs pair with the zinc-finger transcription factor ZFP423 to drive ventral mesoderm specification in *Xenopus*[99] and co-occupy the genome with C/EBPα and GATA1 to drive myeloid and erythroid differentiation in hematopoietic progenitors.[100] Genome occupancy by SMADs is also determined by signal-driven transcription factors (SDTFs) as in the case of the RAS-MAPK responsive factor RREB1 discussed below, and by the accessibility of the chromatin at potential SMAD target loci.[101] SMADs additionally collaborate with factors that bind poised chromatin marks for gene activation, as is the case of TRIM33 and Nodal-activated SMADs in mesendoderm progenitors.[102,103]

TGF-β and immune regulation

Fine tuning of adaptive and innate immunity is critical to the maintenance of organismal integrity. Perturbations in this control contribute to disease pathology, and reversing these perturbations is a common therapeutical goal. TGF-β is a key modulator of innate and adaptive immunity, acting as a general enforcer of immune tolerance and a suppressor of inflammation. These functions are fundamental in TGF-β biology. An excess of TGF-β activity causes immunosuppression which supports tumorigenesis, whereas a deficit can result in inflammation leading to fibrosis. These roles were apparent in *Tgfb1* knockout mice, which die of multiorgan inflammation early in life.[104] This phenotype is substantially rescued by loss of MHC class II,[105] suggesting a critical role for TGF-β in constraining adaptive immune responses. T cell–specific deletion of *Tgfbr2* early in development causes a similar phenotype,[106,107] suggesting that TGF-β acts directly on T cells to suppress excessive adaptive immunity during early post-natal life. TGF-β appears to have a more limited role in the homeostatic regulation of T cells in adult mice. After the immediate perinatal period, TGF-β signaling in T cells is dedicated to dampening responses to pathologic stimuli.[108] However, the effects of TGF-β on immune cells are context specific and include cases of enhanced immune cell activity. For example, mice lacking TGFBR2 in T cells have reduced numbers of peripheral naive $CD4^+$ T cells.[109] TGF-β suppresses the proliferation and activation of B cells yet it stimulates their IgA class switching function.[110] The profound effects of TGF-β signaling on the immune system has been comprehensively reviewed.[8,15,111] Here we highlight the most prominent effects of TGF-β on the main components of the immune system (Figure 3).

Dendritic cells.

Various subsets of dendritic cells (DCs) play central roles in antigen presentation to $CD4^+$ T cells (by DC2 dendritic cells) and $CD8^+$ T cells (by DC1 dendritic cells) for priming of cytotoxic effector functions as well as regulation of the balance between T helper (T_H) and regulatory T cells (T_{reg})[112] (Figure 3). TGF-β regulates the function of these DC subsets. Deletion of *Tgfbr2* from DCs in mice leads to multiorgan inflammation and death by 15 weeks of age.[113] DCs lacking TGFBR2 express normal levels of MHC class II and costimulatory molecules but produce more interferon γ (IFN-γ), which reduces their ability to induce T_{reg} cells. Adoptive transfer of wild type T_{reg} cells, or inhibition of IFN-γ each only partially rescue this phenotype, suggesting that additional mechanisms are also at play. TGF-β is also important for the development of a subset of skin DCs called Langerhans cells. Targeted deletion of *Tgfbr2* of *Tgfb1* by *langerin*-Cre prevents the development of Langerhans cells.[114] In contrast to the role of DCs as T cell activators, a subtype of RORγt $^+$ antigen-presenting cells, called thetis cells (TC), induce pT_{reg} differentiation in intestinal lymph nodes during early life and require TGF-β-activating integrin αvβ8 for intestinal pT_{reg} differentiation. Loss of *Itgb8* in these cells causes colitis, suggesting that this population plays an essential role in tolerogenic antigen presentation.[115]

T helper cells.

TGF-β plays fundamental roles in regulating and balancing the differentiation of naïve T cells into specific effector subsets (Figure 3). $CD4^+$ T helper (T_H) cells support the development and function of $CD8^+$ effector T cells and fall into two subtypes distinguished by their driving transcription factors and secreted cytokines. T-bet and STAT4 drive the differentiation of naïve $CD4^+$ into T_H1 cells, which produce IFN-γ and IL-2, and support $CD8^+$ cytotoxic T cells and macrophages. TGF-β-SMAD signaling potently inhibits T_H1 differentiation through coordinated effects on at least three levels: inhibition of IL-12 receptors required for T_H1 differentiation, inhibition of the expression of T-bet and STAT4; and, inhibition of IFN-γ production by natural killer (NK) cells, thereby interfering with a positive feedback loop through which NK cell-derived IFN-γ amplifies T_H1 differentiation.[116] T_H2 cells are driven by GATA3 and STAT6, and produce IL-4, IL-5 and IL-13 to support B cells and other effector cells. TGF-β inhibits differentiation of T_H2 cells by down-regulating GATA3,[117] and inhibiting GATA3 function indirectly by inducing the expression of SOX4.[118] Balanced inhibition of both T_H1 and T_H2 cells by TGF-β is important. Although tissue inflammation and damage due to loss of TGF-β signaling in T cells is predominantly mediated by T_H1 cells, a loss of the T_H1-inducing T-bet led to multiorgan inflammation associated with enhanced T_H2 cell differentiation.[107] Disabling TGF-β signaling in $CD4^+$ cells in mammary tumor-bearing mice augmented IL-4 production in T_H2 cells (but not IFN-γ production in T_H1 cells) leading to tumor regression.[119]

One exception to the general rule of TGF-β as an inhibitor of adaptive immune responses is that TGF-β-activated SMADs cooperate with RORγt to induce the T helper 17 (T_H17) phenotype.[120] T_H17 cells, are important in immune responses to bacteria and fungi and in the development of autoimmunity. Mice lacking TGFBR2 on T cells and mice lacking the TGF-β activating integrin αvβ8 on DCs have reduced numbers of T_H17 cells and are protected from developing Experimental Autoimmune Encephalomyelitis (EAE), a disease

model that depends on T_H17 cells.[121] TGF-β-blocking antibody also protects against EAE while overexpression of active TGF-β by T cells exacerbates CNS inflammation and EAE.[122]

Regulatory T cells.

T_{reg} cells are a subset of T cells that suppress immune responses to enforce tolerance.[123] T_{reg} cells perform this role through multiple specialized effects on T helper and effector cells. The transcription factor Foxp3 drives differentiation of naive $CD4^+$ T cells into T_{reg} cells. There are two major subtypes of T_{reg} cells: natural T_{reg} (nT_{reg}), produced in the thymus in early life, and T_{reg} derived from naïve $CD4^+$ T cells in the periphery (pT_{reg}).

TGF-β positively regulates T_{reg} differentiation and activity. TGF-β enhances survival of nT_{reg} cells by suppression of proapoptotic proteins and upregulation of the antiapoptotic protein Bcl2.[124] TGF-β-activated SMADs cooperate with STAT5 and nuclear factor of activated T cells (NFAT) to induce expression of FOXP3 in naive $CD4^+$ T cells[125] (Figure 3). Mice lacking TGF-β1 or TGFBR2 in T cells display marked reductions in $FOXP3^+$ T_{reg} cell numbers in the periphery, consistent with a role for TGF-β in maintenance of these cells.[106,107,126] TGF-β can also promote retention of pT_{reg} cells in specific peripheral tissues such as the large intestine.[127] Mice lacking a particular *Foxp3* enhancer that is required for TGF-β-mediated pT_{reg} induction do not develop the severe, early-onset multiorgan inflammation seen in mice lacking all T_{reg} cells, suggesting that nT_{reg} cells are sufficient to prevent this phenotype. However, older mice lacking pT_{reg} cells develop T_H2-mediated pathology in lung and intestine,[128] indicating that pT_{reg} cells do play important roles in controlling immune responses in some peripheral tissues.

Differentiation of naïve $CD4^+$ T cells into pT_{reg} cells or T_H17 cells leads to drastically different effects on tissue inflammation. Upon initial sensing of TGF-β, naïve $CD4^+$ T cells upregulate both Foxp3 (critical for T_{reg} differentiation) and RORγt (critical for T_H17 cell differentiation). Both the local concentration of active TGF-β and the presence of additional extracellular factors are important determinants of the commitment to either pT_{reg} or T_H17 cell fate. Low TGF-β concentrations inhibit the expression of the IL-23 receptor and favor Foxp3 expression, while high concentrations in conjunction with IL-6 upregulate the IL-23 receptor and favor RORγt expression and T_H17 cell induction.[120] Foxp3 inhibits RORγt function and prevents IL-17 induction, and this is counterbalanced by IL-6, IL-21, and IL-23 to facilitate the formation of T_H17 cells. Thus, the $T_{reg,}$ T_H17, T_H1 and T_H2 states are tied to each other through a mutual regulation of their generation and function with TGF-β as a central balancing signal.

Cytotoxic T lymphocytes.

$CD8^+$ T cells mature into effector T cells, also called cytotoxic T lymphocytes (CTLs), which eliminate cancer cells and pathogen-infected cells through the release of cytolytic mediators. TGF-β dampens the proliferation and cytolytic functions of CTLs (Figure 3).[8] *In vivo*, expression of a CD2-driven or CD4-driven dominant-negative TGFBR2 construct, which reduces TGF-β signaling, leads to expansion of $CD8^+$ T cells. However, complete loss of TGF-β signaling in T cells inhibits $CD8^+$ T cell development,[107] likely due to

the requirement for TGF-β to induce the IL-7 receptor on $CD8^+$ T cells.[129] Thus, distinct effects of TGF-β signaling on $CD8^+$ T cell induction, expansion and activation appear to be quantitatively regulated, with low levels of TGF-β signaling acting as an initiator of development in the thymus, but higher levels acting as a brake to inhibit inappropriate expansion and activation in the periphery.

Consistent with a role of TGF-β as a brake on excessive $CD8^+$ T cell function, TGF-β suppresses multiple effector functions of cytotoxic T cells, inhibiting expression of perforin, IFN-γ and granzymes A and B through SMADs in partnership with ATF1.[130] Impairment of TGF-β signaling in T cells, or *in vivo* treatment with inhibitors of TGF-β activation have been consistently shown to enhance $CD8^+$ T cell killing of tumor cells and to reduce tumor growth in multiple *in vivo* models. TGF-β signaling in $CD8^+$ T cells is important in promoting apoptosis in short-lived effector cells.[122] TGF-β also induces a specialized subset of $CD8^+$ T cells residing within the single cell epithelial layer of the intestine that is important for the integrity of mucosal immune responses.[131]

Natural killer cells.

Natural killer (NK) cells are cytotoxic cells of the innate immune system. NK cells recognize target cells expressing NK cell chemotactic signals and NK receptor ligands upon viral infection or cancer-related genomic alterations. TGF-β blunts innate responses to viral infection and tumors through suppression of NK cell functions.[8,111] In addition to inhibiting IFN-γ production by NK cells, TGF-β inhibits the expression of cell-surface receptors NKG2D and NKp30, which NK cells use to recognize and kill stressed and malignant cells.[132,133] TGF-β also induces the expression mir-183 in NK cells, which reduces expression of the adaptor protein, DAP12, thus inhibiting responses to cytotoxic NK receptors including NKG2D.[134] TGF-β further suppresses NK cell activity by inhibiting activating responses to IL-15.[135] Moreover, TGF-β can contribute to immune evasion by inducing trans-differentiation of NK cells into type 1 innate lymphoid cells, which are not cytotoxic.[136,137]

Neutrophils and macrophages.

Neutrophils (also known as polymorphonuclear leukocytes) are highly prevalent among white blood cells and are responsive to infection and cancer.[138] Neutrophils can adopt an anti-tumor phenotype but also a TGF-β-dependent pro-tumorigenic phenotype (tumor associated neutrophils, TAN) that significantly impacts tumor growth and the response to immunotherapy[139] (Figure 3).

TGF-β signaling also has dramatic effects on macrophages. *In vitro*, incubation of tissue macrophages with TGF-β inhibits expression of multiple pro-inflammatory genes, including TNF, IL-12, and inducible nitric oxide synthase which are characteristic of inflammatory macrophages. In parallel, TGF-β induces expression of a suite of genes, including arginase 1 and IL-10 which are characteristic of tumor associated macrophages (TAM). Mice lacking TGFBR2 in myeloid cells demonstrate increased anti-tumor immunity, decreased tumor growth and metastasis and an increased predisposition to stroke.[140,141] Although the mechanisms underlying these events differ among models, the decrease in metastases

and predisposition to strokes both appear to be explained by increased production of pro-inflammatory cytokines by macrophages that are unable to respond to TGF-β. The persistent stimulation of myelopoiesis that accompanies chronic infection, inflammation, and cancer is associated with the emergence of myeloid-derived suppressor cells (MDSC) displaying TGF-β dependent immunosuppressive ability.[142]

Microglia are the resident macrophages of the central nervous system. Deletion of αvβ8 from neuroepithelial cells or deletion of *Tgfbr2* or *Tgfb1* in microglia each lead to the same phenotype of profound and progressive motor defects and persistence of dysmature microglia.[47] The motor defects and many of the associated anatomic abnormalities in the brains of these mice can be rescued by post-natal deletion of microglia. This phenotype seems to depend on loss of TGF-β signaling during a limited developmental window, since deletion of *Tgfbr2* from macrophages of adult mice results in many of the same changes in macrophage gene expression without dramatic functional impairment.[143]

TGF-β and fibroblast regulation

TGF-β regulates fibroblast activity in virtually all phases of the early tissue response to injury and the eventual return to normal homeostasis (Figure 4). Fibroblasts are the main producers of connective tissue matrix and play a key role in tissue repair. Fibroblasts are defined by morphological traits combined with a lack of markers for other lineages, and expression of vimentin or platelet-derived growth factor receptor-α.[144] Recent data from single cell RNA sequencing show that fibroblasts are markedly heterogeneous with distinct molecular profiles that allow these cells to perform distinct roles in different organs, and different anatomic locations within organs.[145,146] Their responses facilitate maintenance of tissue integrity and repair, but when they trigger feed-forward circuits involving TGF-β in response to chronic inflammation, fibroblasts become major contributors to pathologic tissue scarring and organ failure. Here we highlight the shared effects of TGF-β on fibroblasts in these various contexts.

Fibroblast activation.

In response to tissue injury, fibroblast subsets undergo profound changes in gene expression that either enhance or inhibit tissue inflammation, regeneration, and scarring.[145,146] Normally, fibroblast activation results in short term accumulation of fibrillar collagens and other ECM components, together with a coordinated regulation of epithelial, immune, and endothelial cells through immunomodulatory and angiogenic signals.[144] This initial response is followed by fibroblast apoptosis and removal of excess collagen to restore normal tissue architecture.[147]

TGF-β is a potent activator of different fibroblast subsets (Figure 4A). In cell culture, TGF-β induces a highly contractile phenotype associated with the expression of α-smooth muscle actin (αSMA, also known as ACTA2), multiple ECM components, and the enzymes and chaperones required for ECM assembly.[13] In this state, fibroblasts are often called “myofibroblasts”, although the expression of ECM proteins and contractile proteins is not highly correlated *in vivo*. Besides producing and assembling ECM, activated fibroblasts establish paracrine communication with epithelial cells, promote angiogenesis through

production of vascular endothelial growth factor A (VEGFA), and mobilize local innate and adaptive immune functions through the secretion of chemokines.[144,148] Thus, TGF-β-activated fibroblasts are hubs of ECM production and remodeling and of regulatory signals for epithelial, immune, and endothelial cells.

Coordinated ECM production.

Collagens are comprised of three polypeptide chains organized into a triple helical conformation. Four (of 28) mammalian collagens, (I, II, III and VI) form densely packed fibrils by covalent head-to-tail cross-linking of monomers. After injury, fibroblast-derived collagens I and III are the principal collagens that restore tensile strength and tissue integrity. Fibrillar collagens are produced abundantly in TGF-β activated fibroblasts and have a high content (10%) of proline. TGF-β supports the bioenergetic demands of collagen production by increasing the mitochondrial oxidation of glucose and glutamine. Mitochondrial redox generation promotes proline biosynthesis from glutamine for collagen production while preventing the generation of deleterious reactive oxygen species.[149] Collagen monomers undergo extensive lysine and proline hydroxylation for proper folding and assembly. Procollagen-lysine,2-oxoglutarate 5-deoxygenase 2 (PLOD2) catalyzes lysine hydroxylation and prolyl-4-hydroxylase 3 (P4HA3) catalyzes proline hydroxylation. After collagen multimers assemble, the protein folding chaperone HSP47 prevents collagen denaturation or premature fibril formation. TGF-β potently induces expression of each of these fibrillar collagens, enzymes, and chaperones[13] (Figure 4B).

After secretion and further proteolytic processing, fibrillar collagens form polymeric fibrils requiring oxidation of lysine residues for fibril cross-linking and stabilization. This step is mediated by a family of five lysyl oxidases, which are all strongly induced by TGF-β.[13] TGF-β also induces the expression of plasminogen activator inhibitor 1 (also known as serpin E1) and tissue inhibitor of metalloproteinase 3, which prevent collagen degradation by extracellular proteases. The organization of collagen fibrils is further determined by other TGF-β inducible ECM components including fibronectin, osteopontin, periostin, and biglycan.[127] Single cell RNA sequencing data suggest that the genes can be coordinately upregulated in pro-fibrotic fibroblasts in the setting of tissue fibrosis.[145,146]

Besides modulating ECM production, TGF-β increases expression of integrins on fibroblasts[150] and epithelial cells.[151] Integrins are the main receptors that cells use to detect and respond to ECM components, providing another example of TGF-β coordinating multi-cellular responses in tissue injury. TGF-β also upregulates expression of the TGF-β activating integrin, αvβ6, a process that may rapidly amplify local TGF-β signaling where needed, but that also contributes to a pathologic feed-forward circuit.

TGF-β and fibrosis.

Tissue fibrosis, characterized by chronic inflammation and accumulation of ECM components impairing organ function in kidney, lungs, liver, colon, and other organs, is a leading cause of morbidity and mortality worldwide.[152] The normal production and turnover of ECM is a complex process that requires coordination of inputs from epithelial cells, endothelial cells, innate and adaptive immune cells, and nerves (Figure 4B). Perturbation of

inputs from any of these cells can contribute to fibrotic pathology. However, it is primarily through effects on fibroblasts and epithelial cells that TGF-β participates as a prominent player in the initiation, progression, and persistence of fibrosis.

Fibrotic effects through fibroblasts.

Tissue fibrosis results from exaggerated production of collagens and other components of the ECM by tissue resident fibroblasts, often coupled with a reduction in ECM degradation and recycling by these cells. TGF-β promotes fibronectin and collagen production by both mesenchymal and epithelial cells.[153] Injection of TGF-β1 into the skin or transgenic or adenovirus-mediated overexpression of TGF-β in the lung cause extensive tissue fibrosis.[154,155] TGF-β-blocking antibodies prevent fibrosis in the skin, liver, lung, and kidney.[13] TGF-β additionally inhibits multiple secreted proteases that contribute to ECM protein degradation. Recent data from single cell RNA sequencing has identified a subset of fibroblasts that emerge in many tissues in the setting of pathologic fibrosis and are characterized by the highest levels of expression of genes encoding fibrillar collagens and other components of the pathologic ECM.[145,146] TGF-β signaling is a major upstream regulator of the gene expression signature that characterizes these cells.

Feed-forward loops involving TGF-β often contribute to fibrosis pathogenesis by exaggerating normal physiologic responses, driving their chronic persistence, and triggering inflammation (Figure 4A). By increasing both ECM production and collagen cross-linking, TGF-β increases tissue stiffness which in turn favors increased collagen production and expression of contractile proteins.[156] Activated TGF-β can drive further expression of TGF-β in both autocrine and paracrine fashions. TGF-β is also a potent inducer of the TGF-β activating integrin, αvβ6.[151] Furthermore, fibroblasts migrate toward and accumulate at regions of increased stiffness, a process that has been termed durotaxis.[157] Increased stiffness facilitates TGF-β activation through αvβ6 on epithelial cells and αvβ1 on fibroblasts, since both activate TGF-β through contraction-dependent effects on the conformation of the latent complex and this process is facilitated when cells are tethered to a stiff substrate.[44]

Fibrogenic effects through epithelial cells.

Effects of TGF-β on epithelial cells also contribute to fibrosis, as demonstrated by the observation that deletion of TGF-β receptors from epithelial cells inhibits pulmonary fibrosis induced by intratracheal delivery of bleomycin.[158] TGF-β stimulates the expression of fibrogenic factors in normal and malignant epithelial cells, which is associated with strong intratumor fibrosis in models of lung metastasis.[101] The effects of TGF-β on expression of integrin αvβ6, inhibition of epithelial cell proliferation, and induction of epithelial cell senescence and death may also facilitate fibrosis and perturb normal regeneration in injured epithelial organs.

Considerable attention has been paid to the potential role of epithelial cell senescence as a driver of tissue fibrosis, especially in lung fibrosis. Balanced cell senescence and apoptosis mediate the removal of unwanted cells during homeostasis. However, excessive senescence or apoptosis with a persistent senescence-associated secretory phenotype (SASP)

creates an inflammatory microenvironment leading to pathological repair that progresses to fibrosis.[159,160]

TGF-β in epithelial cell regulation

Epithelial barriers protect against noxious agents and fluid loss while supporting respiration, metabolite traffic, secretion, and other specialized functions. Preserving the integrity of epithelial barriers is paramount to metazoan organisms. Epithelial homeostasis and repair involve coordinated interactions between epithelial progenitors and fibroblasts, immune cells, vascular structures, and other stromal components. Adult epithelia and other tissues harbor rare pluripotent progenitors that are poised to proliferate and differentiate to replace the programmed loss of older progeny or accidental losses due to injury. TGF-β regulates the phenotypic plasticity and proliferation of epithelial progenitors and their interactions with other cell types both in health and disease conditions (Figure 5).

Regulation of phenotypic plasticity.

Phenotypic plasticity refers to the ability of biological systems to change morphology and function in response to environmental and developmental cues. Progenitor cells are adept at responding to such cues during development and injury. TGF-β profoundly influences the phenotypic plasticity of epithelial progenitors, regulating their differentiation and phenotypic transitions during tissue development, morphogenesis, and repair. TGF-β frequently exerts these effects in counterbalance with other inputs, principally from the WNT, BMP, and RAS pathways (Figure 5A). For example, during early development, Nodal-activated SMAD transcriptional complexes and WNT-activated TCF complexes bind to shared target enhancers, activating mesendoderm specification transcription factors.[161,162] Postnatal development and adult homeostasis of epithelial tissues provide numerous examples of progenitor differentiation under the control of TGF-β in combination with WNT, BMP, and RAS signaling, such as in mammary ductal differentiation and branching morphogenesis during puberty, pregnancy and lactation; and in lung and kidney morphogenesis, liver regeneration; and intestinal epithelium homeostasis.[163-166]

Epithelial-mesenchymal transitions.

Another manifestation of phenotypic plasticity is the ability of epithelial progenitors to undergo EMTs. EMTs play critical roles during development, injury repair, and disease.[167,168] In an EMT, epithelial cells lose apicobasal polarity and adhesive contacts while gaining actin stress fibers, anteroposterior polarity, motility, and remodeled contacts with neighboring cells and the ECM. EMTs are driven by transcription factors (EMT-TFs) including the zinc-finger proteins Snail (encoded by *SNAI1*), Slug (*SNAI2*), ZEB1 and ZEB2, the basic helix-loop-helix proteins Twist1 and Twist2, among others. EMT-TFs cooperatively repress epithelial genes and induce mesenchymal markers. The extent of the mesenchymal traits gained by a cell during an EMT – that is, the “completeness” of an EMT – depends on the range of phenotypic states that a particular epithelial progenitor is programed to access. After undergoing an EMT, cells can revert to an epithelial state through a mesenchymal-to-epithelial transition (MET). However, epithelial progenitors may undergo

differentiation during an EMT–MET cycle, emerging from it in a distinct developmental stage.

EMTs are triggered by cell-extrinsic signals, TGF-β being the most widespread and potent of these.[167] TGF-β induces EMTs in epithelial cells in mammary, pulmonary, renal, hepatic, and other tissues during development, injury repair, fibrosis, and cancer, whereas Nodal drives EMT in epiblast cells during gastrulation.[14] To trigger an EMT, signal-activated SMADs induce the expression of SNAI1/2 and ZEB1/2 to repress epithelial junction proteins such as E-cadherin, occludin and claudin-3, and of epithelial transcription factors such as KLF5.

Developmental and regenerative EMT programs.

TGF-β triggers EMTs as part of broad programs that include coordinated changes in cell proliferation, differentiation, and survival.[101,169] In mouse epiblast progenitors, Nodal induces the expression of EMT-TFs and mesendoderm specification transcription factors coordinating EMT and differentiation during gastrulation.[170] In adult mammary cells and in lung, breast, and pancreatic carcinoma cells, TGF-β induces the expression of EMT-TFs (e.g. Snail) and fibroblast-activating cytokines (e.g. IL-11, PDGFB), thereby coupling EMT and fibrogenesis.[101] Thus, EMTs induced by TGF-β are associated with multiple programs and outcomes in different contexts: mesendodermal differentiation in epiblast progenitors and fibrogenesis in adult epithelial cells and carcinoma cells.

In all these cases, the effects of TGF-β depend on RAS-MAPK activity.[82,171-174] The RAS effector RREB1 (RAS-responsive element binding protein 1) plays a central role in this process.[101] MAPK-phosphorylates RREB1 in the N-terminal domain to enable its binding to cognate DNA sites in target loci. RREB1 target loci include EMT-TF genes and either mesendoderm specification genes in epiblast cells or fibrogenic genes in adult epithelial and adenocarcinoma cells, depending on cell-specific chromatin accessibility patterns. The MAPK-activated, pre-bound RREB1 then enables TGF-β-activated SMADs to drive expression of these target genes (Figure 5B). RREB1 functions both as a nexus between the TGF-β-SMAD and RAS-MAPK pathways and as a link between EMT and developmental or fibrogenic gene expression programs depending on the cell context. Why these SMAD target genes and not others require RREB1, and what function RREB1 provides to enable transcription of these genes remain open questions.

Growth inhibition and cell senescence.

A strong antiproliferative effect on lung epithelial cell cultures was one of the first identified activities of TGF-β. Dissection of the mechanism led to the identification of the CDK inhibitors p27KIP1,[175] p57KIP2,[176] and p15INK4B,[177] and an interplay between p27KIP1, p15INK4B, and p21CIP1 as TGF-β regulated inhibitors of the cell cycle in lung epithelial cells.[178] TGF-β-activated SMADs partner with FOXO transcription factors to activate the expression of p15INK4B and p21CIP1 in keratinocytes and neuroepithelial cells.[179,180] Other antiproliferative responses such as the down-regulation of the pleiotropic growth promoting transcription factor MYC frequently accompany the induction of CDK inhibitors by TGF-β.[181] The extent of the growth inhibitory effect of TGF-β on epithelial cells

depends on the cell type. TGF-β induces a complete arrest of cell cycle in lung epithelial cells in culture, which allowed the isolation of TGF-β receptor mutants to elucidate the mechanism of receptor activation.[182-184] However, other epithelial cell types show only mild growth inhibitory responses to TGF-β, and mice with conditional ablation of *Tgfbr2* in the skin and gastrointestinal tract do not show extensive hyperplasia except at sites of tissue stress.[185]

TGF-β induces senescence of epithelial cells in various contexts. Senescence is a process by which cells irreversibly cease to proliferate and typically acquire altered secretory profiles.[186] Senescent cells undergo dramatic transcriptional changes including expression of a suite of secreted proteins, including SASP cytokines, growth factors and proteases. Senescence involves expression of the cell cycle inhibitor p21CIP1, which is induced by TGF-β.[187]

TGF-β duality in cancer: Tumor suppression

TGF-β has been implicated in the progression of many types of cancer, but the most detailed body of knowledge comes from the analysis of its dual role in breast, colorectal, pancreatic, and lung adenocarcinomas (Figure 6). In these tumors, TGF-β can suppress or promote carcinoma progression depending on the stage of the disease. TGF-β induces apoptosis in early-stage epithelial progenitors harboring oncogenic RAS mutations, but it promotes tumorigenic immunosuppression in cancer cell clones that escape the suppressive effects of TGF-β by inactivating this pathway or decoupling it from apoptosis. Moreover, carcinoma cells that decouple the TGF-β pathway from apoptosis can respond to TGF-β with invasion, dissemination, and metastasis, thus leading to tumor progression instead of tumor suppression.

Tumor suppressive apoptosis.

Conditional ablation of *Tgfbr2* or *Smad4* in pancreatic, intestinal, oral mucosa, and skin epithelia in mice does not interfere with the development of these tissues and causes only a mild hyperplasia. However, loss of *Tgfbr2* accelerates tumor progression when these tissues harbor *KRAS* or *HRAS* oncogenes.[83,185,188,189] Genetically engineered mouse models of pancreatic ductal adenocarcinoma (PDAC)[83,188] and CRC[190-192] showed that the TGF-β pathway interferes with the transition of pre-malignant cells to the carcinoma stage during malignant progression. In line with these observations, pre-malignant pancreatic, intestinal, and skin epithelial progenitors with *KRAS* or *HRAS* mutations undergo apoptosis in response to TGF-β, accounting for the tumor suppressive role in these cancer models.[82,83,185]

TGF-β receptors are not directly coupled to apoptosis effector molecules. How TGF-β becomes a potent inducer of apoptosis was illuminated by studies in normal and malignant pancreatic epithelial progenitors.[82,101] In normal progenitors, TGF-β induces expression of SOX4, which pairs with KLF5 as a transcriptional partner to specify a pancreatic epithelial progenitor state. In pre-malignant pancreatic cells with mutant *Kras*, the dysregulated MAPKs strongly activate RREB1, which pairs with TGF-β-activated SMADs to trigger an intense induction of SNAIL expression and an EMT (Figure 5B). As a transcriptional repressor of epithelial genes, SNAIL inhibits KLF5 expression, driving SOX4 to activate

the expression of *Bim* and other pro-apoptotic genes. Here, an otherwise normal TGF-β/RAS dependent EMT becomes pro-apoptotic owing to a conflict of cell fate signals: a pro-epithelial SOX4 and an overexpressed pro-mesenchymal SNAIL. Of note, *RREB1* is frequently downregulated or genetically inactivated in PDAC.[193,194]

Prevention of tumorigenic inflammation.

In addition to these direct tumor suppressive effects on pre-malignant cells, TGF-β acts as an indirect tumor suppressor by preventing inflammatory responses that cause cancer predisposition. TGF-β is a key suppressor of inflammatory responses through coordinated effects on different immune cell types.[15] Disruption of this function can lead to chronic inflammation, which predisposes pre-malignant cells to progress to tumor formation. This is particularly evident in the gastrointestinal tract. TGF-β prevents intestinal inflammatory reactions to the commensal microbiota by exerting tolerogenic effects on immune and epithelial cells.[195] Dysregulation of TGF-β signaling is linked to the pathogenesis of ulcerative colitis, a cancer predisposition condition.[196] *Tgfb1* mutant mice develop a lethal multifocal inflammatory disease as a prominent phenotype and are highly susceptible to developing colitis.[104,197] Mice with TGF-β pathway mutations in T cells or dendritic cells also show inflammatory phenotypes[8,111] and a higher incidence of gastrointestinal carcinomas.[198,199]

Is growth inhibition tumor suppressive?

The cytostatic effects of TGF-β through the expression of CDK inhibitors and other factors in epithelial cells have been viewed as tumor suppressive effects. However, this notion is challenged by different lines of evidence. In mouse models of PDAC, the tumor suppressive effect of TGF-β is based on apoptosis, not growth arrest of KRAS-mutant progenitors.[83] In mouse models of HER2+ breast cancer, expression of a constitutively active *Tgfbr1* transgene in mammary epithelial cells decreased the growth rate of mammary tumors but accelerated lung metastasis from these tumors.[200] The growth inhibitory effects of TGF-β on epithelial cells are reversible and unlikely to provide an effective mechanism for the sustained suppression of tumor growth. In fact, disseminated breast cancer cells[201] and lung cancer cells[202] enter a growth arrested state in response to TGF-β in models of metastatic dormancy. As we discuss below, metastatic dormancy protects stem-like progenitor cells from immune surveillance, preserving these cells for eventual relapse. In effect, TGF-β-mediated immune evasive growth arrest is a strategy for metastatic progression in these models. Although the antiproliferative effects of TGF-β may dampen the growth of some tumors, there is no compelling evidence that these effects suppress tumor development or progression.

Escaping tumor suppression.

Loss-of-function mutations in *TGFBR2* were identified in human colon cancer cells with microsatellite instability,[203] deletions in *SMAD4* in PDAC,[204] and missense mutations in *SMAD2* in colorectal carcinomas (CRC)[205] shortly following the identification of these genes. Subsequent studies identified recurrent genetic alterations in other core components of this pathway, including *TGFBR1*, *SMAD3*, and the Activin receptors *ACVR1B* and *ACVR2A* which signal through the same SMADs. Analysis of pathway mutations, copy-

number changes, and other genetic alterations in 9,125 tumors profiled by The Cancer Genome Atlas in 33 cancer types showed that these alterations are frequent in carcinomas of the pancreas, gastrointestinal tract, lungs, breast, bladder, prostate, endometrium, and head and neck, as well as in low-grade gliomas and diffuse large B-cell lymphomas.[206] Most of these alterations are loss-of-function events selected during tumor progression. Gastrointestinal carcinomas and uterine carcinosarcomas with microsatellite instability (MSI) and high mutational loads frequently harbor *TGFBR2* and *ACVR2A* inactivating mutations in prone microsatellite-like sequences.[203,206]

In the microsatellite-stable common subtype of CRCs, which are initiated by mutations in the WNT pathway and *KRAS,* alterations in *SMAD4* and *TGFBR2* occur late during adenoma-carcinoma progression.[207] Similarly, *SMAD4* inactivating mutations during PDAC progression accumulate after the emergence of early-stage lesions containing *KRAS* mutations which are indispensable for PDAC initiation.[208] This is consistent with the evidence from mouse models of CRC and PDAC mentioned above, showing that TGF-β interferes with the transition to the carcinoma stage during tumorigenesis.

Restoring SMAD4 expression in PDAC tumor cells that developed with *Smad4* loss makes these cells undergo massive apoptosis in response to TGF-β.[82,209] Although many PDACs elude TGFβ-induced tumor suppression through inactivating mutations in TGF-β receptors and SMADs, nearly half of PDAC tumors and larger proportions of other tumors retain a functional TGF-β pathway. PDACs with intact TGF-β signaling components lack apoptotic responses to TGF-β owing to alterations in the expression of ID1 family members, which are core transcriptional regulators in PDAC progenitors. In these cells, TGF-β-SMAD signaling upregulates ID1 expression, which decouples EMT from apoptosis by neutralizing the effects of SOX4. PI3K/AKT signaling and mechanisms linked to low-frequency genetic events additionally converge with ID1 to prevent TGF-β-dependent apoptosis in PDAC.[209]

In contrast, human head and neck squamous cell carcinomas (HNSCCs) show loss of *SMAD4* at an early-stage, and mice with *Smad4* deletion develop spontaneous HNSCCs and genomic instability, suggesting that loss of SMAD4 is a tumor-initiating event in this context.[210] In these tumors, SMAD4 loss is associated with decreased expression of BRCA1 and an elevated mutational burden accompanied with sensitivity to PARP inhibitors. These findings, which are also supported by results in a mouse model, suggest that SMAD4-dependent signaling suppresses the emergence of HNSCC tumors by enhancing BRCA1-dependent repair of double-strand DNA breaks.[210,211] Of interest, germline *SMAD4* mutations give rise to a juvenile polyposis syndrome with inflammatory gastrointestinal polyps that may progress to carcinoma.[212,213] *SMAD4* haploinsufficiency in this context may predispose to tumorigenic inflammation and/or lead to tumor progression through the eventual loss of the wild type *SMAD4* allele.

TGF-β duality in cancer: Tumor progression and metastasis

Cancer cells with the TGF-β pathway decoupled from tumor suppressive effects can respond to TGF-β with effects that promote various phases of the metastatic process, from invasion, dissemination, immune evasive dormancy, and organ-specific colonization

(Figure 6). Moreover, independently of the status of this pathway in the cancer cells, TGF-β can additionally promote tumor progression and relapse through effects on the stroma, notably immunosuppressive and desmoplastic effects, which limit the effectiveness of immunotherapy. The effect of TGF-β on these functions varies depending on the tumor type. The extant knowledge comes from mechanistic analysis of mouse models and correlative evidence in human patient samples, showing that TGF-β promotes tumor progression, metastasis, and resistance to therapy.

Immunosuppressive tumor microenvironments.

TGF-β has a multitude of effects on virtually all the adaptive and innate immune cell types and most of these effects enforce tolerance and prevent autoimmune responses. Not surprisingly, one after the other, these effects have been implicated in the generation of an immunosuppressive tumor microenvironment (TME) that favors tumor progression and metastasis and limits the effectiveness of immunotherapy.[8,15,111] Immune checkpoint inhibitors, which unleash endogenous anti-tumor immunity by inhibiting pathways that normally restrain $CD8^+$ T cell-mediated tumor cell killing, have revolutionized treatment of a wide variety of cancers, but this approach remains ineffective for most patients. Approximately half of solid tumors can be characterized histologically as “immune excluded”, in which $CD8^+$ T cells accumulate around the periphery of the tumor in a dense collagen-containing band but fail to infiltrate the tumor itself. These tumors are generally resistant to checkpoint inhibitors and exhibit a gene expression pattern suggestive of increased TGF-β signaling. Recent evidence from syngeneic murine models suggests that immune checkpoint sensitivity can be induced in some immune-excluded tumors by treatment with TGF-β inhibitors. This treatment promotes $CD8^+$ T cell infiltration into the core of the tumor, increases granzyme B and interferon production by infiltrating T cells and in many cases leads to induction of long-term anti-tumor immunity.[192,214,215] Similar effects can be caused by inhibition of the TGF-β-activating αvβ8 integrin,[216-218] which can be expressed on tumor cells themselves or on $CD4^+$ T cells in the tumors.[216-218] The mechanisms underlying synergy between checkpoint inhibitors and blockade of TGF-β signaling or activation are the focus of current investigation, and these pre-clinical results have led to active clinical trials evaluating TGF-β or αvβ8 integrin inhibitors in patients with cancer.

Tumorigenic effects through CAFs.

Fibroblasts present in tumors are called cancer-associated fibroblasts (CAFs). CAFs are an important component of the TME and influence tumor progression through ECM remodeling and paracrine signaling which are characteristic of activated fibroblasts. There are no specific markers distinguishing CAFs from the activated fibroblasts participating in wound healing or fibrosis. The consensus is that most CAFs result from the activation, likely dysfunctional, of local fibroblasts.[144] Distinct CAF populations distinguished by means of single-cell analysis exhibit either a TGF-β-driven matrix-producing contractile phenotype or an immunomodulating secretome.[219-221] Similar to TGF-β activated fibroblasts in wound healing and fibrotic diseases, CAFs are highly effective at producing and remodeling ECM within the TME. This process generates intra-tumoral fibrosis and ECM stiffness, known enhancers of carcinoma cell growth and invasion.[222-224] Additionally, CAFs are a source

of IL-6 and TGF-β itself, which are immunosuppressive in the TME, and VEGF which drives tumor angiogenesis.[144] Beyond these effects on CAFs in carcinomas, TGF-β has been shown to promote growth of other tumor types such as gliomas through the induction of autocrine and paracrine secretomes.[225]

Metastatic dissemination.

In cancer cells that retain a functional TGF-β pathway, TGF-β can induce stimulate migration into, and out from blood capillaries for metastatic dissemination (Figure 6). Induction of an EMT by TGF-β-SMAD signaling accompanied with increased motility, invasiveness, and metastasis has been noted in HRAS-driven cutaneous squamous cell carcinomas induced by carcinogens[226] or by genetic engineering in mice.[227] TGF-β signaling in cancer cells in an orthotopic mammary tumor model promoted local invasion and hematogenous dissemination by inducing EMT and a switch from cohesive cell motility to single-cell motility.[228] TGF-β can also function as a promoter of extravasation of circulating tumor cells. The activation of TGF-β stored in blood platelets coating colon cancer cells induced EMT and extravasation of cancer cell in the lungs of mice.[229] To be determined is Whether TGF-β-induced EMTs promote extravasation by maintaining circulating carcinoma cells in an EMT state or by inducing an EMT in circulating epithelioid carcinoma cell clusters that lodge in capillaries remains to be determined. Additionally, TGF-β-rich breast primary tumors release cancer cells expressing angiopoietin-like 4, a TGF-β-inducible mediator of extravasation and lung metastasis.[230,231]

Immune evasive dormancy.

Disseminated cancer cells suffer extensive attrition due to immune attack, physical barriers, and metabolic stresses.[232] Metastasis typically develops after a dormancy period lasting from months to decades, implying that cancer cells that survive the stress of dissemination enter a period of dormancy.[233] In mouse models of metastatic dormancy, disseminated tumor cells localize to perivascular regions where they are able to remain dormant for many months.[201,234] Developmentally, these cells correspond to a stem-like early progenitor stage (e.g., SOX2+ stage in the case of LUAD) and are primed to enter quiescence in response to TGF-β and autocrine WNT inhibition.[202,234,235] During the dormant phase, these metastasis-initiating progenitors are in equilibrium between an immune-privileged quiescent state and a proliferative state liable to immune-mediated clearance (Figure 6). Quiescent cells downregulate MHC class I molecules,[236,237] NK receptor ligands[234] and STING (stimulator of interferon genes)[202] to evade elimination by the immune system. Dormant cancer cells reentering the cycle re-express these mediators of immune recognition and consequently are cleared by the combined action of T cells and NK cells. Metastatic outbreaks succeed when proliferative clones elude immunity or when immune surveillance subsides.[202] Entry into proliferative quiescence in response to TGF-β appears to be a specific property of early-stage malignant progenitors and not of their developmentally more advanced progeny that constitute the bulk of the tumor mass. Thus, TGF-β-induced dormancy may protect disseminated metastatic progenitors from immune surveillance, preserving these cells for long-term relapse. Interestingly, an immune evasive state is also manifest in normal stem cells in hair follicles and muscle, which remain quiescent, but not in intestinal, mammary, or ovarian proliferative stem cells.[238] Evolutionarily, immune

evasive quiescence of adult stem cells may serve to protect longevity of these cells as they accumulate neoantigens during the organism's lifespan.

Stromal cooption during metastatic colonization.

After infiltrating distant organs and resisting during dormancy, disseminated cancer cells may initiate metastatic outbreaks. As the metastatic tumors develop, TGF-β in the TME may resume its pro-tumorigenic roles as a mediator of immune suppression, EMT, invasion, and further dissemination.

In each tumor type metastasis follows a stereotypic pattern of affected organs and timing.[239] The organ distribution of metastasis is a function of many factors including circulation patterns, intrinsic resilience of disseminated cancer cells to diverse tissue microenvironments and their resident immunity, and the ability to express and select for organ-specific colonization traits. Such traits provide cancer cells with the ability to adapt to different metabolic environments, avert hostile surveillance, extract survival signals from the TME, or coopt the host stroma for aggressive outgrowth as a metastatic colony. In the primary tumor and during prolonged dormancy in host organs upon dissemination, the TME selects for organ-specific colonization traits that a cancer cell population may be able to express as a function of its origin. The emergence of organ-specific metastatic traits largely arises from non-genetic changes, although these changes may indirectly result from mutations in epigenetic regulators.[240]

TGF-β augments the expression of various mediators of organ colonization in cancer cells. This is particularly evident in the case of osteolytic bone metastasis from triple-negative breast cancer, which is driven by TGF-β released during bone matrix resorption.[241] TGF-β-SMAD signaling in bone-tropic breast cancer cells stimulates the expression of several mediators of osteolytic bone metastasis[242] including parathyroid-related protein,[243] IL-11[244] and Jagged 1.[245] In the lungs, TGF-β- and RAS-dependent activation of a fibrogenic EMT in LUAD cells stimulates lung metastasis,[101] whereas TGF-β-dependent expression of ID1 in disseminated breast cancer cells favors the reentry of these cells into the cell cycle.[246]

Approaches and challenges to therapeutically targeting TGF-β

Pharmaceutical companies have been working for many years to develop inhibitors of TGF-β expression, activation or TGF-β signaling for treatment of a variety of diseases, including cancer, immune dysregulation, fibrosis, and developmental disorders. Numerous TGF-β inhibitors are being tested in ongoing clinical trials and others are in various stages of preclinical development. These inhibitors fall into several classes (Figure 7) including antibodies that prevent the activation of latent TGF-β; antibodies and receptor ectodomain proteins that trap TGF-β or block the TGF-β receptors; small molecule inhibitors of TGF-β receptor kinases. Other approaches seek to mitigate TGF-β interference with immunotherapy by incorporating a dominant-negative TGFBR2 construct or TGF-β antisense oligonucleotides into engineered autologous CTL vaccines or chimeric antigen receptor (CAR) T cells. To specifically target TGF-β near cells of interest, fusion proteins have been created that consist of a TGF-β trapping receptor ectodomains fused to anti-CD4 antibody (to block TGF-β around T cells) or to antibodies against immune checkpoint

molecules such as PDL1, CTLA4 or CD37. Detailed accounts on the development and status of these various therapeutic agents are available.[8,14,225,247]

Targeting TGF-β ligands and receptors.

Many of the molecules targeting TGF-β or its receptors are highly effective at blocking TGF-β signaling. However, obstacles need to be overcome to realize the therapeutic potential of these strategies. Some obstacles relate to specificity. The small-molecule inhibitors of TGF-β receptors primarily target the ATP-binding pocket of the receptor protein kinase domain. As a result, these inhibitors present the challenge of achieving specificity for TGF-β receptors versus other members of this receptor kinase family. Other obstacles relate to pleiotropy. Because of the many critical roles TGF-β family members play in normal development and tissue homeostasis, potent inhibition of all TGF-β signaling would likely lead to unacceptable toxicity. This possibility is underscored by the embryonic or early post-natal lethal phenotypes of mice with inactivating mutations in each of the three mammalian TGF-β isoforms, and by the severe auto-immune phenotypes of mice with severely impaired TGF-β signaling in T cells or dendritic cells. These dramatic effects might all be consequences of critical developmental roles for TGF-β signaling and do not necessarily preclude treatment of adults with TGF-β inhibitors. Indeed, some of the TGF-β inhibitors currently under development have been given to hundreds of patients without signs of severe toxicity.[15,225]

Nevertheless, monkeys, rats and mice have developed thickening of cardiac valves and some patients treated with TGF-β inhibitors developed low grade skin cancers, consistent with the known effects of TGF-β in valve development and as a brake on epithelial carcinogenesis, raising concerns about the safety of this approach. Furthermore, because latent forms of each TGF-β isoform are expressed at high concentrations in many tissues of healthy adults, antibodies or other biologics targeting TGF-β isoforms could be limited in effectiveness because of the likelihood they would be sequestered by irrelevant TGF-β tissue stores. This might be one reason for the apparent lack of serious toxicity for several anti-TGF-β biologics that entered clinical trials thus far and for their surprisingly limited efficacy. Cell-permeable small-molecule inhibitors of TGF-β receptor kinases could overcome the challenges of large extracellular stores of latent TGF-β, but those developed so far have generally had quite short *in vivo* half-lives and would not, in any case, overcome the challenges of the many roles TGF-β signaling plays in maintaining normal tissue homeostasis.

Targeting TGF-β activation.

Renewed confidence on the viability of therapeutically targeting TGF-β comes from the increasing knowledge about the specific mechanisms of TGF-β activation, and the ability to target TGF-β inhibition to a specific cellular context or specific downstream responses. Several strategies to get around these problems are in various stages of development, most aimed at increasing the precision for inhibiting specific pathologic functions of TGF-β without targeting beneficial homeostatic effects. One such strategy has focused on the pathways for integrin-mediated TGF-β activation. As noted above, three integrins, αvβ1, αvβ6 and αvβ8 are involved in the activation of latent TGF-β stored in the ECM or

on cell surface.[27,34,41] TGF-β can also be activated by integrin-independent pathways, such as binding to thrombospondin, narrowing the scope, and thus the potential toxicity of therapeutic targeting. Importantly, each of these integrins is expressed in a distinct, limited number of cells and at low copy number, further overcoming the challenges of indiscriminately targeting all TGF-β isoforms in every tissue. For example, αvβ6 is restricted in its expression to epithelial cell,[32] and is generally expressed at very low levels in healthy epithelia but dramatically upregulated in response to injury and at sites of fibrosis.[248] Currently, there are at least three drugs targeting TGF-β activating integrins in active clinical trials: a dual small molecule inhibitor of αvβ1 and αvβ6 in phase 2 clinical trials for treatment of pulmonary fibrosis and primary sclerosing cholangitis, a small molecule inhibitor of avβ1 in a phase 1 trial for liver fibrosis in the setting of non-alcoholic steatohepatitis, and a humanized monoclonal antibody targeting the αvβ8 integrin in a phase 1 study for enhancing responses to immune checkpoint inhibition in cancer. All these studies are in early stages, but recently released data for a 12-week phase 2 study of the αvβ1/αvβ6 inhibitor in 67 patients with idiopathic pulmonary fibrosis showed no significant on-target toxicity and an apparent dose dependent efficacy in slowing the rate of loss of lung function and progression of radiographic evidence of fibrosis. However, other drugs targeting TGF-β activating integrins have been withdrawn because of pre-clinical or clinical adverse events, so conclusions about the safety and efficacy of this approach will need to await the results of longer and larger clinical trials.

Targeting downstream mediators.

Other strategies are based on more precisely targeting inhibition of TGF-β signaling to the cells that drive specific disease pathology. One encouraging example of this approach was the identification of epigallocatechin gallate (EGCG) as a natural product found in several fruits and green teas which protects mice from bleomycin-induced pulmonary fibrosis.[249] Efforts to identify its mechanism of action showed that when EGCG covalently inhibits lysyl oxidase homolog 2 (LOXL2), the compound itself is converted into a potent, irreversible inhibitor of TGFBR2. Importantly, LOXL2 is not broadly expressed, with substantial expression in normal tissues restricted to fibroblasts. EGCG is thus effectively a specific inhibitor of TGF-β signaling in fibroblasts. Biopsies from patients who received EGCG showed significant reductions in extractable collagen and other indicators of active fibrosis compared to patients treated with placebo.[250]

Several efforts are underway to treat TGF-β-driven diseases more precisely by targeting key steps downstream of TGF-β signaling that might contribute more to pathology than to normal homeostatic functions. One example is a monoclonal antibody that blocks connective tissue growth factor, a TGF-β-induced protein that has been proposed to mediate some of the pro-fibrotic effects of TGF-β.[251] Another is the development of drugs targeting nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX4), a TGF-β-induced protein that catalyzes the reduction of molecular oxygen to hydrogen peroxide. NOX4 expression is increased in activated fibroblasts and inhibition of NOX4 reduces collagen production from these cells *in vitro* and inhibits pulmonary fibrosis *in vivo*.[252] Pulmonary fibrosis is a disease associated with aging and *in vivo* studies in mice suggest that NOX4 upregulation persists longer in fibroblasts from bleomycin-treated aged mice. Aged mice also exhibit more prolonged

fibrosis after a single dose of bleomycin than young mice do, and inhibition of NOX4 inhibits fibrosis persistence in aged mice.[253] Based on these findings, NOX4 inhibitors are currently under development for treatment of pulmonary fibrosis.

Enhancing cell therapies.

Finally, the advancement of cellular therapeutics opens new avenues to translate what has been learned about TGF-β signaling into novel therapeutic interventions. For example, incorporation of a dominant negative TGFBR2 into CAR-T cells holds promise to overcome the suppressive effects of TGF-β on immune responses within the TME, while minimizing adverse effects of inhibiting the homeostatic roles that TGF-β plays to suppress pathologic immune responses in tissues unaffected by the tumor. Recent early reports suggest that such an approach might be feasible for both hematologic[254] and solid tumors.[255] Similarly, new advances in synthetic biology, such as the development of synthetic Notch receptors that allow localized and tightly regulated delivery of genetically encoded therapeutics at precise sites of tissue pathology,[256] open the possibility for locally presenting TGF-β inhibitors at sites of non-malignant TGF-β-driven diseases such as tissue fibrosis.

Summation and perspectives

We have highlighted how TGF-β functions as a central regulator of tissue homeostasis throughout the lifespan of metazoan organisms. To effectively play this role, the TGF-β pathway engages many other regulatory inputs in distinct cellular contexts, generating an array of molecular and behavioral outputs. Although in most situations these outputs play critical roles in healthy development and maintenance of normal organ function and the effective repair of tissue injury. However, the centrality of TGF-β signaling also sets it up for deviant feed-forward circuits that contribute to progressive pathology and disease. Continued focus on understanding the operating logic of the TGF-β system will identify additional effective strategies to precisely target pathologic roles of TGF-β while preserving its many critical functions.

Recent progress in this field teaches us that the overall response of a tissue to TGF-β is defined by the integrated TGF-β responses of its constituent cell types. The TGF-β response of each cell type in turn consists of multiple coordinated effects on diverse cellular functions –for example, effects on collagen biosynthesis and cytokine production coupled with phenotypic activation and metabolic adaptation in fibroblasts; effects on EMT coupled with fibrogenic signaling, growth inhibition, and differentiation in epithelial progenitors; and, effects on differentiation coupled with immune regulatory functions in T cells. Moreover, variables such as aging, metabolism, endocrine signals, and microbiomes, which impact immunity, inflammation, and stem cell pools, likely influence how tissues read TGF-β signals. How TGF-β response programs are integrated and affected by these variables warrants further investigation.

Out of necessity, this review has focused on fibroblasts, epithelial and immune cells as the most abundant TGF-β target cells, and on fibrosis and cancer as the most common diseases of the TGF-β system. However, in so doing, we have sidestepped other important TGF-β target cells and the diseases that result from defective TGF-β signaling in these cell types

(Figure 1). The rare if serious congenital diseases linked to TGF-β and other cytokines in this family (Table 2) substantiate this point. As components of TGF-β target tissues, endothelial, neural, skeletal, connective tissue, and smooth muscle cells also participate in integrated multicellular responses to TGF-β. These cell types and their responses are subject to the same principles discussed above. Advances in understanding the roles of TGF-β in common diseases such as fibrosis and cancer and their potential treatments might be relevant to these other pathologies as well.

The growing understanding of TGF-β signaling in health and disease has created opportunities for intervention in difficult to treat diseases that remain major sources of morbidity and mortality throughout the world. Although systemically administered drugs that broadly inhibit TGF-β signaling are challenging to develop because of the narrow window between efficacy and toxicity, many promising strategies have emerged that use these new biologic insights to more precisely target pathologic TGF-β functions. These strategies nurture considerable optimism about the potential impact of targeted inhibitors of TGF-β activation and signaling for treatment of multiple currently challenging diseases.

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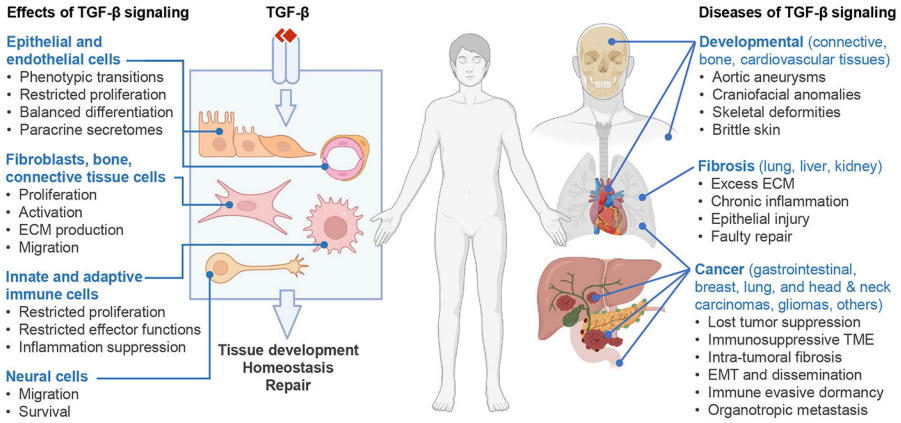

Figure 1. TGF-β in health and disease

TGF-β guards tissue homeostasis through multiple effects on different cell types. Although TGF-β signals through a common receptor and a set of transcription factors in all cells, it triggers different effects on diverse cellular functions depending on the cell type and tissue environment. Epithelial cells, fibroblasts, immune, vascular, connective, and neural cells are important TGF-β targets, and their coordinated responses determine the overall effect of TGF-β on a tissue. The whole tissue, more than any of the constituent cell types, is the target of TGF-β, and preserving tissue integrity is the ultimate output. TGF-β response programs drive embryo development and promote tissue homeostasis and injury repair in the adult. Congenital defects in TGF-β signaling cause rare yet serious developmental syndromes, and somatic alterations of this pathway underly common forms of fibrosis and cancer.

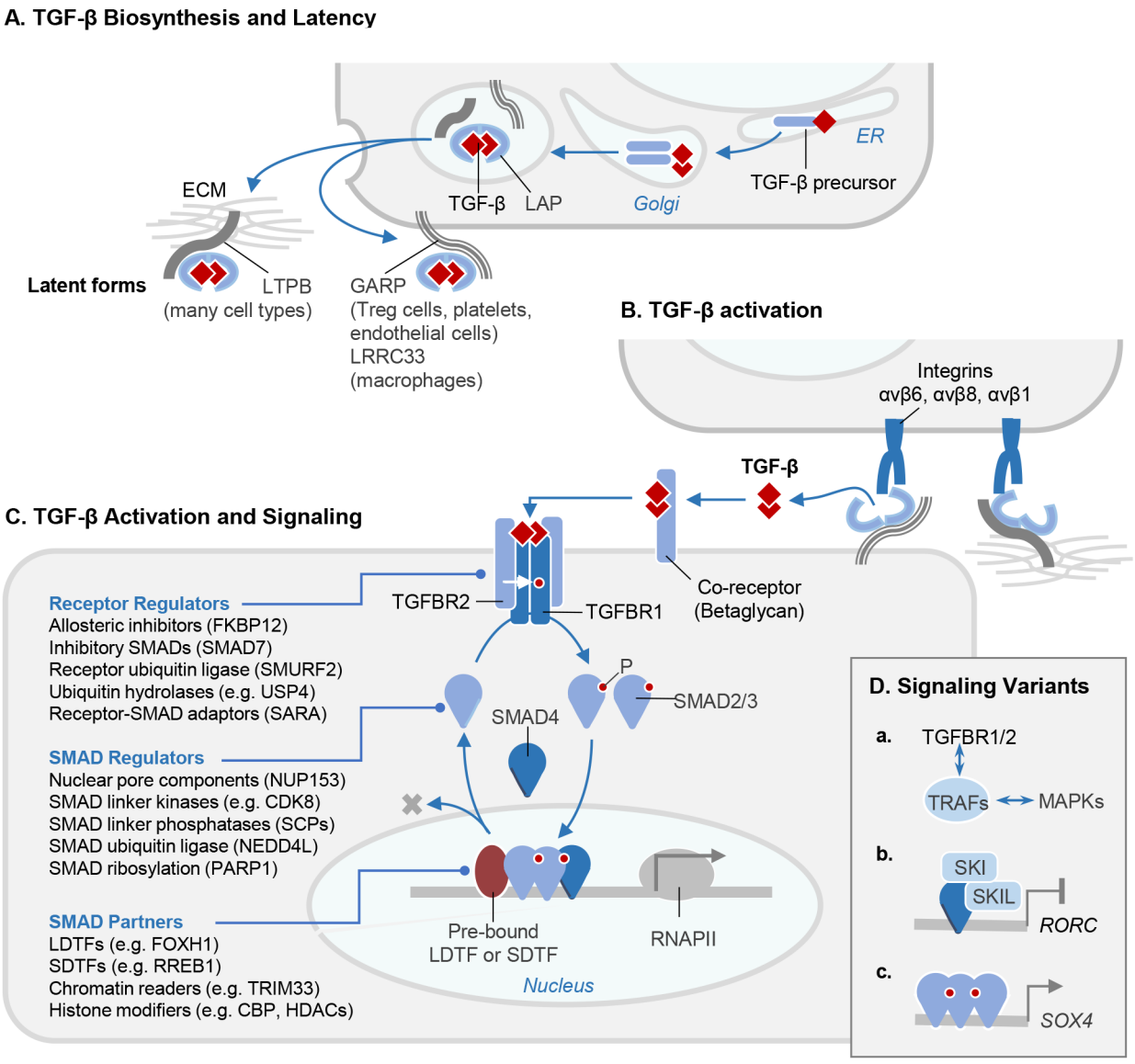

Figure 2. The TGF-β signaling pathway

A. TGF-β cytokines are generated by cleavage of the dimeric C-terminal domain of a biosynthetic precursor in the Golgi. The mature cytokine remains sequestered by non-covalent binding to the N-terminal domain of the precursor, or latency-associated peptide (LAP). LAP in this complex becomes disulfide-linked to the latent TGF-β binding protein (LTBP), which is deposited in the extracellular matrix (ECM) after secretion. Alternatively, in the indicated cell types, LAP in the TGF-β complex becomes disulfide-linked to the membrane-anchored proteins GARP or LRRC33 and retained on the cell surface. **B.** Activation of latent TGF-β involves binding of LAP to αv integrins on adjacent cells, leading to a conformational change that releases the captive TGF-β for binding to receptors. **C.** The membrane proteoglycan Betaglycan functions as a co-receptor that collects TGF-β for presentation to signaling receptors. TGF-β binds to two pairs of transmembrane serine/threonine protein kinases known as TGFBR1 (type I receptor) and TGFBR2 (type II

receptor), to assemble the receptor complex. In this complex, TGFBR2 phosphorylates and activates the TGFBR1 kinase, which binds and phosphorylates (P) the transcription factors SMAD2 and SMAD3. On phosphorylation, these SMADs form trimeric complexes with SMAD4 and accumulate in the nucleus to bind and transcriptionally activate target loci. Recognition of these loci by the SMAD complex frequently requires molecular interaction with lineage-determining transcription factors (LDTF) or signal-driven transcription factors (SDTF). The signaling cycle ends with SMAD dephosphorylation and dissociation from DNA for another round of signaling, or with SMAD polyubiquitination and degradation. Each step in the pathway is controlled by different classes of regulators, the most prominent of which are listed (with examples). **D.** Variant versions of this pathway include: (**a**) TGF-β receptors links with MAPKs through TRAF adaptor proteins; (**b**) SMAD4 recruitment of a SKI-SKIL repressor complex to certain target genes (e.g. *RORC* in T_H17 helper T cells) to prevent leaky transcription in the absence of TGF-β; and (**c**) SMAD4-independent activation of certain target genes (e.g. *SOX4* in pancreatic epithelial progenitors) by SMAD2 and SMAD3.

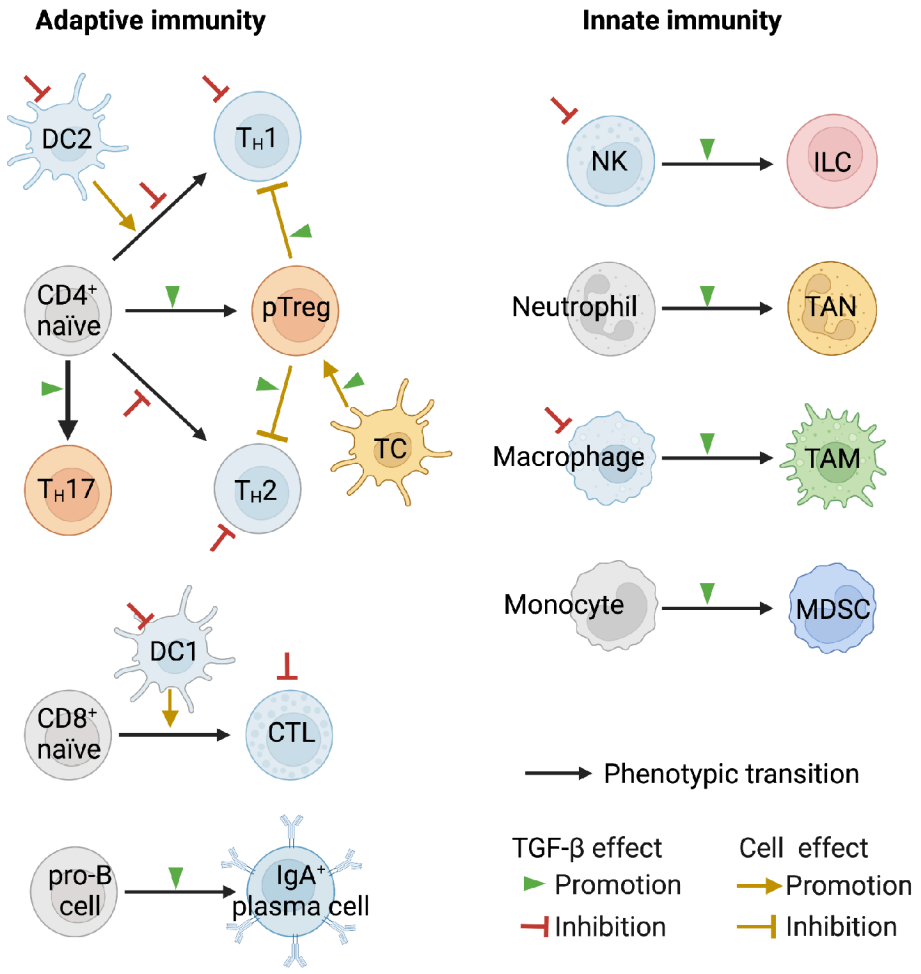

Figure 3. TGF-β and immune regulation

Scheme of the main classes of immune cells and their regulation by TGF-β in the adult. TGF-β is a critical modulator of both adaptive and innate immunity arms, acting as a general enforcer of immune tolerance and a suppressor of inflammation. In the adaptive arm, TGF-β inhibits the maturation of naïve $CD4^+$ T cells into T_H1 and T_H2 T helper cells and of naïve $CD8^+$ T cell into cytotoxic T lymphocytes (CTL). TGF-β exerts these effects through direct inhibition of $CD4^+$ and $CD8^+$ maturation and through inhibition of dendritic cell subsets (DC1, DC2) that drive naïve these maturation steps. TGF-β additionally inhibits the helper functions of T_H1 and T_H2, and the effector functions of CTL cells, and it can do so by acting directly on these cells as well as by promoting the differentiation of $CD4^+$ T cells into peripheral regulatory T cells (pT_{reg}), which inhibit T_H1 and T_H2 cells partly through TGF-β. A specialized $ROR\gamma t^+$ antigen-presenting cell (TC) activates pT_{reg} cells in the intestinal lymph nodes. TGF-β inhibits B cell proliferation but stimulates IgA class switching in B cells. In the innate immunity arm, TGF-β blunts the effector functions of natural killer (NK) cells, and the inflammatory functions of neutrophils and macrophages while favoring, in the context of tumors, the adoption of tumor-associated neutrophil (TAN) and macrophage (TAM) states which support tumor progression. In chronic infection, inflammation, and cancer, the persistent myelopoiesis includes production of myeloid-derived suppressor cells

(MDSC) with TGF-β dependent immunosuppressive functions. These regulatory effects of TGF-β on the immune system occur to different extents in different tissue contexts and depending on whether the circumstance is homeostasis, acute injury or infection, or chronic inflammation, fibrosis, or cancer.

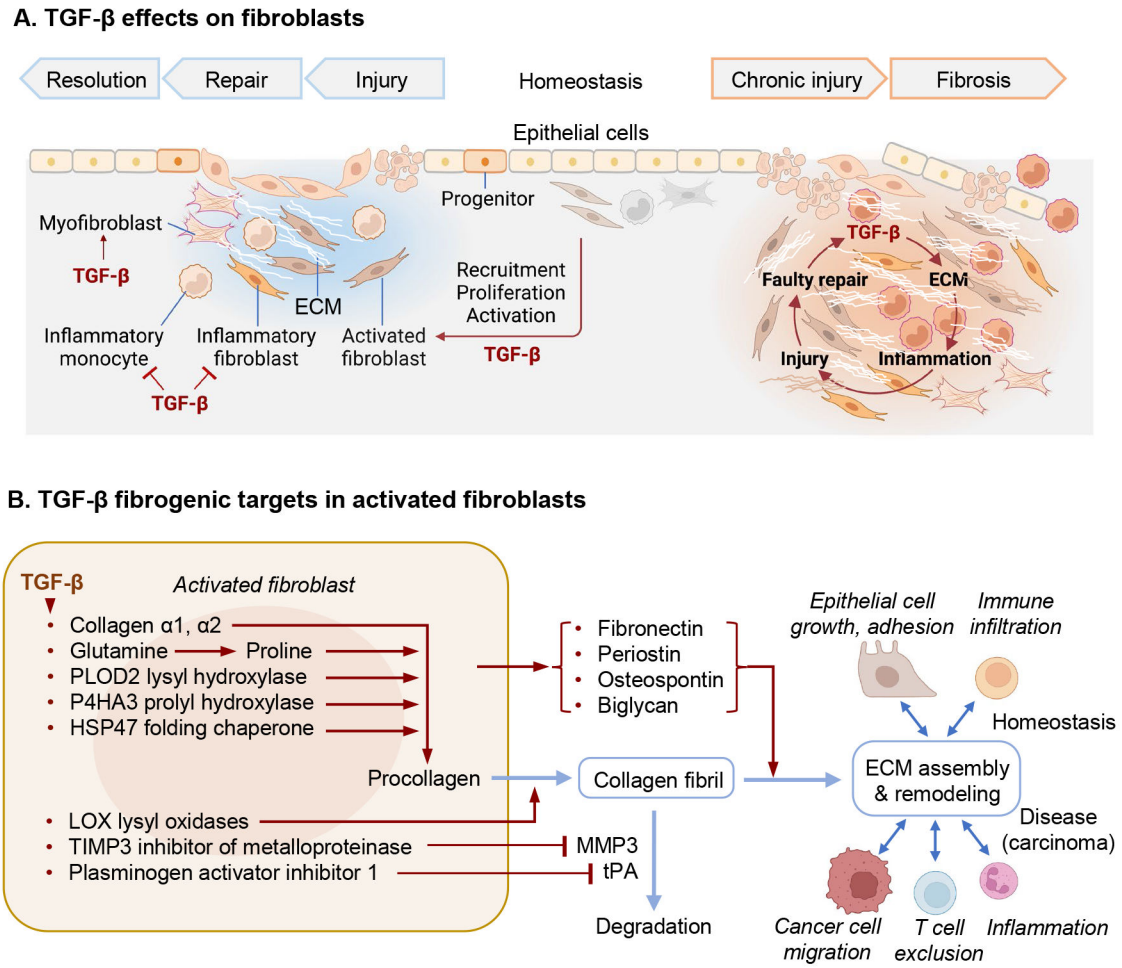

Figure 4. TGF-β regulation of fibroblasts in health and disease

A. Main effects of TGF-β on fibroblasts during injury repair and chronic fibrosis, and impact on epithelial and immune cells. TGF-β regulates fibroblast activity throughout the tissue response to injury and the return to homeostasis (*left side*) as well as during chronic fibrosis (*right side*). TGF-β potently induces the recruitment, proliferation and activation of fibroblast that produce collagens, fibronectin, and other components required for ECM assembly, as well as integrins that mediate cell adhesion to the ECM. Activated fibroblasts additionally establish paracrine communication with epithelial cells, angiogenic progenitors, and local innate and adaptive immune functions. TGF-β also induces a highly contractile myofibroblast phenotype expressing α-smooth muscle actin. These phenotypes appear to emerge at the expense of a pro-inflammatory fibroblast phenotype, while TGF-β additionally restricts inflammatory monocytes. ECM deposition and remodeling is essential for epithelial progenitors to reconstitute the barrier tissue after injury. Tissue fibrosis, characterized by chronic inflammation and accumulation of fibrillar collagens and other ECM components resulting from imbalanced production of ECM by tissue resident fibroblasts. Feed-forward loops involving TGF-β contribute to fibrosis by exaggerating normal physiologic responses and triggering further epithelial injury and inflammation. **B.** TGF-β potently induces expression of fibrillar collagens as well as the

metabolic adaptations, enzymes, and chaperones required for the biosynthesis and ECM deposition of collagen fibrils. TGF-β induces expression of additional ECM components in fibroblasts and epithelial cells. The production and turnover of ECM is a complex process requiring inputs from epithelial cells, innate and adaptive immune cells, and other cell types. Intratumoral fibrosis contributes to the exclusion of T cells from tumors. PLOD2, procollagen-lysine,2-oxoglutarate 5-deoxygenase 2; P4HA3, prolyl-4-hydroxylase 3, catalyzes proline hydroxylation; HSP47, heat-shock protein 47; LOX, lysyl oxidase; TIMP3, tissue inhibitor of metalloproteinase 3.

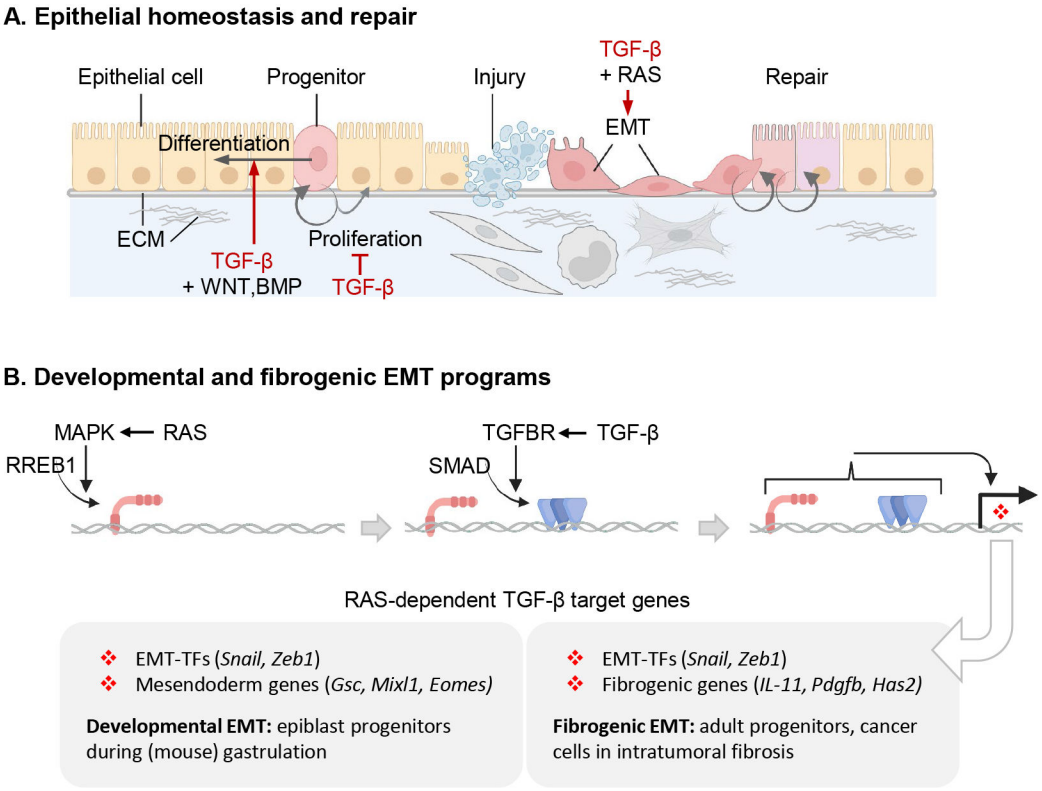

Figure 5. TGF-β in epithelial cell regulation

A. TGF-β regulates the phenotypic plasticity of epithelial progenitors and their interactions with other cell types. TGF-β derived from fibroblasts, immune cells, and from the epithelial cells themselves modulates the proliferation of epithelial progenitors and regulates their differentiation, frequently with countervailing WNT, BMP and other signals. In response to injury, epithelial progenitors undergo EMT for migration to niches that provide appropriate basal lamina ECM support and signals to orchestrate injury repair and eventual resolution. TGF-β is a major inducer of EMTs, which frequently requires the cooperation of RAS-activated MAPK signals. **B.** RREB1 (RAS-responsive element binding protein 1) links the TGF-β-SMAD and RAS-MARK pathways and coordinates the expression of developmental and fibrogenic EMT programs. MAPK-activated RREB1 binds to target loci including in EMT-TF genes and either mesendoderm specification genes in epiblast cells or fibrogenic genes in adult epithelial progenitors and adenocarcinoma cells. DNA-bound RREB1 then enables TGF-β receptor-activated SMADs to drive expression of these genes.

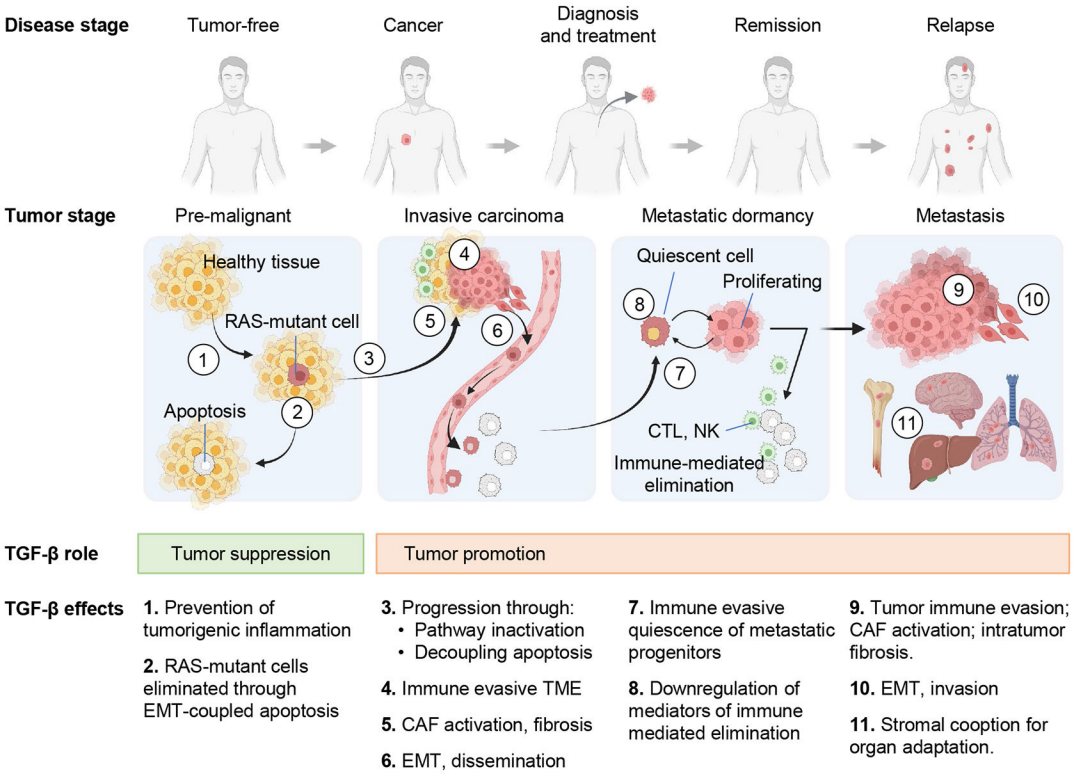

Figure 6. Roles of TGF-β in cancer

During the early stages of carcinogenesis, TGF-β exerts tumor suppressive effects by inhibiting tumorigenic inflammation (**1** in the graphic) or triggering EMT-coupled apoptosis in pre-malignant progenitors harboring RAS mutations (**2**). To escape TGF-β dependent apoptosis (**3**), RAS-mutant cells must acquire TGF-β pathway inactivating mutations or alterations that decouple TGF-β-dependent EMT from apoptosis. This enables carcinoma progression and turns TGF-β into a tumor promoting agonist as the disease progresses. The tumor promoting effects of TGF-β include: (**4**) generation of an immune evasive TME by excluding or suppressing cytotoxic T cells and NK cells and turning macrophages into TAMs and neutrophils into TANs; (**5**) activation of CAF fibrogenic and paracrine activities, which favor cancer cell growth, invasion, immune evasion, and angiogenesis; (**6**) induction of cancer cell EMTs which increase tumor invasion, entry into, and exit from the circulation for tumor dissemination; (**7**) induction of immune evasive dormancy in disseminated metastatic progenitors; (**8**) downregulation of mediators of immune clearance in dormant cancer cells; (**9**, **10**) repeated generation of an immune evasive TME, activation of CAFs, and induction of fibrogenic EMT in dormant metastatic progenitors that resume proliferative and survive elimination by the immune system; (**11**) promotion of metastatic outgrowth by stimulating organ-specific cancer cell-stroma interactions. The cancer cell-intrinsic tumorigenic effects of TGF-β (effects 6, 7, 8, 10 and, partly, 11) are available to carcinoma cells that retain an active TGF-β pathway (though decoupled from apoptosis). The TME effects of TGF-β (effects 4, 5, 9 and, partly, 11) are available to carcinoma cells regardless of how the tumor suppressive effects of TGF-β are cancelled.

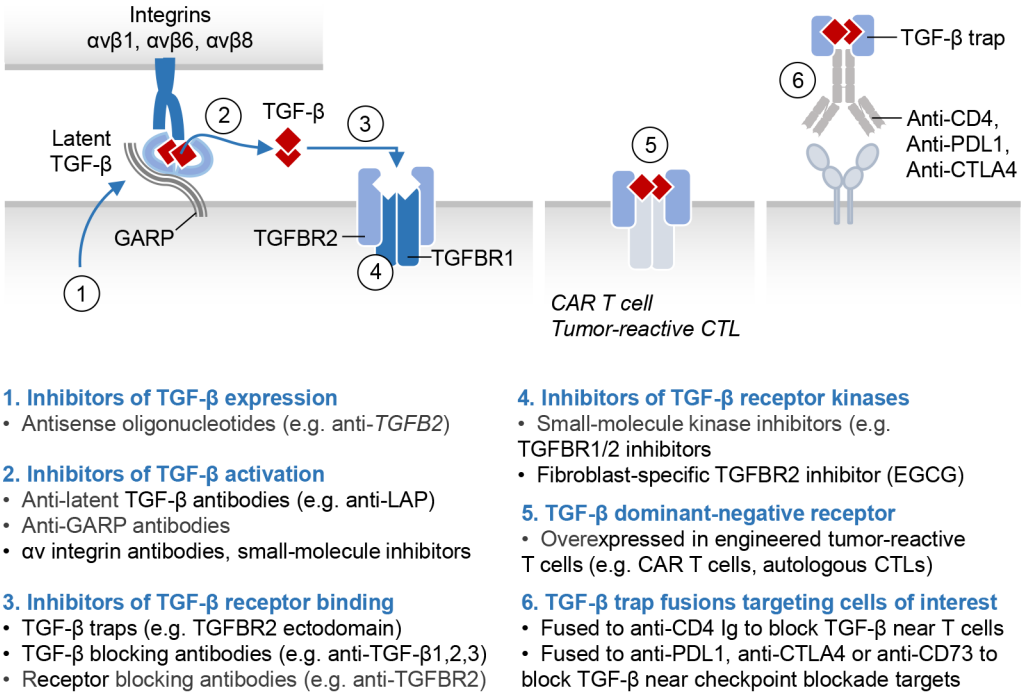

Figure 7. Approaches to therapeutically targeting TGF-β.

The image summarizes the main points of the TGF-β production, activation, and signaling being targeted by various agents currently under development to treat cancer, fibrosis, and other diseases. TGF-β inhibitory agents include antisense oligonucleotides targeting TGF-β expression, antibodies targeting latent TGF-β, TGF-β-activating integrins, active TGF-β or TGF-β receptors, and small-molecule compounds targeting TGF-β-activating integrins and TGF-β receptors. TGF-β receptor ectodomains fused to immune checkpoint antibodies are engineered to increase the efficacy of immunotherapeutic agents by trapping TGF-β near target cells. For the same purpose, dominant-negative TGF-β receptor constructs are overexpressed in engineered various types of anti-cancer T cells (CAR T cells, autologous CTLs).

Table 1.
Mammalian TGF-β family members and receptors

(*) TGFBR1 is also known as TβRI or ALK5; ACVR1A as ActR1A or ALK2; ACVR1B as ActR1B or ALK4; ACVR1C as ALK7; BMPR1A and BMPR1B as ALK3 and ALK6, respectively; ACVR2 and ACVR2B as ActRII and ActRIIB, respectively; and ACVRL1 as ALK1 or TSR1. (**) Inhibin, lefty, and BMP3 block the receptors for Activins, Nodal, and BMPs, respectively. Not included is GDF15, a distant member of the TGF-β family that binds to GDNF receptor α-like (GFRAL). GFRAL and related receptors for artemin, neurturin, persephin, and glial-derived neurotrophic factor (GDNF) signal through the receptor tyrosine kinase RET[257]

Ligand	Type I Receptor	Type II Receptor	Co-receptor	Smad
TGFβ-1	TGFBR1 *	TGFBR2	Betaglycan	SMAD2/3
TGFβ-2	TGFBR1	TGFBR2	Betaglycan	SMAD2/3
TGFβ-3	TGFBR1	TGFBR2	Betaglycan	SMAD2/3
Activin A	ACVR1B, ACVR1C	ACVR2A, ACVR2B		SMAD2/3
Activin B	ACVR1B, ACVR1C	ACVR2A, ACVR2B		SMAD2/3
Activin C	ACVR1B, ACVR1C	ACVR2A, ACVR2B		SMAD2/3
Activin E	ACVR1B, ACVR1C	ACVR2B		SMAD2/3
Nodal	ACVR1B, ACVR1C	ACVR2A, ACVR2B	Cripto, Cryptic	SMAD2/3
GDF1	ACVR1B, ACVR1C	ACVR2A, ACVR2B	Cripto, Cryptic	SMAD2/3
GDF3	ACVR1B, ACVR1C	ACVR2A, ACVR2B	Cripto, Cryptic	SMAD2/3
GDF8/Myostatin	ACVR1B, ACVR1C	ACVR2A		SMAD2/3
GDF9	ACVR1B	BMPR2		SMAD2/3
GDF11	ACVR1B, TGFBR1	ACVR2A, ACVR2B		SMAD2/3
Inhibin **	—	ACVR2A	Betaglycan	—
Lefty-1 **	—	—	Cripto, Cryptic	—
Lefty-2 **	—	—	Cripto, Cryptic	—
BMP2	BMPR1A BMPR1B	ACVR2A, ACVR2B, BMPR2	RGM	SMAD1/5
BMP4	BMPR1A BMPR1B	ACVR2A, ACVR2B, BMPR2		SMAD1/5
BMP5	ACVR1A, BMPR1A, BMPR1B	ACVR2A, ACVR2B, BMPR2		SMAD1/5
BMP6	ACVR1A, BMPR1A, BMPR1B	ACVR2A, ACVR2B, BMPR2	RGM	SMAD1/5
BPM7	ACVR1A, BMPR1A, BMPR1B	ACVR2A, ACVR2B, BMPR2		SMAD1/5
BPM8	ACVR1A, BMPR1A, BMPR1B	ACVR2A, ACVR2B, BMPR2		SMAD1/5
BPM8B	BMPR1A, BMPR1B	ACVR2A, BMPR2		SMAD1/5
BMP9/GDF2	ACVRL1	ACVR2, BMPR2	Endoglin	SMAD1/5
BMP10	ACVRL1	ACVR2, BMPR2	Endoglin	SMAD1/5
BMP15	BMPR1B	BMPR2		SMAD1/5
GDF5	BMPR1A, BMPR1B	ACVR2, ACVR2B, BMPR2		SMAD1/5
GDF6	BMPR1A, BMPR1B	ACVR2, ACVR2B, BMPR2		SMAD1/5
GDF7	BMPR1A, BMPR1B	ACVR2, ACVR2B, BMPR2		SMAD1/5
GDF10	BMPR1A, BMPR1B	ACVR2, ACVR2B, BMPR2		SMAD1/5
AMH	ACVR1A, BMPR1A,	AMHR2		SMAD1/5
BMP3 **	—	ACVR2B		—

Table 2.
Congenital conditions associated with TGF-β pathway mutations

Mutant gene	Condition	Refs.
Ligands		
TGFB1	Camurati-Engelmann disease	68
TGFB2	Loeys-Dietz aortic aneurysm syndrome type 4	258
TGFB3	Loeys-Dietz aortic aneurysm syndrome type 5, arrhythmogenic ventricular dysplasia	259
INHA	Male infertility; Premature ovarian failure	260,261
NODAL	Heterotaxy	262
BMP2	Brachydactyly	263
BMP6	Iron overload	264
BMP10	Pulmonary arterial hypertension	265
BMP15	Ovarian dysgenesis	266
GDF1	Congenital cardiovascular malformations	267
GDF2 (BMP9)	Hereditary hemorrhagic telangiectasia type 5	268-270
GDF3	Microphthalmia, coloboma, skeletal abnormalities	271
GDF5	Chondrodysplasia, brachydactyly, symphalangism, acromesomelic dysplasia	272-274
GDF6	Klippel-Feil syndrome, microphthalmia, Leber congenital amaurosis	275,276
MSTN (GDF8)	Increased skeletal muscle mass	277
GDF9	Polycystic ovary syndrome	278
AMH	Persistent Mullerian duct syndrome type 1	279
Receptors		
TGFBR1	Loeys-Dietz aortic aneurysm syndrome type 1	280
TGFBR2	Loeys-Dietz aortic aneurysm syndrome type 2, Marfan syndrome type 2	280,281
ACVR1A	Fibrodysplasia ossificans progressiva	282
ACVR2A	Pre-eclampsia	283
ACVR2B	Left-right axis malformations	284
ACVRL1	Hereditary hemorrhagic telangiectasia type 2, pulmonary arterial hypertension	285,286
BMPR1B	Pulmonary arterial hypertension, acromesomelic dysplasia, juvenile polyposis	287-289
BMPR2	Pulmonary arterial hypertension, pulmonary veno-occlusive disease	290-292
AMHR2	Persistent Mullerian duct syndrome type 2	293
Co-receptors		
ENG (Endoglin)	Hereditary hemorrhagic telangiectasia type 1, pulmonary arterial hypertension	286,294
TDGF1 (Cripto)	Forebrain defects	295
CFC1 (Cryptic)	Autosomal visceral heterotaxy, congenital heart disease	296,297
SMADs		
SMAD1	Pulmonary arterial hypertension	298
SMAD3	Loeys-Dietz aortic aneurysm syndrome type 3	299

Mutant gene	Condition	Refs.
SMAD4	Juvenile polyposis–hereditary hemorrhagic telangiectasia syndrome	212,298,300
SMAD8	Pulmonary arterial hypertension	298