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Extracellular matrix as a contextual determinant of transforming growth factor-β signaling in epithelial-mesenchymal transition and in cancer

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Extracellular matrix (ECM) provides both structural support and contextual information to cells within tissues and organs. The combination of biochemical and biomechanical signals from the ECM modulates responses to extracellular signals toward differentiation, proliferation, or apoptosis; alterations in the ECM are necessary for development and remodeling processes, but aberrations in the composition and organization of ECM are associated with disease pathology and can predispose to development of cancer. The primary cell surface sensors of the ECM are the integrins, which provide the physical connection between the ECM and the cytoskeleton and also convey biochemical information about the composition of the ECM. Transforming growth factor-β (TGF-β) is an extracellular signaling molecule that is a powerful controller of a variety of cellular functions, and that has been found to induce very different outcomes according to cell type and cellular context. It is becoming clear that ECM-mediated signaling through integrins is reciprocally influenced by TGF-β: integrin expression, activation, and responses are affected by cellular exposure to TGF-β, and TGF-β activation and cellular responses are in turn controlled by signaling from the ECM through integrins. Epithelial-mesenchymal transition (EMT), a physiological process that is activated by TGF-β in normal development and in cancer, is also affected by the composition and structure of the ECM. Here, we will outline how signaling from the ECM controls the contextual response to TGF-β, and how this response is selectively modulated during disease, with an emphasis on recent findings, current challenges, and future opportunities.

Basics of ECM and Integrin Signaling

ECM is a dynamic and complex combination of collagens, glycoproteins and proteoglycans.[1,2] It provides structural support in bone, cartilage, and the basement membrane; specific association of cells with the ECM also provides contextual information that controls cellular phenotype, including differentiation, proliferation, or apoptosis.[3,4] ECM also regulates availability and activity of many signaling molecules, including TGF-β, through controlled sequestration, presentation, and release.[5,6] The primary cell surface receptors for the ECM are the integrins, a family of 24 heterodimeric proteins composed of one of 18 α-subunits and 8 β-subunits.[7,8] Integrins bind to motifs present in the ECM though an interaction domain located between the α- and β-subunit; while many integrin-binding motifs have been identified, the best studied is the arginine-glycine-aspartate (RGD) sequence that is present in fibronectin and many other extracellular molecules. Integrins become activated in a process that is regulated both by availability of ECM substrate (outside-in activation) and signals from within the cell (inside-out activation).[9] Activated integrins can bind to the actin cytoskeleton and recruit a variety of cytosolic components into adhesion complexes. Through those interactions integrins can transduce biochemical signaling dependent on ECM composition, as well as directly link physical forces acting on the ECM to the cellular cytoskeleton (**Fig. 1**).[10,11]

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Basics of TGF-β Signaling

Three TGF-β isoforms are present in mammals, TGF-β1, -2, and -3, each encoded by a separate gene, and each playing distinct physiological roles during development.[12] Exposure of cells to an active TGF-β isoform leads to assembly of a TGF-β-ligated tetrameric receptor complex, composed of 2 type I and 2 type II TGF-β receptor subunits (TGFBRI and TGFBRII). TGFBRII then phosphorylates TGFBRI, enabling it to activate downstream signaling responses, which are regulated through canonical and noncanonical signaling pathways (**Fig. 2**).

In the canonical signaling pathway, TGFBRI phosphorylates the receptor SMAD proteins, SMAD2 or SMAD3. Phosphorylated receptor SMADs associate with SMAD4 and the resultant oligomeric complex becomes translocated to the nucleus, where it can bind to a variety of other transcription factors and cofactors. Transcriptional alterations induced by the canonical pathway vary according to the strength and sustained maintenance of the TGF-β receptor signaling, the composition and availability

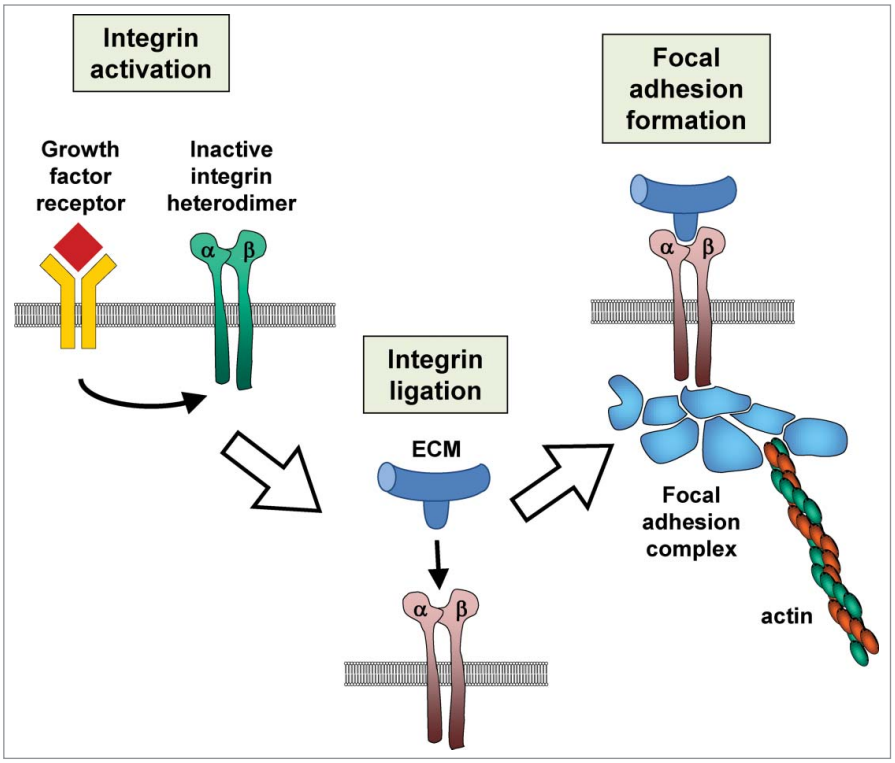

Figure 1. Integrin activation. Stimulation of cellular signaling pathways can lead to increased affinity for binding sites in the ECM. Integrin ligation to the ECM triggers assembly of cytoplasmic molecules that can lead to focal adhesion complex formation and connection to the actin cytoskeleton.

of existing transcriptional cofactors, and the epigenetic landscape of the chromatin at the time of TGF-β pathway activation.[13] The gene expression effects are highly cell type and context dependent; for example, the canonical signaling pathway inhibits expression of the inhibitor of differentiation (ID1) gene in normal mammary epithelial cells, but activates its expression in breast cancer cells.[13-15]

In the noncanonical signaling pathway, the activated TGF-β receptor complex directly regulates non-SMAD-dependent pathways to activate, sustain, or modulate cellular responses.[16,17] Non-canonical pathways include activation of ERK/MAPK signaling through tyrosine phosphorylation of TGFBR1 and recruitment of Grb/Shc, and subsequent activation of Ras,[18,19] which may contribute to TGF-β-dependent induction of senescence and prevention of transformation in normal human mammary epithelial cells.[20] TGF-β can also induce JNK/p38 through a SMAD-independent pathway,[21,22] which can then reinforce SMAD-dependent transcriptional alterations through a reactive oxygen species (ROS)-mediated mechanism.[23] Rho family GTPases can be regulated through SMAD-independent TGF-β signaling: RhoA, Rac1, and Cdc42 can be activated in epithelial cells,[24-26] or RhoA can be targeted for degradation via a pathway initiated by direct phosphorylation of the polarity protein PAR6 by TGFBRII.[27,28] TGF-β can also activate Akt through SMAD-independent induction of PI3K, which can in turn act as a regulator of the canonical pathway through phosphorylation of ERK and consequent activation of SMADs.[29] The principal determinant and mediator of whether TGF-β will signal through the canonical or noncanonical pathway in normal cells is SMAD7, which is able to inhibit phosphorylation of receptor SMADs through multiple mechanisms.[30] SMAD7 is a transcriptional target of the canonical pathway, which constitutes a mechanism for channeling signaling from canonical to noncanonical pathways.“

TGF-β expression has been studied in many tumor types, where it has been found to function both as a tumor suppressor and a tumor promoter. In normal cells and in early stage tumors, TGF-β acts to block cell proliferation through the canonical pathways, including via SMAD-dependent inhibition of MYC, as well as activation of cyclin-dependent kinase inhibitors. Additionally, in premalignant cells which have acquired oncogenic mutations, TGF-β can induce apoptosis. In more advanced tumors, the TGF-β-dependent cytostatic effects are suppressed, and EMT-associated cell invasion and metastasis become dominant.[31,32] The transition from tumor-inhibitory to tumor-promoting behaviors has been described as the TGF-β paradox, and while multiple components of this transition have been discovered, many aspects remain unknown.[33] Additionally, TGF-β can induce a variety of different anti- and pro-tumorigenic effects indirectly by acting on stromal cells in the cancer microenvironment.[34]

TGF-β Regulation of ECM Secretion and Integrin Function

One of the most highly investigated roles of TGF-β in pathology is in the context of development of tissue fibrosis.[35] Under normal circumstances, tissue damage triggers a wound healing response characterized by deposition of transitional ECM, followed by activation and invasion of fibroblasts that remodel and contract the wound ECM; once tissue homeostasis is restored, the fibroblasts undergo apoptosis. Under fibrotic conditions, however, a feedback loop is activated in which excessive ECM deposition leads to increased proliferation and activation of ECM-producing fibroblasts. Maintained for extended periods of time, fibrosis can become a significant problem in its own right, and can also stimulate malignant transformation and promote tumor progression.[36,37] TGF-β has been implicated as a critical player in chronic fibrosis of many organs, including lung, kidney, liver, and skin. TGF-β directly stimulates expression of ECM proteins, including collagen, fibronectin, and proteoglycans.[38-40] TGF-β induces conversion of fibroblasts into myofibroblasts

which can further contract and distort the ECM. TGF-β can also directly stimulate myofibroblast formation from epithelial and endothelial cells through EMT-related processes, and this function is essential for development of fibrosis in several organs.[41-44] The relative amount of epithelial- vs fibroblast-derived myofibroblasts is a current point of debate and is likely to be highly tissue specific.

In addition to inducing integrin signaling through increased production of ECM, TGF-β can also regulate integrin function directly. TGF-β can control expression of αv-, β3-, and β1-integrin subunits through both canonical and noncanonical pathways, according to cell type.[45-48] TGF-β signaling can also directly phosphorylate and activate β1-integrin, stimulating cell invasion and facilitating tissue regeneration.[49,50] TGF-β can also induce cross-talk between integrins and growth factor receptors, including via activation of focal adhesion kinase (FAK)-dependent clustering of ErbB2 (HER2) and integrins α6, β1, and β4 through a pathway initiated by EGFR-dependent phosphorylation and activation of SRC; the overall effect of this pathway is increased cell migration and survival.[51]

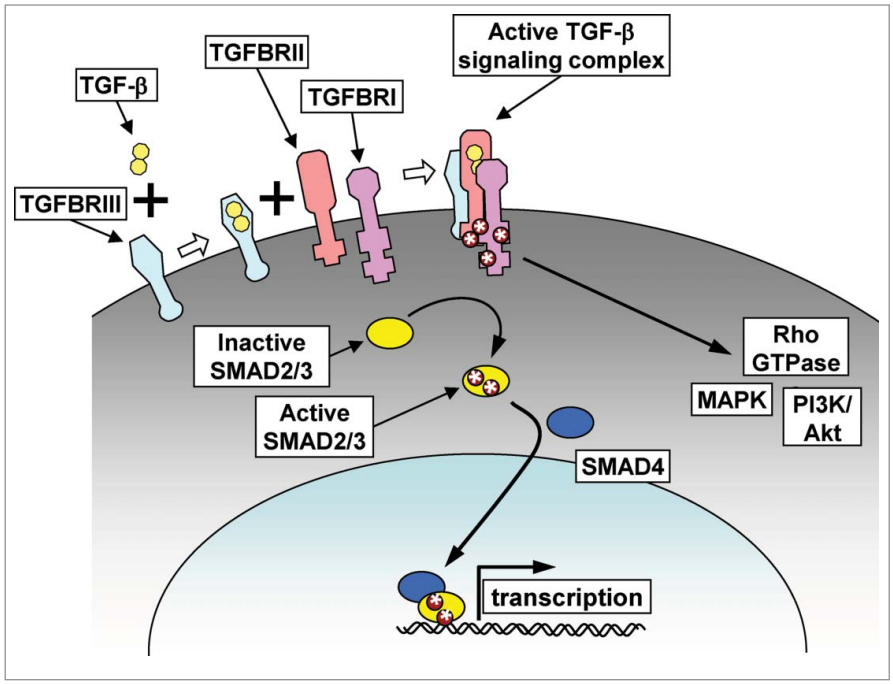

Figure 2. TGF-β signaling. TGF-β signaling can be initiated by binding of TGF-β to TGF-β receptor type III (TGFBRIII), which can then assemble with TGF-β receptors types II and I (TGFBRII, TGFBRI) to form an active signaling complex. In the canonical signaling pathway, the active signaling complex phosphorylates the receptor SMADs (SMAD2 and SMAD3), which then associate with SMAD4, translocate to the nucleus and affect gene transcription. In the noncanonical pathway, phosphorylation of the TGF-β receptor complex leads to activation of cytosolic signaling pathways, including MAPK, PI3K/Akt, and Rho GTPases.

Integrin Regulation of TGF-β Activation and Signaling

Integrins are directly involved in the activation of TGF-β (**Fig. 3**).[52,53] TGF-β isoforms are translated as preproproteins that contain a 25–30 kDa latency associated peptide (LAP) and the 13 kDa TGF-β molecule. LAP-TGF-β homodimers linked by disulfide bonds are formed in the endoplasmic reticulum (ER), followed by protoelytic cleavage of LAP from TGF-β in the Golgi; the resultant homodimers of TGF-β and LAP remain noncovalently associated following secretion as an inactive protein complex called the small latency complex (SLC). Many cell types also produce latent TGF-β-binding protein (LTBP), which can covalently bind to the SLC, producing the large latency complex (LLC), which can become associated with fibrillar ECM molecules.[52,53] Outside of the cell, TGF-β can be activated following release from the SLC, which can occur through selective proteolytic digestion, exposure to ROS, or through direct interaction with ECM molecules.[54-56] Recent studies have identified a process by which TGF-β1 and TGF-β3 can be released from their LAP-TGF-β complex through a force-dependent conformational shift induced by association of RGD motifs in their respective LAP proteins with integrins; all αv-containing integrins (αvβ1, αvβ3, αvβ5, αvβ6, and αvβ8) as well as α8β1 integrin have been shown to bind LAP, although whether αvβ1 and α8β1 can activate latent TGF-β has not been determined.[57-59] The relevance of αv integrin-mediated activation of latent TGF-β was demonstrated by studies showing that transgenic mice with mutations in the RGD motif of the TGF-β1-associated LAP protein recapitulate the phenotype of the TGF-β1 knockout mouse; similar developmental alterations are seen in mice lacking a functional αv-integrin gene.[60-62] Of αv-containing integrins that can activate latent TGF-β, αvβ6- and αvβ8-integrins have been shown to play a critical role in TGF-β activation during development and in immune homeostasis, while αvβ3 and αvβ5 may play a more important role in TGF-β activation during fibrosis.[6,63,64]

In addition to controlling TGF-β activation, integrins can also affect signaling downstream of the TGF-β receptor.[6] This can occur through integrin-mediated activation of the TGF-β receptor, through stimulation of canonical and noncanonical signaling pathways, and through increased transcription of genes encoding TGF-β and TGF-β receptor isoforms.[6,65] Recent studies have shown that the pathways regulating these effects vary in different cell and tissue types.[66] Activity of β1-integrins and the integrin signaling mediator integrin-linked kinase (ILK) are required for TGF-β-induced EMT in mammary epithelial cells.[67,68] Integrin α3β1 is necessary for TGF-β-induced EMT of alveolar epithelial cells and development of pulmonary fibrosis,[69] and integrin α1β1 is required for TGF-β-induced kidney fibrosis.[70] Integrin αvβ5 and ILK activity are a prerequisite for TGF-β-dependent activation of dermal fibroblasts.[71-73]

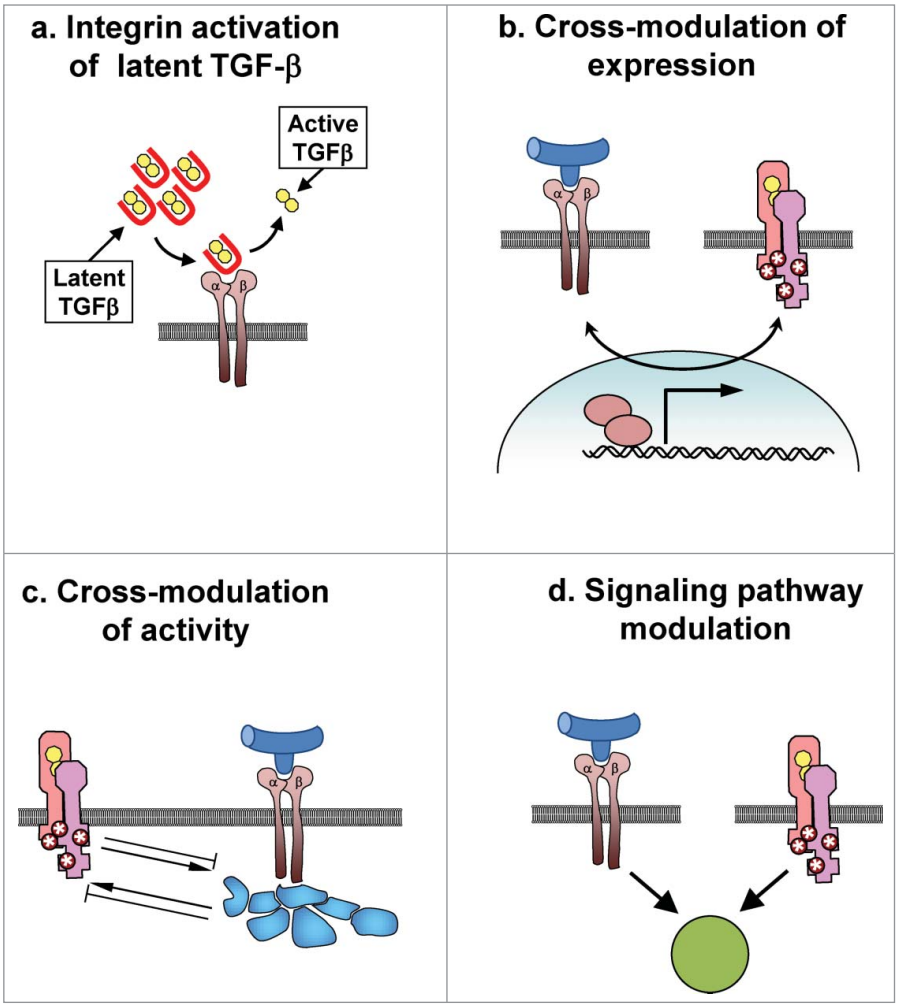

Figure 3. Interaction of integrins and TGF-β signaling pathways. (**a**) Integrin binding to latent TGF-β complexes can lead to release of active TGF-β. (**b**) Signaling from integrins or TGF-β receptors can stimulate expression of receptors or effectors of the other signaling pathway. (**c**) Activation of integrins or TGF-β receptors can lead to activation or inhibition of the other signaling receptor. (**d**) Cooperative signaling from integrins and TGF-β receptor may be necessary to stimulate phenotypic outcomes, including EMT.

Furthermore, the response of the TGFβ receptor can be potentiated through binding of specific integrins to TGFBRII, leading to phosphorylation of the receptor in a FAK- and SRC- dependent manner.[73-78] These effects can be quite cell-type specific: in mammary epithelial cells, the activation of TGFBRII by interaction with integrin β3 is inhibited by signaling from integrin β1, while in chondrocytes, integrin β1 preferentially associates with and activates TGFBRII.[76,77]

As an additional layer of control, the strength of the signal from the TGF-β receptor complex depends on which TGF-β ligand is bound, as the ligands vary in their ability to bind to TGFBRI and to assemble the active tetrameric receptor complex with TGFBRII; the co-receptor, TGFBRIII, can further modulate the ligand-dependent assembly and activation of the complex.[6,34] While a role for integrin-mediated regulation of TGF-β and receptor isoform expression is relatively unexplored, it is known that activation of integrin α5β1 stimulates TGFBRII expression, while expression of both TGFBRI and TGFBRII can be repressed by integrin αvβ3.[79,80]

Approaches to Therapeutic Intervention

As TGF-β has been found to play a central role in promotion of tumor cell invasion and metastasis, stimulation of pathological EMT, and induction of cancer-promoting microenvironmental changes, it is unsurprising that there has been considerable effort to develop inhibitors targeting the TGF-β pathway as potential cancer therapeutics. To date, multiple inhibitors of the TGF-β signaling have been developed and tested in animal models and clinical trials.[34,81,82] These include monoclonal antibody-based inhibitors of TGF-β signaling, for example fresolimumab (GC1008), a fully human monoclonal antibody against TGF-β-1, -2 and -3, currently tested in clinical trials for combinatorial treatment with radiation therapy in metastatic breast cancer (ClinicalTrials.gov identifier: NCT01401062) and in glioma (ClinicalTrials.gov identifier: NCT01472731). Another avenue for TGF-β pathway inhibition is through synthetic antisense oligonucleotides, such as trabedersen (AP 12009), targeting TGF-β2, currently being tested for treatment of glioma,[83] pancreatic cancer, melanoma and colorectal cancer.[84] Other types of TGF-β pathway inhibitors include small molecule TGF-β receptor kinase inhibitors which bind to and directly block receptor signaling, as well as peptide aptamers which bind to and inhibit downstream mediators of the TGF-β pathway, such as SMADs.[34,81,85]

An alternative way to interfere with TGF-β signaling would be to target αv integrin subunit integrins required for activation of latent TGF-β. Multiple anti-integrin αv small molecule synthetic inhibitors and monoclonal anitbodies have been developed and are being tested in preclinical and clinical trials.[8,86,87] Among these, the monoclonal antibody intentumumab[88,89] is currently being tested in patients with melanoma (ClinicalTrials.gov identifier: NCT00246012) and prostate cancer (ClinicalTrials.gov identifier: NCT00537381). It should be noted that, given the pleiotropic effects of TGF-β and potential development of resistance, the current paradigms for targeting TGF-β signaling in cancer treatment have focused on short term dosing in combination with other therapies.

Conclusions

TGF-β is a multi-functional cytokine that regulates virtually all cellular processes. It can act as a tumor suppressor, blocking proliferation and inhibiting stromal mitogens, as well as a tumor promoter and an inducer of cancer-associated EMT, allowing evasion of immune surveillance and stimulating invasion and metastatic spread. Those opposing consequences of TGF-β signaling are highly cell type dependent and regulated by the cellular context. ECM is a key regulator of both initiation of TGF-β signaling and a determinant of its outcomes. Integrins, acting as bidirectional signal transducers between the cellular microenvironment and the cell itself, play crucial roles in this process. Often, the presence of certain integrins is required for activation of latent TGF-β and thus induction of downstream signaling pathways; integrins can also lead to ligand-independent signaling via activation of TGF-β receptor. The complexity and dual nature of TGF-β signaling effects reinforce the need to specifically study the context and determinants of those different responses. Moreover, while it is the paradigmatic view that in early stages of tumor development TGF-β acts to inhibit proliferation, whereas in later stages that aspect of signaling is lost in favor of promotion of EMT and invasiveness, we should be mindful that this phenotypic transition from cytostasis to motility is unlikely to occur in every cell within a tumor, and that competition between pathways responsible for these different phenotypes likely leads to complex and sometimes unexpected outcomes. Most importantly, if TGF-β pathway is to be targeted in cancer therapy, the challenge remains to predict the types and stages of tumors in which TGF-β signaling inhibition would prevent metastasis without facilitating growth of the primary tumor.

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