TGF-β-induced EMT: mechanisms and implications for fibrotic lung disease

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Willis BC, Borok Z. TGF-β-induced EMT: mechanisms and implications for fibrotic lung disease. Am J Physiol Lung Cell Mol Physiol 293: L525–L534, 2007. First published July 13, 2007; doi:10.1152/ajplung.00163.2007.—Epithelial-mesenchymal transition (EMT), a process whereby fully differentiated epithelial cells undergo transition to a mesenchymal phenotype giving rise to fibroblasts and myofibroblasts, is increasingly recognized as playing an important role in repair and scar formation following epithelial injury. The extent to which this process contributes to fibrosis following injury in the lung is a subject of active investigation. Recently, it was demonstrated that transforming growth factor (TGF)-β induces EMT in alveolar epithelial cells (AEC) in vitro and in vivo, and epithelial and mesenchymal markers have been colocalized to hyperplastic type II (AT2) cells in lung tissue from patients with idiopathic pulmonary fibrosis (IPF), suggesting that AEC may exhibit extreme plasticity and serve as a source of fibroblasts and/or myofibroblasts in lung fibrosis. In this review, we describe the characteristic features of EMT and its mechanistic underpinnings. We further describe the contribution of EMT to fibrosis in adult tissues following injury, focusing especially on the critical role of TGF-β and its downstream mediators in this process. Finally, we highlight recent descriptions of EMT in the lung and the potential implications of this process for the treatment of fibrotic lung disease. Treatment for fibrosis of the lung in diseases such as IPF has heretofore focused largely on amelioration of potential inciting processes such as inflammation. It is hoped that this review will stimulate further consideration of the cellular mechanisms of fibrogenesis in the lung and especially the role of the epithelium in this process, potentially leading to innovative avenues of investigation and treatment.

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The highly specialized morphology, function, and polarity of epithelial cells commonly lead to the assumption that their differentiated state is relatively immutable. It is conceptually difficult to imagine that a highly differentiated cell type, such as a renal tubular or alveolar epithelial cell, with a wide array of characteristic secretory, transport, and structural functions, could radically modify its genetic program and “transform” into another cell type. However, the recent explosion of knowledge in the biology of cellular differentiation has highlighted the fact that seemingly any cell type can become any other, given the correct set of transcription factors and cellular environment (94). Dramatic changes in cellular phenotype are well described in oncogenic transformation, and the loss of epithelial characteristics and subsequent acquisition of mesenchymal characteristics is a critical process to metastasis formation and tumor invasion by epithelium-derived tumors (67, 96). Whereas complete changes in phenotype across embryonic lineages have classically been regarded as unlikely in terminally differentiated epithelia, transdifferentiation of various cell types into other differentiated cell types has been extensively observed. Hepatocytes, retinal pigment cells, and pancreatic acinar cells transdifferentiate into other types of epithelia (35). In the lung, alveolar epithelial type II (AT2) cells are believed to serve as progenitors for repair of the alveolar epithelium following injury, being capable of both self-renewal and of giving rise to alveolar epithelial type I (AT1) cells (2). More recently, the possibility that epithelial cells [including alveolar epithelial cells (AEC)] may exhibit greater phenotypic plasticity and are capable of undergoing even more dramatic changes to give rise to a mesenchymal phenotype has been suggested (35, 106, 115). Epithelial-mesenchymal transition or EMT, a process whereby fully differentiated epithelial cells undergo transition to a mesenchymal phenotype giving rise to fibroblasts and myofibroblasts, is increasingly recognized as playing an integral role in the process of repair and scar formation following epithelial injury in a number of tissues (35, 55).

In this review, we describe the characteristic features of EMT and the role of EMT in fibrosis of adult tissues following injury. We focus especially on the critical role of transforming growth factor (TGF)-β in this process and the mechanisms underlying TGF-β-mediated EMT. Finally, we highlight recent descriptions of EMT in the lung and potential implications of this process for the treatment of fibrotic lung disease. Treatment for fibrosis of the lung in diseases such as idiopathic pulmonary fibrosis (IPF) has largely focused on amelioration...
of potential inciting processes such as inflammation. It may be that the relative lack of success of these treatments is due to a lack of appreciation for, and understanding of, the role of the epithelium and the process of EMT in the generation of fibroblasts and production of extracellular matrix in adult tissues. It is hoped that this review will stimulate further consideration of the cellular mechanisms of fibrogenesis in the lung, potentially leading to innovative avenues of investigation and treatment.

**EMT**

As mentioned above, EMT has long been known to play a role in cellular differentiation during development and tumor invasion (23, 67, 96). EMT enables the development of mesoderm from epithelium during gastrulation and is involved in the development of the heart (formation of endocardial cushions of the atrioventricular canal) and palate (fusion of medial epithelial cells) (11, 26, 66). In this context, EMT affects tissues as a coordinated unit in the process of organogenesis. Oncogenic EMT refers to acquisition of a migratory phenotype by malignant cells and is associated specifically with tumor invasiveness. Oncogenic EMT occurs in combination with other abnormalities intrinsic to malignant cells, such as the ability to evade apoptosis and anoikis, and is generally accepted as a mechanism underlying metastasis, particularly in the context of Ras activation (32, 69). Interactions between TGF-β and Ras cooperate to maintain EMT in a variety of epithelial cell types through autocrine induction of TGF-β and may also contribute to tumor invasiveness in vivo (32, 69). An essential role for TGF-β-mediated Smad signaling has been demonstrated in EMT associated with tumor progression and development, with differential involvement of Smad2 or Smad3 depending on cellular context. In tumor progression, Smad proteins and Ras cooperate to induce EMT and metastasis formation (32, 69). A role for Smad 2 signaling has been demonstrated in TGF-β-induced EMT in cancerous lung epithelial A549 cells (37), whereas inhibition of Smad3 signaling decreases the metastatic potential of xenografted breast cancer cell lines (98, 99). In contrast, during development, EMT is unimpaired in Smad3 knockout mice, whereas Smad2 is essential (67).

EMT can be viewed as a manifestation of extreme epithelial cell plasticity, characterized by loss of polarity, loss of epithelial markers (including junctional and cell-cell adhesion proteins [e.g., zona occludens-1 (ZO-1)] and E-cadherin due to disassembly of cell-cell contacts), cytoskeletal reorganization, and transition to a spindle-shaped morphology concomitant with acquisition of mesenchymal markers [including α-smooth muscle actin (α-SMA)] and an invasive phenotype (35, 115, 118). A requirement for acquisition of a migratory phenotype to define EMT is somewhat controversial, especially in the context of fibrosis (67). Investigation of EMT requires the use of panels of markers that describe an EMT profile. Loss of the epithelial phenotype can be clearly defined by loss of expression of specific epithelial proteins, including junction-associated proteins (e.g., ZO-1, E-cadherin), cytokeratins, and apical actin-binding transmembrane protein-1 (MUC-1). In particular, loss of E-cadherin is a universal feature of EMT, regardless of initiating stimulus (26), and in some instances, reversal of the invasive mesenchymal phenotype can be observed if E-cadherin is constitutively produced (103). However, acquisition of a mesenchymal phenotype has been more difficult to define due to lack of specificity of many available phenotypic markers (115). Markers used to define the mesenchymal phenotype include vimentin, α-SMA, fibroblast-specific protein-1 (FSP-1), desmin, (pro)-collagen, fibronectin, connective tissue growth factor (CTGF), N-cadherin, the transcription factors Snail and Slug, and expression of matrix metalloproteinases (MMPs) (52). However, vimentin is not absolutely specific for fibroblastoid cells since it is also present in leukocytes and endothelial cells and is not expressed by all fibroblasts (70), whereas α-SMA expression is limited only to myofibroblasts that constitute a subset of activated fibroblasts (29, 92). Similarly, collagen I is expressed by only a subset of fibroblasts, whereas FSP-1 (S100A4) is also expressed by inflammatory cells as well as vascular smooth muscle and endothelium (22, 27, 51, 95). This lack of marker specificity makes it necessary to simultaneously evaluate a panel of probes to define the mesenchymal phenotype in the context of EMT.

**EMT in Cellular Injury and Fibrosis**

It is increasingly being recognized that EMT can be observed in response to epithelial stress/injury in many adult tissues (e.g., kidney and eye) (35, 55, 86). Injury to lens epithelial cells and retinal pigment epithelial cells leads to EMT with resultant fibrosis (25, 86, 122). EMT has been observed in animal models of renal fibrosis as well as in biopsies of diseased human kidneys (29, 81, 95, 118). The most convincing evidence for EMT as a source of myofibroblasts in vivo was derived from a study in which genetically tagged proximal tubular epithelial cells gave rise to up to 36% of interstitial fibroblasts via EMT following unilateral ureteral obstruction, a model of acute renal injury (29). EMT has been frequently observed in vitro in renal epithelia in response to environmental stresses such as hypoxia (58), reactive oxygen species (80, 82), exposure to advanced glycation end products (71), and treatment with a variety of cytokines and growth factors. Depending on the precise physiological context, EMT can be induced by a number of extracellular mediators individually or in combination, including TGF-β, fibroblast growth factor-2 (FGF-2), epidermal growth factor (EGF), CTGF, insulin-like growth factor-2 (IGF-II), interleukin-1 (IL-1), hepatocyte growth factor (HGF), and Wnt ligands (115). Effects of these mediators can be augmented by proteolytic degradation of basement membranes (13), whereas overexpression of MMPs alone leading to the disruption of integrin/matrix interactions can induce EMT, in some instances involving activation of TGF-β (80). In turn, TGF-β and other cytokines and growth factors can induce MMPs (76). The main inducers of EMT can also promote apoptosis, suggesting that EMT could potentially serve as a pathway for escape from cell death depending on the particular cytokine milieu (4, 68). In this regard, treatment with EGF was shown to inhibit proapoptotic effects of TGF-β without preventing its EMT-inducing effects and facilitating survival of cells undergoing EMT (18). EMT is least understood in the context of epithelial injury and fibrosis, and, as noted above, much that is known of the mechanisms underlying EMT has been derived from studies of development and carcinogenesis. Differences among the mechanisms underlying EMT in these diverse situations are not well understood.
and have largely not been addressed in the literature to date. Although some of the mediators may be similar, the exact mechanisms are likely different depending on the specific cellular and physiological context.

**TGF-β and EMT**

TGF-β is a multifunctional cytokine that regulates tissue morphogenesis and differentiation through effects on cell proliferation, differentiation, apoptosis, and extracellular matrix production (16). TGF-β has been implicated as a “master switch” in induction of fibrosis in many tissues including the lung (93). In this regard, TGF-β is upregulated in lungs of patients with IPF, and expression of active TGF-β in lungs of rats induces a dramatic fibrotic response, whereas the inability to respond to TGF-β1 affords protection from bleomycin-induced fibrosis (120).

TGF-β is a major inducer of EMT in development, carcinogenesis, and fibrosis with different isoforms mediating various effects depending on specific cellular context (67). TGF-β1 was first described as an inducer of EMT in normal mammary epithelial cells (62) and has since been shown to mediate EMT in vitro in a number of different epithelial cells, including renal proximal tubular, lens, and most recently alveolar epithelial cells (19, 25, 37, 86, 106). TGF-β is considered to be the prototypical cytokine for induction of EMT, whereas the effects of other mediators are often context-dependent and variable (e.g., HGF can either promote or inhibit EMT, depending on cellular context) (110). Modulation of the TGF-β-dependent Smad pathway in animal models has provided strong evidence for a role for TGF-β in fibrotic EMT in vivo. As discussed further below, EMT is ameliorated in Smad3 knockout mice (86, 88), and Smad7, an antagonist of TGF-β signaling, or bone morphogenetic protein-7 (BMP-7) acting in a Smad-dependent manner, can reverse or delay fibrosis in renal and lens epithelia (85, 117). A direct role for TGF-β in EMT in lung in vivo was demonstrated recently by overexpression of active TGF-β1 using an adenoviral vector in triple transgenic mice in which AT2 cells permanently express β-galactosidase. In this model, X-gal-positive cells that expressed the myofibroblast marker α-SMA were identified in injured lungs demonstrating EMT in situ. One-third of cells identified as fibroblasts by the expression of vimentin were X-gal-positive, indicating an epithelial origin, and X-gal-positive cells accounted for most of the increase in vimentin-positive cells 21 days after instillation of TGF-β1 (45). Together, these data demonstrate the central importance of TGF-β in EMT both in vitro and in vivo.

**Role for Smad-dependent signaling in EMT.** EMT in response to TGF-β1 and in fibrosis is mediated predominantly via Smad-dependent (mainly Smad3) pathways, although as discussed further below, non-Smad signaling has also been implicated under certain circumstances (20). In the Smad-mediated pathway, TGF-β1 signals are transduced by transmembrane serine/threonine kinase type II and type I receptors. Upon TGF-β1 stimulation, the receptors are internalized into early endosomes where Smad anchor for receptor activation (SARA) modulates formation of complexes with Smad2 or Smad3. Smad2 and Smad3 are then phosphorylated at serine residues by the type I receptor. Phosphorylation induces their association with Smad4 and translocation to the nucleus where they interact with other transcription factors to regulate the transcription of TGF-β-responsive genes including CTGF, α-SMA, collagen 1A2, and plasminogen activator inhibitor-1 (PAI-1) by interacting with Smad-binding elements (16, 17) (Fig. 1).

A requirement for Smad signaling in mediating EMT in vitro was demonstrated by overexpression of a TGF-β receptor type I mutant defective in Smad activation but with retained kinase activity. In mouse NMuMG cells, a nontransformed mammary epithelial cell line, the majority of TGF-β-induced responses, including those related to EMT (e.g., stress fiber formation and fibronectin production), were inhibited in the presence of the mutant receptor indicating a requirement for Smad signaling (28). Similarly, overexpression of Smad3 together with constitutively active type I TGF-β receptor (ALK-5) synergistically enhanced the EMT response of NMuMG epithelial cells (79). The involvement of Smad3 in fibrotic EMT has been clearly demonstrated in vitro and in vivo in the eye and kidney (86, 88). EMT of lens epithelial cells in vivo following injury is completely prevented in Smad3 null mice, while primary cultures of Smad3−/− lens epithelial cells treated with TGF-β are protected from EMT (86). Similarly, in the kidney, Smad3 null mice are protected from experimentally induced tubulointerstitial fibrosis and show reduced EMT and collagen accumulation, whereas cultures of renal tubular epithelial cells from Smad3−/− animals show a block in EMT and a reduction in autoinduction of TGF-β1 (88).

Differential roles for Smad2 and Smad3 have been demonstrated in different cell types. For example, using primary cells from mice with hepatocyte-specific double knockout of Smad2 and Smad3, it was demonstrated that Smad3 but not Smad2 was required for a number of TGF-β-induced functions including induction of EMT (34). In contrast, in human proximal tubular epithelial cells, increased CTGF and decreased E-cadherin were Smad3-dependent, increased MMP-2 was Smad2-dependent, and increases in α-SMA were dependent on both (78). Together, these results suggest that the precise pathway activated may depend on the particular cellular context. A recent transcriptomic analysis of TGF-β-induced EMT in normal mouse and human epithelial cells demonstrated, using a dominant negative approach, that Smad signaling was critical for regulation of all tested target genes (102).

Integrin-linked kinase (ILK), an intracellular serine/threonine kinase that interacts with the cytoplasmic domains of β-integrins and cytoskeletal proteins, has been identified as a potential downstream mediator of Smad-mediated TGF-β1 signaling and may play an important role in EMT (53). ILK is induced in renal tubular epithelial cells in response to TGF-β1 in vitro in a Smad-dependent manner, whereas ectopic expression of ILK suppresses E-cadherin, induces production of the extracellular matrix protein fibronectin, and enhances cell migration. ILK regulates E-cadherin at the transcriptional level, and repression is likely mediated via the transcriptional repressor SNAI-1 in some cell types (72). In addition, ILK phosphorylates Akt (24) and glycogen synthase kinase (GSK) (44), both of which have been implicated in EMT in malignant cells and during development, respectively. While the direct relevance of these findings to EMT during fibrosis is unclear, phosphorylation of GSK-3β in these examples during development and tumor progression leads to its inactivation. Inactivation of GSK-3β results in accumulation of β-catenin and
activation of the Wnt signaling pathway, which has also been strongly implicated in EMT. The potential role of cross talk between TGF-β and Wnt signaling pathways in mediating EMT in response to TGF-β in nonmalignant epithelial cells and in the context of fibrotic EMT remains to be determined.

Role for Smad-independent signaling in EMT. Although less well established than the Smad-dependent pathways in the induction of EMT, there is substantial, although almost entirely in vitro, evidence for TGF-β activation of non-Smad-dependent signaling in some aspects of this process (Fig. 1). However, the distinction between Smad-dependent and nondependent mechanisms can be difficult as there may be significant cross talk between these pathways with non-Smad proteins modulating Smad activity and vice versa. Non-Smad-dependent pathways implicated in TGF-β-dependent EMT include RhoA, Ras, MAPK, PI3 kinase, Notch, and Wnt signaling pathways. In most cases, stimulation of these cooperative pathways provides the context for induction and specification of EMT within a particular tissue, with Smads representing the dominant pathway, which in some instances may be necessary but not sufficient for induction of full EMT (115).

The small GTPase RhoA is involved in TGF-β-induced EMT in a number of cell types including NMuMG mammary epithelial cells and mink lung epithelial (Mv1Lu) cells and appears to play a role particularly in the regulation of cytoskeletal and adherens junction rearrangement (9). However, transient overexpression of constitutively active RhoA constructs did not result in spontaneous EMT indicating that RhoA activation may be necessary but not sufficient for induction of EMT in these cells (9). In addition to its role in cytoskeletal remodeling, Rho has been shown to play a role in TGF-β-mediated activation of the α-SMA promoter during EMT in LLCPK1 cells, making it difficult to conclude that the effects of RhoA in EMT are limited to morphological changes and cytoskeletal rearrangements (60). A recent report suggests a novel mechanism whereby TGF-β regulates EMT through recruitment of the TGF-β receptor to tight junctions where it interacts with the polarity protein Par6 (73). Upon TGF-β signaling, Par6 becomes phosphorylated, leading to recruitment of the ubiquitin ligase Smurf1, resulting in ubiquitination and degradation of RhoA. This leads to dissolution of tight junctions and disassembly of the actin cytoskeleton. There is
also evidence for cross talk between TGF-β and MAPK Erk in vitro. TGF-β stimulates Erk activity in culture models of EMT (human keratinocytes, NMuMG mammary epithelial cells, and mouse cortical tubule epithelial cells) (15, 108, 114). Erk activity was required for disassembly of adherens junctions and induction of cell motility (114), and blockade of Erk inhibited key morphological features of EMT in mammary gland epithelial cells (107). In keratinocytes, EMT was inhibited by blockade of both MAPK and Smad pathways (15). Interactions between TGF-β and p38 MAPK appear to occur in a limited cell type-dependent fashion (mammary epithelial cells and colon cancer cells) in vitro (113), where it appears to confirm a migratory phenotype. TGF-β activation of JNK has also recently been implicated in EMT in transformed keratinocytes, although a previous study did not find evidence for JNK activation (15, 87). There is limited evidence for involvement of PI3K in a Rho-dependent fashion leading to cytoskeletal changes but not full EMT in TGF-β-treated NMuMG mammary epithelial cells (6) and for induction of EMT in lens epithelial cells in a Sna1-dependent fashion (14), whereas PI3K is not required for Ras-mediated EMT (32). Involvement of the Rho, MAPK, and PI3K pathways has mainly been shown following in vitro treatment of cell lines, and it has been suggested that, in derivation and passaging, these cell lines may already have transited Smad-dependent steps required for early induction of EMT (50). Differences in responses between primary cells and cell lines in terms of pathways involved have largely not been addressed. Cross talk has also been demonstrated between TGF-β and Notch signaling, although the downstream mediators of EMT are unknown, and a role for this pathway in fibrosis has not been established (116). There is increasing evidence for a role of the Wnt/b-catenin pathway in regulating EMT (44) and for interactions between TGF-β and b-catenin pathways in EMT, particularly during development (67). A role in fibrosis is less well established. Overall, the predominant pathway involved in TGF-β-mediated EMT appears to be highly cell type and context dependent (64). The complexity of dissecting the pathways involved in mediating TGF-β-induced EMT is demonstrated by a recent report indicating that loss of tight junctions and to some extent E-cadherin in MDCKII cells was Smad independent, whereas complete loss of E-cadherin and transformation to a mesenchymal phenotype were dependent on Smad signaling (61), suggesting that different aspects of EMT can be dissociated and that a clear distinction between the roles of Smad-dependent and Smad-independent pathways may be difficult. The role of any of these pathways in EMT in lung is largely unknown.

Effects of TGF-β on downstream transcription factors. TGF-β also regulates expression of a number of downstream transcription factors involved in EMT via both Smad-dependent and -independent pathways (Fig. 1). Snail1 (SNAI1) and Snail2 (SNAI2) (previously known as Snail and Slug, respectively) are zinc finger proteins that function as repressors of E-cadherin transcription in cultured epithelial cells (12), and both SNAI1 and SNAI2 can be activated by TGF-β via both Smad-dependent and -independent pathways in a cell type-dependent fashion in cultured cells (77). Repression of E-cadherin leads to dissolution of adherens junctions. Differential expression of SNAI1 and SNAI2 is observed in TGF-β-induced EMT in keratinocytes, renal tubular, and mammary epithelial cells, suggesting that they are regulated in a cell-specific fashion. SNAI1 mRNA is increased in renal tubular epithelial cells induced to undergo EMT by mechanical stretch in a TGF-β-dependent fashion (88). SNAI1 activation induces renal fibrosis in transgenic mice and is upregulated in human fibrotic kidneys suggesting a role for SNAI1 in promoting EMT of both tubular and collecting duct cells inducing fibrosis (10). A microarray-based screen of epithelial responses to TGF-β1 identified helix-loop-helix inhibitors of differentiation, Id2 and Id3, as early response targets involved in EMT. Id proteins maintain epithelial phenotype by inhibiting E2A function as a repressor of E-cadherin. TGF-β represses Id proteins, and ectopic expression of Id2 inhibits features of EMT in lens and mammary epithelial cells (46, 48). A recent microarray analysis of TGF-β1-mediated EMT in human proximal tubular epithelial cells identified Id1 as being rapidly induced following TGF-β treatment in a Smad-dependent fashion. Id1 repressed the E-cadherin promoter, but overexpression of Id1 failed to induce α-SMA, fibronectin, MMP-2, or ILK, indicating its inability to confer full EMT and further suggesting that EMT is a multistep process in which loss of epithelial adhesion does not necessarily lead to full transition to a mesenchymal phenotype (54). TGF-β1 induces a subset of Notch target genes of the Hey1 family at the onset of EMT, and EMT can be prevented by the inactivation of Notch or the silencing of Hey1 and Jagged1 in primary kidney tubular epithelial cells (116). However, the precise pathways linking Notch signaling to EMT remain to be identified. High mobility group (HMG) A2 has also been found to be upregulated in TGF-β-induced EMT in a Smad-dependent manner and to in turn regulate SNAI1, SNAI2, Twist, and Id2, all key regulators of EMT, suggesting HMG A2 as a major mediator of EMT (97). HMG A2 is highly expressed in a number of tumors, suggesting a role in tumor invasion, but its role in fibrosis is unknown.

Reversal of TGF-β-induced EMT. A number of interventions have been demonstrated to lead to the reversal of EMT. BMP-7 reversed TGF-β1-induced EMT in adult tubular epithelial cells by directly counteracting TGF-β1-induced Smad3-dependent EMT, and evidence for reversal of renal fibrosis occurring via EMT has been shown in vivo (119). BMP-7 was able to delay EMT in lens epithelium in association with downregulation of Smad2, whereas overexpression of inhibitory Smad7 prevented EMT and decreased nuclear translocation of Smads2 and -3 (85). Furthermore, HGF blocks EMT in human kidney epithelial cells by upregulation of the Smad transcriptional corepressor SnoN, which leads to formation of a transcriptionally inactive SnoN/Smad complex, thereby blocking the effects of TGF-β1 (110). These studies suggest the feasibility of modulating Smad activity as a strategy for counteracting actions of TGF-β to induce EMT. Knowledge of the precise molecular mechanisms mediating TGF-β-induced EMT and its interactions with other signaling pathways will be important for developing strategies to inhibit/reverse EMT without disrupting the beneficial effects of TGF-β signaling.

EMT in Lung

The precise role of EMT during the response to injury and pathogenesis of fibrosis in the adult lung remains to be identified. However, there is increasing evidence that it may play a substantial role in a variety of pathogenic processes, and, in fact, it may be a major source of pathogenic mesenchymal cell...
types, such as myofibroblasts, during pulmonary fibrogenesis. EMT has been identified in the alveolar epithelium and airway epithelium, and we will consider each in turn.

A new focus on the alveolar epithelium as a key pathogenic mediator of fibrosis in the lung has recently been proposed (89, 91, 105). AEC have the capacity to both produce and respond to profibrotic mediators, regulate function and differentiation of fibroblasts through the release of a variety of mediators, and modify cell morphology and gene expression in response to injury (5, 21, 36, 40–42, 47, 56, 57, 74). In addition, epithelial injury and blunted repair in mouse lung explants is sufficient to promote pulmonary fibrosis in the absence of inflammation, and the presence of an intact epithelial layer suppressed fibroblast proliferation and matrix deposition (1, 3). AEC in IPF are morphologically abnormal, with hyperplastic pneumocytes and reactive elongated cells overlying fibroblastic foci, the presumed sites of active fibrogenesis (38, 39, 89, 101). Patterns of reactive elongated cells overlying fibroblastic foci, the pre-morphologically abnormal, with hyperplastic pneumocytes and EMT has been identified in the alveolar epithelium and airway types, such as myofibroblasts, during pulmonary fibrogenesis. EMT has been identified in the alveolar epithelium and airway epithelium, and we will consider each in turn.

A new focus on the alveolar epithelium as a key pathogenic mediator of fibrosis in the lung has recently been proposed (89, 91, 105). AEC have the capacity to both produce and respond to profibrotic mediators, regulate function and differentiation of fibroblasts through the release of a variety of mediators, and modify cell morphology and gene expression in response to injury (5, 21, 36, 40–42, 47, 56, 57, 74). In addition, epithelial injury and blunted repair in mouse lung explants is sufficient to promote pulmonary fibrosis in the absence of inflammation, and the presence of an intact epithelial layer suppressed fibroblast proliferation and matrix deposition (1, 3). AEC in IPF are morphologically abnormal, with hyperplastic pneumocytes and reactive elongated cells overlying fibroblastic foci, the presumed sites of active fibrogenesis (38, 39, 89, 101). Patterns of cytokeratin expression are altered (30), and AEC apoptosis increases adjacent to fibroblastic foci (7, 49, 101). Activated AEC in IPF synthesize a variety of procoagulant factors (e.g., PAI-1 and PAI-2) (47) and fibrogenic cytokines [e.g., PDGF (5), TGF-β (40–42), TNF-α (65), endothelin-1 (21), and CTGF (74)], allowing for bidirectional signaling between AEC and fibroblasts whereby each cell type influences the proliferation/survival of the other. AEC also produce matrix MMPs, suggesting that AEC contribute significantly to extracellular matrix remodeling (90, 123).

Given the apparently critical role for the alveolar epithelium during injury and its potential for plasticity, we recently evaluated the potential for AEC to undergo EMT in vitro and in vivo. Following extended exposure to TGF-β1 in culture, both primary rat AEC and an AT2 cell line (RLE-6TN) undergo EMT as evidenced by loss of epithelial markers [aquaporin-5 (AQP5), cytokeratin, and ZO-1] and upregulation of mesenchymal markers (α-SMA, vimentin, desmin, and type I collagen) concurrent with transition to a fibroblast-like morphology (106). Effects of TGF-β were augmented by TNF-α. Detailed analysis as a function of time in culture demonstrated localized colocalization of cytoplasmic α-SMA and nuclear Nkx2.1 [also known as thyroid transcription factor-1 (TTF-1)], a transcription factor that is normally expressed in distal lung epithelial cells, during transition to a fibroblast phenotype. Importantly, we were also able to colocalize epithelial (Nkx2.1 and prosurfactant protein B) and mesenchymal (α-SMA) markers in more than 80% of hyperplastic epithelial cells overlying fibroblastic foci in lung biopsies of IPF patients, suggesting a new paradigm in which EMT contributes to pulmonary fibrosis in vivo. Our in vivo findings were recently confirmed by colocalization of prosurfactant protein C (pro-SPC), an epithelial marker, and N-cadherin, a mesenchymal cadherin, in IPF lung tissue (45).

Interestingly, in earlier studies, although not interpreted as being indicative of EMT, TGF-β was shown to influence AEC phenotype by stimulating fibronectin and collagen production, downregulating SPC expression, and inducing ECM components including proteoglycans (56, 57, 84). Similar changes in epithelial morphology to a fibroblast-like phenotype were observed after transduction of rat lung explants with a retroviral vector encoding TGF-β1 (109). Consistent with our recent findings using isolated rat AT2 cells in primary culture, Yao et al. (111) demonstrated that treatment of rat AT2 cells with TGF-β resulted in cytoskeletal rearrangements, assumption of a fibroblast-like morphology, downregulation of E-cadherin, increased collagen I production, and induction of α-SMA suggestive of EMT. Although A549 cells have also been shown to undergo EMT in response to TGF-β1, caution should be used in interpreting these results in the context of primary AEC given the malignant origin of these cells (37).

Additional in vivo studies have recently emerged that confirm the importance of EMT in pulmonary fibrosis. Using a Cre-LOX approach for lineage tagging with β-galactosidase (β-gal), lung AT2 cells were shown to express vimentin and undergo EMT in response to overexpression of active TGF-β1 (45). The increase in vimentin-positive cells within injured lungs was nearly all β-gal positive, implicating epithelial cells as a major source of mesenchymal cells in this model. Additionally, a very recent report demonstrated that AEC from the fibrotic lungs of mice overexpressing IGFBP-5 coexpressed epithelial markers and α-SMA, suggesting EMT (112). However, the relative contribution of EMT of AEC to production of intrapulmonary fibroblasts/myofibroblasts during IPF and other forms of human fibrosis remains to be determined. Given that 1) EMT contributes at least one-third of fibroblasts during fibrosis in other organs (29), 2) AEC-fibroblast cross talk and interactions are involved in fibrosis in the lung, 3) AEC undergo EMT in response to TGF-β1, and 4) TGF-β1 is expressed at sites of epithelial injury and adjacent fibrosis in vivo, it seems likely that conversion of AEC to fibroblasts and activated myofibroblasts may contribute significantly to lung fibrosis in vivo. Similar to kidney epithelial cells, AEC appear to have a unique property of plasticity that enables conversion between epithelial and mesenchymal phenotypes. Whether multi- or at least pluripotency is a property of all AEC, specific to type II cells, or specific only to subsets of AEC [e.g., pluripotent subpopulations recently identified as “bronchoalveolar stem cells” (43)], is currently unknown but warrants further elucidation. However, this new paradigm suggests that the epithelium should be viewed as an active participant in fibrosis, serving as a progenitor with plasticity and the capacity to participate in alternate pathways, depending in part on the degree and nature of injury: re-epithelialization to restore normal architecture, apoptosis, or fibrogenesis.

Beyond the alveolar epithelium, the airway epithelium is also beginning to be investigated as a potentially important contributor to intrapulmonary fibroblast and myofibroblast accumulation after injury. Fibrotic obstruction of small and large airways is a key pathological finding in a variety of disorders, including asthma (83) and obliterative bronchiolitis (75). Mechanical injury to pseudostratified airway epithelial cells cocultured with fibroblasts resulted in the induction of myofibroblasts in a guinea pig amniotic membrane model, resulting in increased TGF-β1 release and ECM deposition (63). In the only study to date of its kind, Ward et al. (104) found that airway epithelial cells taken from clinically stable lung transplant recipients exhibited features suggesting EMT. Long-term survival and graft function is largely limited by progressive deterioration in lung function due to airway remodeling from obliterative bronchiolitis posttransplant (75), and there has
been no substantial improvement in the documented incidence of this disease in more than 10 ten years. This may simply be due to a failure to understand the basic pathophysiological mechanisms underlying the induction of fibrotic airway remodeling, similar to the lack of understanding of the pathogenesis of IPF. In the study by Ward et al., 15% of the sampled epithelium stained positive for PSP1 (S100A4 in humans), and epithelial cell cultures from these patients became motile and penetrated collagen-coated filters following stimulation with TGF-β1. Importantly, these changes were noted before the development of detectable clinical deterioration of lung function. Speculatively, this suggests that early intervention designed to inhibit EMT in the airways of transplant recipients could lead to reductions in airway remodeling. Further investigation into the prevalence and mechanisms of EMT in airway fibrosis and potential interventions to inhibit it are needed.

Conclusions

EMT has long been shown to play a role in cellular transdifferentiation during development and tumor invasion. EMT can be viewed as an extreme form of cell plasticity characterized by loss of epithelial markers, cytoskeletal reorganization, and transition to a spindle-shaped morphology concurrent with acquisition of mesenchymal markers. It is increasingly being recognized that, following epithelial stress/injury, epithelial cells can give rise to fibroblasts and thereby contribute to the pathogenesis of fibrosis by undergoing EMT. TGF-β has been implicated as a master switch in induction of fibrosis in many organs, including the lung, and is a major mediator of EMT in a number of physiological contexts, including tissue fibrosis, largely via Smad-dependent pathways. The relative contribution of Smad-dependent vs. -independent pathways appears to be largely context dependent. Recently, it has been demonstrated that TGF-β induces EMT in AEC in vitro, and epithelial and mesenchymal markers have been colocalized to hyperplastic AT2 cells in IPF tissue, suggesting that AEC may exhibit extreme plasticity and serve as a source of (myo)fibroblasts in lung fibrosis. The relative contribution of EMT to fibrosis in IPF and other fibrotic lung disorders remains to be determined. Molecular profiling of the fibroblast phenotype in various fibrotic disorders may identify distinct fibroblast populations in the lung and offer insights into whether these populations are perhaps derived from different sources. Although much more preliminary, there is also evidence that airway epithelium can undergo a transition to a mesenchymal phenotype and contribute directly to airway fibrosis. Understanding the precise molecular interactions that lead to EMT will hopefully lead to the identification of novel therapeutic targets for fibrosis in the lung and elsewhere.

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