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### Spotlight

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## Epithelial-mesenchymal transition and its implications for fibrosis

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Epithelial to mesenchymal transition (EMT) is a central mechanism for diversifying the cells found in complex tissues. This dynamic process helps organize the formation of the body plan, and while EMT is well studied in the context of embryonic development, it also plays a role in the genesis of fibroblasts during organ fibrosis in adult tissues. Emerging evidence from studies of renal fibrosis suggests that more than a third of all disease-related fibroblasts originate from tubular epithelia at the site of injury. This review highlights recent advances in the process of EMT signaling in health and disease and how it may be attenuated or reversed by selective cytokines and growth factors.

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For one hundred and forty-five years, biologists have known that cells come from cells (1). This concept is so fundamental today that we accept it implicitly; cells either divide asymmetrically to preserve stem cell progenitors, partition into sister cells, differentiate along fate pathways, or undergo oncogenesis following the formation of normal tissues. Specification and diversification of cell lineages are initiated by genetic programs under the control of morphogenic cues (2–4). These lineages evolve in a hierarchical manner conforming to developmental boundaries and oscillating biological clocks until reaching terminal differentiation (5). Epithelia from metazoans are emblematic of this process, and at maturity cover outer surfaces (6, 7) or line hollow cavities formed by tubular structures in complex tissues (8–10). Since epithelia typically serve specialized functions (11–13), it is assumed that a state of terminal differentiation is necessary and protected once development is complete.

In recent years, however, this formidable notion has been challenged by observations that mature epithelia change their phenotype following morphogenic pressure from injured tissue. Since the phenomenon of epithelial plasticity was described before it had a firm

biochemical basis (14, 15), one is confronted with a plethora of seemingly interchangeable vocabulary. Today, the terms “epithelial-mesenchymal transformation, interactions, or transition” are comingled inappropriately with the term “epithelial-mesenchymal transdifferentiation.” “Transformation” classically describes the oncogenic conversion of epithelia. Likewise, the induction of bone marrow stem cells to form somatic cells probably should be considered differentiation rather than transdifferentiation (16). Epithelial-mesenchymal interaction refers to proximate paracrine cross-talk between tissue epithelia and stromal fibroblasts and is completely different from the concept of epithelial-mesenchymal transition (EMT). EMT is a variant of transdifferentiation and a well-recognized mechanism for dispersing cells in vertebrate embryos (17), forming fibroblasts in injured tissues (18, 19), or initiating metastases in epithelial cancer (20–23). We prefer the term “transition” to describe this conversion instead of “epithelial-mesenchymal transdifferentiation” because “transdifferentiation” classically refers to differentiated cells changing into other differentiated cells (24). Transdifferentiation has been observed in retinal pigmented cells that become lens epithelia (25, 26), in the conversion from white to brown adipocytes (27), endothelial cells that become vascular smooth muscle cells (28), lactotrophs that interconvert to somatotrophs in the pituitary (29), pancreatic acinar cells that become ductal epithelium (30, 31) or hepatocytes (32, 33), and hepatocytes that morph into pancreatic ductal cells (34). Although many investigators fail to make the distinction between transdifferentiation and transition, it may be time to do so. It is not yet clear whether the fibroblast transition of EMT is an expected middle phase of transdifferentiating epithelium or whether EMT producing fibroblasts is an arrested

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**Nonstandard abbreviations used:** epithelial-mesenchymal transition (EMT); bone morphogenic protein 7 (BMP-7); integrin-linked kinase (ILK); lymphoid enhancer factor (LEF); glycogen synthase kinase (GSK); fibroblast-specific protein-1 (FSP1);  $\alpha$ -smooth muscle actin ( $\alpha$ SMA); tubular basement membrane (TBM); tissue plasminogen activator (tPA).

form of transdifferentiation (24). EMT of terminally differentiated epithelium, in its purest sense, produces a tissue fibroblast (19).

Developmental biologists have also known for decades that epiblasts undergo EMT to form primary mesenchyme in the creation of triploblastic germ layers (17, 35–37; Figure 1). In the mesoderm this is followed by mesenchymal-epithelial transitions to create secondary epithelium as part of somitogenesis (38, 39) and the further commitment and diversification of cells forming mesoendodermal structures (40–42). Secondary epithelium in mature or adult tissues can also undergo EMT following epithelial stress, such as inflammation (18, 19) or wounding (17, 43) that leads to fibroblast production and fibrogenesis. Epithelia forming tumors also use EMT when carcinomas become metastatic (23, 44, 45).

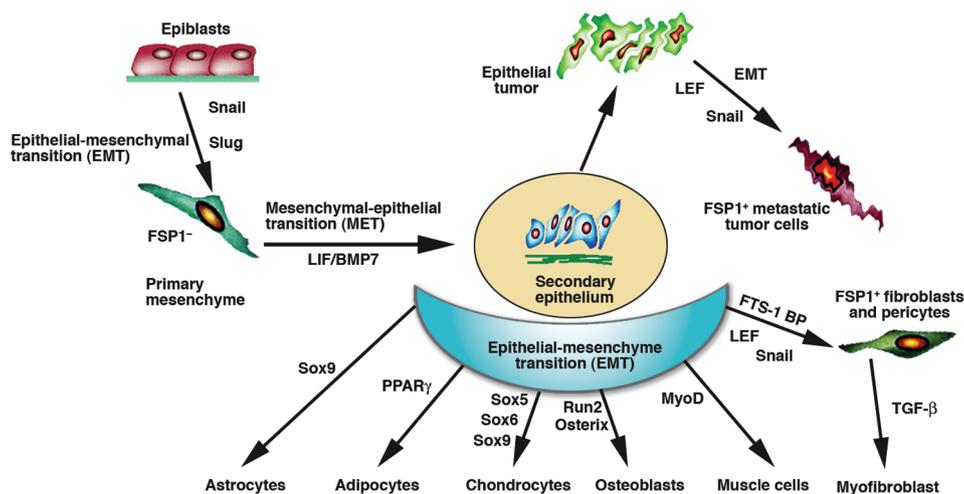
We review here recent observations regarding the mechanism of EMT in culture and during fibrogenesis, especially associated with kidney disease. The problem of tissue fibrosis is that epithelial units are overtaken by scarification and lose their morphogenic cues, leaving involved organs to fail. While traditional studies of fibrosis have focused on the production of extracellular matrix, recent information now suggests that epithelia contribute to the problem by creating new fibroblasts. Experiments demonstrating the reversibility of organ fibrosis also highlight the need to consider cellular mechanisms of fibrogenesis and the basic biology that will, one hopes, contribute new molecules as useful therapeutics.

### The mechanism of EMT

From a general perspective, EMT is about disaggregating epithelial units and reshaping epithelia for move-

ment. Epithelium in transition lose polarity, adherens junctions, tight junctions, desmosomes, and cytokeratin intermediate filaments in order to rearrange their F-actin stress fibers and express filopodia and lamellipodia. This phenotypic conversion requires the molecular reprogramming of epithelium with new biochemical instructions. Much of this conversion has been studied fractionally, during experiments that expose new transduction and signaling pathways, in epithelia that transition in culture, and more recently in fibrogenic tissues. Below we describe an enlarging picture of EMT from a broad and increasingly complex literature.

**Induction of EMT.** EMT is easily engaged by a combination of cytokines associated with proteolytic digestion of basement membranes upon which epithelia reside. Metalloproteinases (46, 47) or membrane assembly inhibitors (48) initiate the process by dismantling the local basement membrane. Local expression of TGF- $\beta$ , EGF, IGF-II, or FGF-2 facilitates EMT (Figure 2) by binding epithelial receptors with ligand-inducible intrinsic kinase activity (49–52). The TGF- $\beta$  effect depends on  $\beta$ -integrin transduction (53), Smad3-dependent transcription (54), or Smad-independent p38MAP kinase activation and GTPase-mediated signaling (53, 55, 56). Depending on the tissue, all three isoforms of TGF- $\beta$  may be involved sequentially (57–59). While TGF- $\beta$  is considered prototypical in its induction of EMT (50, 60, 61), there is an increase in epithelial EGF receptors in the EMT microenvironment (62), and EGF can assist in completing the conversion (50). IGF-II also directs the redistribution of  $\beta$ -catenins from the cell surface to the nucleus and facilitates the intracellular degradation of E-cadherin (51), while FGF-2 and TGF- $\beta$  are required for the expression of MMP-2 and MMP-9 to assist in basement membrane degradation (52).



**Figure 1**

Primitive epithelia (epiblasts) form triploblastic germ layers through EMT. The primary mesenchyme that migrates after EMT is reinduced to secondary epithelium by mesenchymal-epithelial transition. Secondary epithelia differentiate to form new epithelial tissues and undergo a second round of EMT to form the cells of connective tissue, including astrocytes, adipocytes, chondrocytes, osteoblasts, muscle cells, and fibroblasts. Mature secondary epithelia that form epithelial organs can also transform into primary tumors that later undergo EMT to metastasize. These processes are regulated by morphogenic cues and a variety of transcription factors, and are potentially plastic in their adaptation to new biologic circumstances.

Combinations of cytokines are generally present in most areas of tissue injury, so it is difficult to assign priorities or hierarchy. Each moiety may contribute a unique inducement to the transition. Furthermore, the role of HGF and FGFs depends on the timed expression and selective distribution of receptors (63, 64). While HGF action through its c-Met/Crk adaptor proteins (65) induces EMT during somitogenesis and endocardial cushion development (66, 67) and modulates the connectivity of intercellular junctions between polarized intestinal and kidney epithelium (68), in the fibrogenic kidney it has the opposite effect of protecting epithelium from EMT (69). In this regard, the expression of bone morphogenic protein 7 (BMP-7) also counterbalances EMT in the kidney (54). Like many biological systems, countervailing processes that modulate EMT effector events are beginning to appear.

*Engagement of protein kinases.* Epithelial signaling that leads to EMT has been studied in a variety of cultured epithelia and seems to have broad generality across numerous phenotypes (Figure 2). As a result of ligand-inducible receptor kinase activation (14), there is a downstream engagement of GTPases from the Ras superfamily (70, 71) or commitment of SH2-SH3 protein domains of the nonreceptor tyrosine kinase (c-Src and Btk) pathways (14, 72) that shift the intracellular balance of small GTPases (Rho, Rac, and Cdc42). Raf/MAP kinases are subsequently activated with several interconnected consequences: engagement of the EMT transcriptome (73) followed by actin rearrangement of the cytoskeleton (70, 74). Integrin-linked kinase (ILK) activation by TGF- $\beta$ -activated Smad proteins (75) or integrin signaling (76) enhances  $\beta$ -catenin/lymphoid enhancer factor (LEF) expression, suppressing E-cadherin. Activation of Src kinases favors the PI3K pathway, stabilization of  $\beta$ -catenin for nuclear import (77), protection from apoptosis, and disruption of  $\beta$ -integrin binding and E-cadherin complexes (21, 53, 78, 79). Many of these pathways collaboratively reinforce EMT.

The nuclear import of LEF proteins with Smad3 or  $\beta$ -catenin from the cytoplasm is beginning to look like one of several key molecular steps in EMT (73, 80). The phosphorylation of  $\beta$ -catenin by glycogen synthase kinase- (GSK-3 $\beta$ ) allows it to form a complex with APC suppressor protein and Axin (81). p53 activation of APC-dependent pathways also forms a complex with  $\beta$ -catenin (82), and both of these pathways lead to direct loss of free  $\beta$ -catenin through ubiquitination (83). If, however, phosphorylation by GSK-3 $\beta$  is inhibited (77), cytoplasmic  $\beta$ -catenin is stabilized by re-entering the E-cadherin complex (84) or binding to the B-box of LEF where together they move into the nucleus to engage the EMT transcriptome (85). Wnt-1 (81), IGF-II (51), Ras (77), and ILK (76) all stabilize cytoplasmic levels of  $\beta$ -catenin to facilitate EMT, perhaps by GSK-3 $\beta$  (or other kinase) inhibition. Smad3 activation by TGF- $\beta$  family members can also activate LEF-1 in the absence of  $\beta$ -catenin (80). These latter findings suggest either a

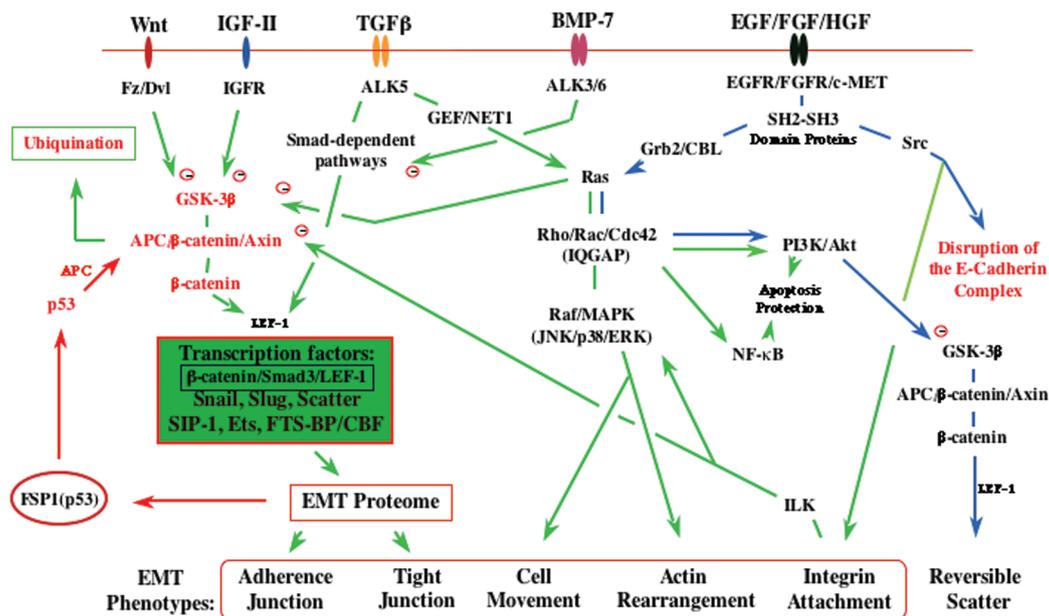
synergistic or independent control of LEF-1 by at least two EMT-linked signaling pathways. While levels of APC suppressor protein in epithelia may protect the state of terminal differentiation from EMT (73, 86), activation of Smad pathways may provide a countervailing leak in this stability.  $\beta$ -catenin and Smad3 also require the engagement of different transcriptional coactivators, depending on the promoter. These differences may regulate the selectivity or availability of the EMT transcriptome.

Recently, there also has been some attempt to distinguish true EMT from an epithelial phenocopy called “reversible scatter” (21, 73). Reversible scatter following cytokine stimulation looks like EMT because the cells assume a spindlelike shape and undergo a brief period of transcription. But because transcription is not sustained on withdrawal of the inducement and/or if the cells are protected from apoptosis, the epithelia return to their original state (21). TGF- $\beta$  and Ras classically produce EMT, while EGF, HGF, and FGF favor scattering (21), but not in all cells (52, 68). A scatter effect may be facilitated by varying levels of cytoplasmic APC suppressor protein (73) or preferred activation of the PI3K pathway (21). Whether scatter reverses or goes on to EMT may really just be a timing issue in the continuum of transition, as it is not clear what biological function reversible scatter serves on its own.

*The EMT proteome.* The EMT proteome reflects a fundamental change in proteins gained, maintained, or lost (Table 1) with the conversion of epiblasts to primary mesenchyme (37, 87), secondary epithelium to fibroblasts (88), or in the transition of tumor epithelia to metastatic cells (89). Many studies have generally focused on only one or two event markers (for example, the changes in E-cadherin or Snail expression) and are

**Table 1**  
The EMT proteome

Proteins gained or maintained:	Proteins attenuated:
Snail	E-cadherin
Slug	$\beta$ -catenin
Scratch	Desmoplakin
SIP1	Muc-1
E47	ZO-1
Ets	Syndecan-1
FTS binding protein	Cytokeratin-18
RhoB	
FSP1	
TGF- $\beta$	
FGF-1,-2,-8	
MMP-2	
MMP-9	
Vimentin	
$\alpha$ SMA	
Fibronectin	
Collagen type I	
Collagen type III	
Thrombospondin	
PAI-1	



**Figure 2**

Epithelial plasticity can lead to classical EMT (loss of cell-cell and cell-substratum attachments, new actin rearrangements, and gain of mobility) or reversible scatter, which looks like EMT but is not enduring and can revert. These events are regulated by ligand-inducible intrinsic kinase receptors on the cell surface, which modulate small GTPases, Smads, PI3Ks, MAP kinases, and the availability of  $\beta$ -catenin to coactivate LEF in the nucleus. Free levels of  $\beta$ -catenin are regulated by E-cadherin or APC/ $\beta$ -catenin/Axin complexes, the latter of which shuttle  $\beta$ -catenin between ubiquitination or utilization in adherens junctions. Activation of nuclear transcription provides new transcriptional regulators (Snail, SIP-1, Ets, and FTS-BP/CarG box binding factor) of the EMT proteome. The EMT proteome comprises proteins listed in Table 1. The variability of receptors, kinases, and the emergence of combined preferences for signaling pathways determine the plasticity unique to each epithelium.

not comprehensive. Most information about the EMT proteome is inferred from proteins found in epithelia but not in fibroblasts or metastatic cancer cells, or is based on apparent targets of transcription factors (15, 73). Although dependent on cell context and ease of growth factor signaling, delamination of epithelia to facilitate movement is also accompanied by a regulatory decrease in apoptosis and mitosis (15, 21).

Cellular plasticity likely requires real-time control by transcriptional networks (90–94). Some models suggest several transcription factors may be key modulators of transitional events. The Snail superfamily of zinc-finger proteins has two evolutionary branches, one for Scratch and the other for Snail and Slug (15). These proteins recognize an E-box binding motif on the promoter for E-cadherin (among others) in competition with the basic helix-loop-helix protein SIP1. Ras/MAPK activates Snail while TGF- $\beta$  regulates Smad-dependent pathways to engage SIP1 and Snail (15, 95). Subsequently E-cadherin, cytokeratin, muc-1, and desmoplakin are repressed, while fibroblast-specific protein-1 (FSP1), fibronectin, vimentin, and Rho are increased (15, 54). Repression of E-cadherin by Snail proteins frees up more cytoplasmic  $\beta$ -catenin, which, as mentioned above, is co-imported with LEF to the nucleus where its activation is strongly associated with EMT (73). Ets transcription factors regulate EMT in the heart (96, 97).

One of the more interesting proteins found in the EMT proteome is FSP1 (18), also known as S100A4 (98).

Support for the notion that EMT is a major source of fibroblasts comes from experiments showing FSP1 expression in cultured epithelium during EMT following exposure to TGF- $\beta$  and EGF (50), histologic evidence that epithelial units expressing FSP1 disaggregate as organ tissues devolve during the early stages of fibrogenesis (18), and direct observations of EMT in transgenic mice carrying marked epithelium (19). Dividing fibroblasts exposed to nucleoside analogues are also selectively eliminated in transgenic mice expressing thymidine kinase under control of the FSP1 promoter (99). Members of the S100 superfamily have been implicated in cytoskeletal-membrane interactions, calcium signal transduction, and cellular growth and differentiation (100). In the presence of calcium, FSP1 dimerizes and binds the c-terminal of p53 in the cytoplasm. In this way, FSP1 may sequester p53 from the APC ubiquitination pathway (101, 102), perhaps raising levels of free  $\beta$ -catenin. We suspect that FSP1 facilitates and may even maintain the EMT phenotype through this mechanism (Figure 2). While the precise function of FSP1 is not entirely clear, its interaction with cytoskeletal moieties and its early role in EMT suggest that FSP1 may fashion mesenchymal cell shape to enable motility (50) and induce angiogenesis (103). The expression of FSP1 indicates the potential presence of a molecular program determining fibroblast phenotype.

The promoter for FSP1 is also part of a transcriptome that shares putative FTS-1/CArG box sites in the early

promoter regions of a group of genes that would be expected in EMT-derived fibroblasts, including those encoding c-myc, c-Fos, H-ras, Slap, TGF- $\beta$ , FGF-1, -2, and -8, FSP1, vimentin,  $\alpha$ -smooth muscle actin ( $\alpha$ SMA), aggrecan, collagen types I and III, thrombospondin I, and matrix metalloproteinases 2 and 9 (19). One hypothesis is that the selective engagement of FTS-1/CArG box sites by a transcriptional complex of proteins may be one of several key regulators of EMT; preliminary evidence suggests a new Kruppel-like zinc finger protein called FTS/CArG box binding factor (FTS-BP/CBF) (refs. 104, 105; and our unpublished observations) may contribute.

*GTPase modulation of cell shape and movement.* Epithelia that undergo EMT during development, inflammation, or carcinogenesis become mesenchymal cells, fibroblasts, or metastatic tumor cells, respectively (19, 72, 106). This conversion of epithelia is dependent on molecular switches under the control of the Ras superfamily of small GTPases (71). Ras and Rho families of GTPases are activated by guanine nucleotide exchange factors (107) and deactivated by GTPase activating proteins (108). GTPases are a signaling link between cell surface receptor activation and the actin cytoskeleton; some GTPases can also cooperatively modulate EMT with cytokine pressure (21, 109). Three of the best-studied small GTPases are Rho, Rac, and Cdc42. The cross-talk between these members suggests they are activated independently or in series: Ras or Cdc42 can activate Rac, and Rac can inhibit or activate Rho (110–113). Rho helps reconfigure actin stress fibers and stimulates actin-myosin contraction in the cell body, Rac induces the assembly of actin surface protrusions called lamellopodia, and Cdc42 promotes the formation of actin-rich finger extensions called filopodia and modulates cellular asymmetry (71). Their differential activation and balance ensure not only epithelialization but also its dissolution. The cellular properties of contraction, migration, proliferation, and phagocytosis are also under GTPase control (70). The cellular actions of these small GTPases engage downstream MAP kinases, alter gene transcription, and are integral to shaping cell phenotype during EMT.

### **Fibroblasts derive from a niche**

Since the original observations of Cohnheim (114), investigators have debated the origin of tissue fibroblasts. Three notions persist regarding their lineage: The longest-held concept is that fibroblasts are simply residual embryonic mesenchymal cells left over from organogenesis. While this hypothesis explains the incorrect but often interchangeable substitution of the term “fibroblast” for “mesenchymal cell,” the idea itself has no proof and is unlikely since primary mesenchymal cells do not express FSP1 (18). A second notion argues that fibroblasts emerge from the bloodstream after release from the bone marrow (115), and a third view suggests that fibroblasts derive locally in tissues following EMT (19). The second and third hypotheses are mechanistically identical; that is, all fibroblasts probably arise from EMT.

Interstitial fibroblasts appear after gastrulation (after E8.5 in mice) (18) and form as a result of EMT from secondary epithelium (19). Support for the notion that EMT is a major source of local fibroblasts comes from experiments described above. FSP1 is also expressed in some endosteal lining cells and marrow stromal cells (19), and about 14–15% of fibroblasts in fibrosing kidney are derived from marrow. Not much is known about the origin of endosteal lining cells (116, 117). Endosteal bone marrow lining cells precede the formation of the marrow cavity and its contents (117), and in some species are separated from medullary hematopoiesis by a marrow sac comprising a mixture of simple epithelium and/or condensed stromal-like cells (118, 119). This sac appears to have a structural and biochemical interface with endosteal lining cells in nodal regions of bone (118, 120), and the collective structure may be an EMT niche for osteogenic precursor cells, indifferent endosteum, fibroblasts, and marrow stromal cells. Recent evidence also suggests it can be a niche for hematopoietic stem cells (121). FSP1<sup>+</sup> cells in the marrow are mostly CD34<sup>-</sup>. CD34<sup>-</sup> progenitor cells cycle their expression of CD34 in the marrow (122–124); both CD34<sup>-</sup> and CD34<sup>+</sup> stromal cells circulate in peripheral blood, and, following bone marrow reconstitution, CD34 cells locate as bone-lining endosteum (116, 124). Since some marrow stromal cells can be released into the circulation (124), CD34<sup>-</sup>, FSP1<sup>+</sup> bone marrow “fibroblasts” might derive from an endosteal EMT niche transitioning to marrow stromal cells (19), which then may evolve into circulating fibrocytes (115). The contribution of CD34<sup>-</sup>, Stro1<sup>+</sup>, CD73<sup>+</sup> mesenchymal stem cells in the accumulation of fibroblasts in this setting is yet unknown.

Most investigators accept with conventional wisdom that fibroblasts represent a cell type of limited diversity. Fibroblast shape, cytoskeletal structure, secretion of interstitial collagens, mobility, participation in tissue fibrosis, and behavior in culture all tend to support this belief. The EMT hypothesis, however, challenges this notion of homogeneity, as do observations that fibroblasts express subtle biochemical differences (125) and phenotypic variability (126–128), and respond differently to cytokines and matrix (129), depending on their tissue of origin. Recent evaluation of the transcriptome from a variety of fibroblasts suggests there is topographic differentiation perhaps based on a “Hox code” (130). Consequently, fibroblasts formed by EMT may differentially express a profile of genes, or a few residual receptors or signaling pathways representative of their previous life as mature epithelium, and, theoretically, can be as heterogeneous as the universe of epithelia.

### **EMT and fibrosis**

The role of EMT during tissue injury leading to organ fibrosis (deposition of collagens, elastin, tenascin, and other matrix molecules) is becoming increasingly clear (Figure 3). A great bulk of such evidence exists for EMT associated with progressive kidney diseases (19), and is probably true for the lung (131) and possibly the liver.

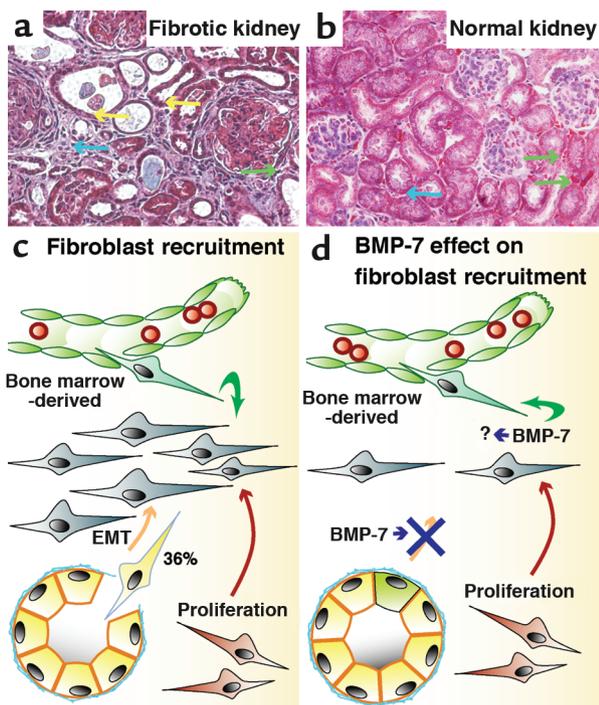
Typical experimental models of kidney fibrosis in mice or rats include progressive glomerulonephritis from anti-glomerular basement membrane disease (132), Alport syndrome (133), or spontaneous lupus nephritis (134), and NOD or db/db nephritic mice (models for diabetic nephropathy) (135), all of which chronically progress at a slow pace, and unilateral ureteral obstruction (136), which progresses to end-stage quickly but leaves the contralateral kidney normal as a control. A number of studies demonstrating EMT during kidney fibrosis correlate with the expression of FSP1 (described above) (18, 50). FSP1 identifies tubular epithelial cells undergoing transition in damaged nephrons trapped by interstitial injury and tracks with increasing numbers of fibroblasts as fibrosis grows worse (50, 137). These FSP1<sup>+</sup> epithelia traverse through damaged tubular basement membrane (TBM) and accumulate in the interstitium of the kidney (138) where they lose their epithelial markers as they gain a fibroblast phenotype (50, 137).

Fibroblasts are not particularly abundant in normal kidneys as they are in lungs, lymphoid nodes, and spleens. When renal fibrogenesis sets in, about 36% of new fibroblasts come from local EMT, about 14–15% from the bone marrow, and the rest from local proliferation (19). This finding reinforces the notion that fibrogenesis is a local epithelial event.

It is worth mentioning that fibroblasts have little in the way of other distinguishing anatomic features, and most of the proteins they express are not highly specific (18). Vimentin is not fibroblast-specific (50, 137), and type I collagen synthesis is generally only detectable in selected subpopulations of fibroblasts (139–142). Some subpopulations of fibroblasts during fibrogenesis express  $\alpha$ SMA (46, 128, 143, 144), a marker of activated fibroblasts (myofibroblasts) (145, 146). While much of the fibrosis literature has relied on this less specific marker,  $\alpha$ SMA is not expressed by all fibroblasts (137), suggesting that it does not define the universe of fibroblasts and potentially also identifies smooth muscle cells separated from local blood vessels during tissue injury (137, 147). The increased number of  $\alpha$ SMA<sup>+</sup> smooth muscle cells in fibrotic tissue may derive from delaminated endothelial cells following endothelial-mesenchymal transition (28).

Why are tubular epithelia susceptible to EMT? Injury to the kidney is associated with many inflammatory cells which can incite EMT using growth factors such as TGF- $\beta$ , EGF, and FGF-2 (52). Under the influence of such growth factors, resident fibroblasts and tubular epithelia induce basement membrane-degrading enzymes such as MMP-2 and MMP-9 (48). Degradation of TBM results in disruption of tubular nephrons, and delaminated epithelial cells either fall off into the tubular fluid or migrate towards the interstitium under the influence of increasing growth factor gradients and chemoattractants (47). This initial recruitment of tubular epithelial cells for EMT can be inhibited by blocking the expression of MMP-9 through the disruption of tissue plasminogen activator (tPA, an activator of MMP-9) (148). Other studies have also demonstrated that HGF can decrease levels of TGF- $\beta$ , restore TGF- $\beta$ -mediated loss of E-cadherin, and potentially decrease amounts of active MMP-9 (149). In this regard, ILK is now identified as a key mediator of TGF- $\beta$ -induced EMT associated with tubular epithelial cells (75).

The relevance of TGF- $\beta$ -induced EMT for progression of kidney fibrosis was recently addressed in studies using BMP-7 as an intracellular competitor of TGF- $\beta$  signaling (54, 150, 151). BMP-7 is the endogenous antagonist of TGF- $\beta$ -induced EMT in the kidney and elsewhere (54, 150, 151). BMP-7 reverses the decrease of E-cadherin caused by TGF- $\beta$  (54). Restoration of E-cadherin by BMP-7 is mediated by its ALK3/6 receptors and Smad5. The capacity of BMP-7 to reverse TGF- $\beta$ -induced EMT in culture is also observed in mouse models of kidney fibrosis. Systemic administration of recombinant BMP-7 in mice with kidney fibrosis following ureteral obstruction results in reversal of EMT



**Figure 3** Origin of fibroblasts during kidney fibrosis. (a) Fibrotic kidney which displays accumulation of numerous fibroblasts (blue arrow), damaged kidney tubules (yellow arrow), and blood vessels (green arrow). (b) Normal kidney with proper tubular structures and very few fibroblasts. (c) Schematic illustration of three possible mechanisms via which fibroblasts can originate during kidney injury. Recent experiments suggest that approximately 14–15% of fibroblasts are from bone marrow, 36% can arise via local EMT involving tubular epithelial cells under inflammatory stress, and the rest are likely contributed by proliferation of fibroblasts from all sources. (d) Systemic treatment of mice with renal fibrosis using recombinant human BMP-7 results in reversal of renal disease due to severe decrease in EMT-derived fibroblasts and potentially bone marrow-derived fibroblasts. Such events likely have a cascade of beneficial effects that decreasing the overall number of fibroblasts in the kidney, and attenuating fibrosis.

and repair of damaged tubular structures with repopulation of healthy tubular epithelial cells (54, 152). This reversal is also associated with return of renal function, a significant decrease in FSP1<sup>+</sup> interstitial fibroblasts and de novo activation of BMP-7 signaling (54). Renal protection from BMP-7 has also been observed in murine models of diabetic nephropathy (153), Alport syndrome, and lupus nephritis (150). Today, TGF- $\beta$  signaling attenuated by BMP-7 is the closest paradigm in EMT arguing in favor of privileged pathways.

## Summary

Progress in understanding EMT has been an exercise in coming to appreciate the level of complexity required for changing cellular identity. The mechanism of transition highlights an integration of nuclear regulation and network signaling with alterations in microenvironment to create a moving cell. Remarkably, differentiating epithelia make these transitions during development, and terminally differentiated epithelia use them for physiologic repair or to advance oncogenesis. EMT is a form of molecular exaptation, a mechanism of economy by which cells reuse known physiologic processes to provide new functions (154). With the foundation established by current studies, new questions regarding the definition and role of pericytes and myofibroblasts can be explored, and a framework for a better understanding of other transitions, like endothelial-mesenchymal transition, is possible. EMT also provides a mechanism for creating ancestral relationships between local cells and may be particularly important in tumor expansion. Lastly, fibroblasts may carry forward remnants of a unique epithelial signature. And if all fibroblasts or tumor cells which arise via EMT are not created equal, then therapies to combat fibrosis or metastatic disease may need more specificity. Nevertheless, the future holds great promise for EMT as a viable therapeutic target.

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