Epithelial–mesenchymal transition in lung development and disease: does it exist and is it important?

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ABSTRACT
Epithelial–mesenchymal transition (EMT) is a process when epithelial cells gradually transform into mesenchymal-like cells losing their epithelial functionality and characteristics. EMT is thought to be involved in the pathogenesis of numerous lung diseases ranging from developmental disorders, fibrotic tissue remodelling to lung cancer. The most important question—namely what is the importance and contribution of EMT in the pathogenesis of several chronic lung conditions (asthma, COPD, bronchiolitis obliterans syndrome and lung fibrosis)—is currently intensely debated. This review gives a brief insight into the mechanism and assessment methods of EMT in various pulmonary diseases and summarises the recent literature highlighting the controversial experimental data and conclusions.

WHAT IS EPITHELIAL–MESENCHYMAL TRANSITION?
Epithelial–mesenchymal transition (EMT) is a process in which epithelial cells gradually acquire a mesenchymal (fibroblast-like) cell phenotype. EMT has been shown to be essential for embryonic development, gastrulation, and the development of the neural crest, heart and other organs. EMT is also implicated in tissue repair, organ fibrosis and cancer progression. EMT is associated with the progression of many type of tumours and is involved in their metastasis and treatment resistance.[1 2]

CELLULAR CHANGES IN EMT
During EMT, epithelial cells undergo profound changes which can be categorised into four broad cellular functions: changes in transcriptional regulation; changes in cytoskeleton and motility; changes in cell adhesion; and changes in the synthesis of extracellular matrix (ECM) components.

Several transcription factors such as Snail, Slug, ZEB-1, ZEB-2, Twist, β-catenin and Tcf/LEF have been identified as key regulators of EMT, and are the 'master switches' important for cell reprogramming (figure 1).[2–5]

Cytokeratins are the characteristic intermediate filaments in epithelial cells. During EMT cells are reprogrammed to express vimentin instead.[2 5] Other cytoskeletal changes involve de novo expression of fibroblast specific protein (FSP)-1 (also called S100A4) in the epithelium, which has been associated with increased migratory capacity, a more invasive phenotype in tumours and also worse prognosis in lung cancer.[6] α-Smooth muscle actin (α-SMA) is also a frequently assessed marker in EMT. Expression of α-SMA is characteristic for myofibroblasts which are laying down an excessive amount of ECM and thus are responsible for tissue remodelling and fibrosis (figure 1).[7]

Early molecular signs of EMT involve the dissociation of cell–cell junctions induced by downregulation and/or disassembly of junctional components, such as occludins, ZO-1, claudins and E-cadherin.[2 5] The gradual loss of E-cadherin—considered a hallmark of EMT—is accompanied by the simultaneous gain of N-cadherin, a mesenchymal marker. This change is commonly termed the 'cadherin switch' (figure 1).[5]

Cells undergoing EMT also begin to synthesise ECM components like fibronectin and collagen type 1.[8 9]

ASSESSMENT OF EMT IN LUNG TISSUE
EMT is thought to be a gradual process, meaning that there are intermediate cellular phenotypes during the transition. An epithelial cell undergoing EMT will express a set of different marker genes during this process. Since changes in gene expression have also been detected during injury, activation or dedifferentiation of epithelial cells, it is obviously rather challenging to identify with certainty epithelial cells undergoing EMT in complex pathological conditions.

It is well established that during the physiological repair process following epithelial injury, loosening of epithelial–epithelial cell contacts (eg, tight junctions and adherent junctions), alteration of cell–ECM contacts, and cytoskeletal activation (migration) and morphological changes (spreading) occur during wound repair.[10] These phenotypic changes are thought to be mediated by EMT transcription factors.[11] However, we have to emphasise that during physiological wound repair mesenchymal markers expressed by epithelial cells do not necessarily mean transdifferentiation to fibroblasts or myofibroblasts. Although acquiring mesenchymal features and molecular markers, migrating epithelial cells in wound repair do not downregulate E-cadherin and tight junction proteins; there is rather an intercellular redistribution of these proteins.[11–13]

There are numerous articles demonstrating that epithelial cells coexpress mesenchymal and epithelial markers in various lung diseases in humans.[14–20] Other methods commonly used to identify EMT are to show the activation of signalling pathways and transcription factors thought to regulate EMT. These mechanisms—for example, the activation of transcription factors of the *Snail* family and the
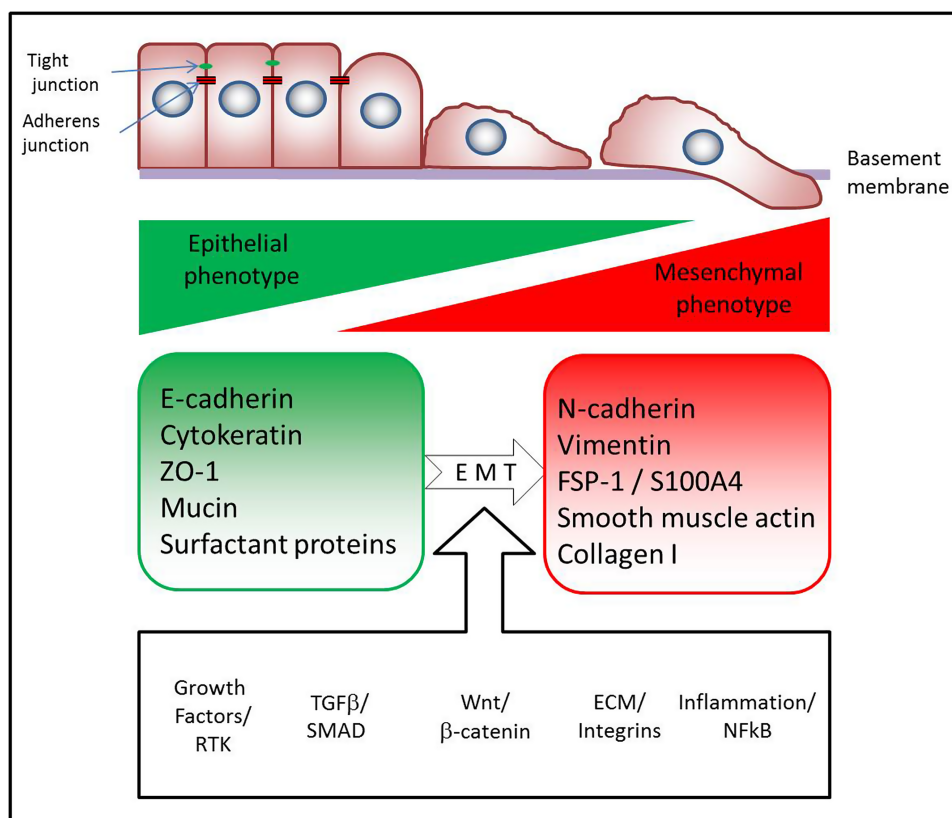
Figure 1 The most important features of epithelial–mesenchymal transition (EMT). Multiple pathways have been found to trigger EMT: tyrosine kinase receptors (epidermal growth factor, fibroblast growth factor, connective tissue growth factor, platelet-derived growth factor, insulin-like growth factor, etc), integrins, Wnt, nuclear factor (NF)-κB and transforming growth factor β (TGF-β) pathways. A common feature of these pathways is that they activate 'master transcription factors' (Snail, Slug, Zeb-1, Twist, etc) which switch on the EMT programme in epithelial cells, namely, downregulating E-cadherin expression and other genes linked to the epithelial phenotype and activating the transcription of genes associated with the mesenchymal phenotype. Canonical TGF-β/SMAD signalling is perhaps the best characterised signalling pathway which contributes to fibrosis (for a recent review, see Fernandez et al[90]). Activation of canonical Wnt signalling and β catenin makes epithelial cells susceptible to EMT caused by TGF-β, by inhibiting the growth arrest which is induced by TGF-β. The extracellular matrix (ECM) also modulates TGF-β signalling through integrin signalling contributing to EMT. Inflammatory cytokines activating NF-κB are thought to further accentuate fibrosis and EMT in the lung.

consequent downregulation of E-cadherin—show striking similarities between fibrosis, developmental programmes involving EMT and carcinogenesis, suggesting a common molecular regulatory background is present.[21–23] However, these experimental methods only provide a 'snapshot' of a process stretched over a period of time and thus are criticised for not being suitable to follow a dynamic process like EMT. To solve this ongoing controversy there is a need for methods which are able to track the transdifferentiation process more reliably, like intravital microscopy or cell fate mapping.

Currently there are no publications available describing the use of intravital microscopy to evaluate EMT of lung epithelial cells in real time. Faulkner et al[24] found that kidney interstitial fibroblasts do not derive from tubular epithelial cells in renal fibrosis and it was described that activation of intrinsic transforming growth factor β (TGF-β) signalling is essential in blood-borne metastasis formation in breast cancer cells using in vivo imaging techniques.[25]

However, the most important question remaining is whether EMT causally contributes to lung disease and, if yes, to what extent? Lung conditions with chronic inflammation and persistent injury result in scarring, fibrosis and tissue remodelling, leading to a decrease of lung function, respiratory failure and death. Recently, experiments using genetic cell fate tracking of lung epithelial cells were performed to address this issue in

mouse models of lung fibrosis[9 26 27] and allergic asthma.[28] These studies found that approximately 30–50% of murine lung fibroblasts in lung fibrosis and allergic asthma were derived from epithelial cells which had undergone EMT as identified by genetic tagging. In these models, β galactosidase was used as a genetic label.[9 26 27] Criticism of this method highlighted that the enzymatic reaction using X-gal and β galactosidase does not allow high-resolution confocal imaging contrary to fluorescent tags. Because of this, the borders between neighbouring cells are obscure and the possibility of false-positive identification of cells cannot be excluded.[29] Additionally, these lineage-tracking studies[9 26 27] used transgenic mice in which the recombination was driven by a 3.7 kb fragment of the human surfactant protein C (Sftpc) promoter,[30] which is somewhat different from the endogenous murine Sftpc promoter which drives the recombination in other animal models.[29] Also in vitro culture of alveolar epithelial cells (AECs) after isolation may result in experimental artefacts, which raises doubt as to whether EMT actually contributes to in vivo fibroblast formation.[8 9 26]

Recently published data have challenged the idea that a significant fraction of myofibroblasts in the diseased lung derive from epithelial cells in animal models[29 31] and humans.[32] These contradictive results from different laboratories have resulted in an intensive debate on lung fibrosis, and kidney[33 34] and liver fibrosis.[35] Rock et al used a transgenic mouse strain with a

fluorescent protein tag (Tomato) under the control of the endogenous *Sftpc* promoter. These experiments suggests that AECs do not transdifferentiate into myofibroblasts in large numbers in the bleomycin lung injury model.[29] A recently published study in mice using multiple genetic tags for cell fate tracking found that myofibroblasts in unilateral urethral obstruction (UUO) kidney fibrosis derive from proliferation of local precursors (50%), bone-marrow-derived fibrocytes (35%), endothelial-mesenchymal transition (10%) and EMT (5%).[34] The study of LeBleu *et al*[34] using the UUO model is a good example, highlighting the multiple origins of myofibroblasts. However, it is difficult to extrapolate from the UUO kidney fibrosis model to the lung as the organ function, the mechanism of parenchymal injury and consequent fibrotic changes are considerably different.

These controversial data illustrate the ongoing intensive scientific debate on the origin of myofibroblasts in fibrotic diseases and tissue remodelling. Elucidating this further is crucial to determine the direction of future research and identification of new therapeutic targets.

EMT IN LUNG DEVELOPMENT AND PAEDIATRIC DISEASES

The first EMT event described occurs in gastrulation—the earliest stage of embryonic development. During this process, the primary mesenchyme is formed, giving the first distinction between epithelial and mesenchymal phenotypes. The epithelial and mesenchymal cell phenotypes are not irreversible, and during embryonic development, cells can convert between the epithelial and mesenchymal states through the process of EMT or mesenchymal-to-epithelial transition (MET).[36 37] Sometimes, several rounds of EMT and MET are required to give a rise to specialised cell types needed for organ formation. During development, EMT provides flexibility in cell fate; however, the development of specialised tissues during embryogenesis requires additional epithelial cell plasticity that slightly differs from classical EMT. Epithelial cell plasticity is essential throughout the process of branching morphogenesis in numerous organs, including the lung.[38 39] Lung-branching morphogenesis requires reciprocal signalling between the epithelium and surrounding mesenchyme that is mediated by coordinated activity of TGF-β/bone morphogenetic protein, Wnt, Sonic hedgehog, fibroblast growth factor (FGF) or retinoic acid signalling.[40] Dysregulation of these signalling pathways is implicated in aberrant lung branching, alveologenesis and pulmonary vascular development, events critical for normal lung development. In addition to genetic factors, pre-natal and post-natal environmental exposures, including epigenetics, play a significant role in programming lung morphogenesis and development of paediatric lung disease.

Bronchopulmonary dysplasia (BPD) is a chronic lung disease that occurs in very premature infants and is characterised by impaired alveologenesis and vascular development. BPD develops as a result of injury or infection on a very immature lung. Interstitial fibrosis is recognised as a prominent feature of BPD as a consequence of repetitive lung injury. Deng and coworkers,[41] using transgenic reporter mice, showed that lung fibroblasts in BPD could arise from bone-marrow-derived ATII cells that have undergone EMT. Noseda and colleagues[42 43] revealed that expression of Notch1 or Notch4 in endothelial cells can cause transition to a mesenchymal phenotype via endothelial-to-mesenchymal transition, a process very similar to EMT. Constitutive Notch1 activation, together with inhibitor studies in lung organ cultures, identified a role for Notch signalling together with TGF-β in mesothelial EMT.[44] Que *et al*[45]

have also shown that mesothelial cells covering the lung surface can migrate into the organ itself and give rise to various cell types, including mesenchymal cells, within the developing lung.

Taken together, these findings have important implications for our understanding of lung developmental defects; however, the casual impact of EMT to BPD has yet to be determined.

EMT IN ASTHMA

Airway remodelling comprises thickening of the basement membrane accompanied by sub-epithelial fibrosis, epithelial shedding and smooth muscle hypertrophy/hyperplasia.[17] Under normal conditions, the damaged epithelium is able to repair itself quickly. However, constant damage inflicted by chronic inflammation in asthma results in a greatly reduced number of ciliated cells, leaving the basal membrane surface only partially covered with basal cells which are more resistant to apoptosis.[17] Loss of E-cadherin as a hallmark of EMT in airway epithelial cells of patients with asthma is also described.[14 46 47] Fibroblasts and myofibroblasts are responsible for the increased production of ECM proteins, for example, collagen and proteoglycans, and also serve as storage for cytokines and growth factors like TGF-β, which play important roles in fibrosis and the regulation of fibroblast–myofibroblast differentiation in inflamed airways.[48 49]

Recent experimental data indicate that T helper 2 dominant chronic inflammation leads to elevated TGF-β1 levels in airways via increased production of TGF-β by eosinophils and macrophages, in addition to fibroblasts, endothelial and smooth muscle cells. TGF-β is stored in the ECM and in turn induces EMT and sub-epithelial fibrosis.[28]

Concerning the pathogenesis of asthma, does EMT contribute to tissue remodelling and sub-epithelial fibrosis? Johnson and colleagues[28] found evidence for this phenomenon in a house dust mite (HDM) allergen sensitised transgenic murine model. They demonstrated that β-galactosidase-tagged large airway epithelial cells gradually lose epithelial characteristics, gain expression of mesenchymal markers, for example, vimentin, and migrate into the sub-epithelial compartment.[28] In vivo cell-fate tracking of genetically labelled epithelial cells provides evidence for epithelial cell transdifferentiation in this mouse model of chronic asthma. Other studies use primary human bronchial epithelial cell (hBEC) cultures to study the role of allergen sensitisation in EMT. HDM sensitisation of the airways synergises with TGF-β1 and results in the loss of E-cadherin expression,[47] caveolin-1 internalisation and consequent loss of epithelial barrier function.[46] Hackett *et al*[14] investigated EMT using air–liquid interface cultures of hBECs isolated from patients with asthma and non-asthmatic controls. The authors found that basal cells of the stratified airway epithelium are the most susceptible to TGF-β1-induced EMT. Interestingly, they found no histological evidence for expression of mesenchymal markers in the bronchial epithelium of matched patients with asthma, rather an increased number of cytokeratin-5+ (KRT5+) positive cells, indicating a differentiation shift towards the basal epithelial cell phenotype.[14] The results discussed above highlight the controversy and discrepancy between research data concerning EMT.

EMT IN BRONCHIOLITIS OBLITERANS SYNDROME

Bronchiolitis obliterans syndrome (BOS) is a major cause of allograft dysfunction in lung transplant recipients. Hodge *et al*[15] found increased expression of mesenchymal proteins by large airway bronchial epithelial cells (BECs) in patients with BOS after lung transplantation to be a clear indicator of the presence

of EMT. Ward et al[50] found that S100A4 and matrix metallo-proteinase (MMP)-2, MMP-7 and MMP-9 expression was present in the airway epithelium of patients who were stable following lung transplant without signs of BOS. Invasive capacity of BECs isolated from these patients was also shown. It is known that allograft infections pose a risk for BOS and has been shown to promote EMT in patient-derived small airway epithelial cells.[51] Inadequate graft microvasculature and consequent graft ischaemia has emerged as a promoting factor for EMT in transplanted lungs.[52 53] Intriguingly, an in vitro study suggests that commonly used immunosuppressive agents may promote EMT in BECs.[54]

EMT IN COPD
COPD is accompanied by inflammation and tissue remodelling,[55] and has been known to be strongly associated with lung cancer. A recent study found a robust genomic link between COPD, lung cancer and Hedgehog signalling, which is also implicated in tobacco-smoke-induced EMT.[56] Tissue remodelling in COPD is characterised by emphysema, and small airway remodelling with peribronchiolar fibrosis. Recent investigations reported that nicotine and tobacco smoke induce EMT in BECs in a Wnt3a/β-catenin/TGF-β-dependent manner.[57] Wang et al[58] described that urokinase plasminogen activator receptor is overexpressed in the airway epithelium of patients with COPD and is involved in mediating the effects of tobacco smoke on EMT in BECs. Studies in mice chronically exposed to tobacco smoke showed increased levels of TGF-β1, connective tissue growth factor (CTGF) and platelet-derived growth factor (PDGF)-B in the treatment group.[59] All these growth factors are known to induce EMT.[2 60 61] Recent clinical investigations in patients with COPD[18 20] showed that cells costaining for epithelial and mesenchymal markers are present in the large airways of asymptomatic smokers and in especially large numbers in current smokers. The authors showed that the reticular basement membrane (Rbm) in people who smoke and those with COPD is highly fragmented, with elongated spaces or cracks termed 'clefts', which is a hallmark of EMT in the airways.[62 63] These clefts in the Rbm usually contained cells positive for mesenchymal markers S100A4, vimentin and MMP-9.[20] Recent findings indicate that similar changes can also be observed in the small (<1 mm) airways of smokers.[64]

EMT IN IDIOPATHIC PULMONARY FIBROSIS
The histological finding characteristic for idiopathic pulmonary fibrosis (IPF) is usual interstitial pneumonia (UIP) with scattered α-SMA-positive fibroblastic foci (FF) in collagen-rich areas, alternating with normal lung areas. The most often used animal model for IPF is the single-dose bleomycin challenge, although it is known that several features of IPF—most importantly being a progressive and chronic disease—are poorly represented. Also, many drugs including corticosteroids proved to be effective in the bleomycin model but are ineffective in IPF.[65] A recently published study suggests that a repetitive bleomycin injury model recapitulates several features of human IPF better than the conventional single-dose model. In this model the authors found that about 50% of S100A4+ fibroblasts are epithelial derived using genetic fate tracking.[27] On the contrary, Rock et al[29] found that epithelial cells do not contribute to fibroblast formation in the single-dose model.

Human immunohistochemistry studies on the role of EMT in the formation of myofibroblasts in IPF are controversial.[66] Harada et al[67] found myofibroblast cells in FF expressing epithelial markers TTF-1, cytokeratin and surfactant protein B.

Chilosi et al[68] identified migratory markers laminin5-γ2, heat shock protein 27 and fascin expression in epithelial cells clustering above FF in UIP samples with special 'sandwich morphology'. On the contrary, Yamada et al[32] did not find histological evidence for EMT in tissue samples from patients diagnosed with IPF (n=15) and non-specific interstitial pneumonia (n=12) using dual immunohistochemistry. In conclusion, the evidence that EMT is involved in the formation of activated myofibroblasts in pulmonary fibrosis remains controversial.

EMT IN LUNG CANCER
EMT is thought to contribute to cancer invasion and metastasis by allowing malignantly transformed epithelial cells to migrate, invade the surrounding stroma, and spread through the blood and lymphatic system to distant sites. Through the process of EMT, cancer cells not only lose their cell–cell adhesions and exhibit elevated motility and invasion, but also gain increased resistance to apoptosis, chemotherapeutic drugs and even develop stem-cell like properties.[69] According to current views, EMT has prognostic significance in various cancers as it is highly correlated with invasive phenotype and metastasis-forming capacity. This so-called 'EMT phenotype' is a primary determinant of prognosis.[70] It has been shown in non-small-cell lung cancer that EMT phenotype is associated with epidermal growth factor receptor mutations and drug resistance,[71] and also with formation of cancer stem cells.[70] In lung cancer, the circulating tumour cells (CTCs) expressing epithelial marker EpCAM are lower compared with other solid tumours.[72] However, when CTC isolation is not based on epithelial markers, the CTC numbers are similar to those of other solid tumours and have strong prognostic value.[73] These data suggest that lung cancer CTCs have lost their epithelial characteristics having undergone EMT. A recent review suggests that anticancer and antimetastasis drugs frequently have side effects associated with defective wound healing, implicating that similar molecular pathways contribute to these processes.[12] However, despite the growing body of evidence, there is still no direct proof in humans that EMT actually happens as a dynamic process in tumour metastasis formation.

WHAT IS THE CLINICAL IMPORTANCE OF EMT?
Two disease groups need to be considered concerning the clinical importance of EMT in the lung: conditions with fibrosis and tissue remodelling, and malignant lung diseases.

It has been recently highlighted that various forms of lung fibrosis and tissue remodelling are present in all chronic respiratory diseases.[74] Current research data highlight that we have to be careful on judging how much EMT contributes to the actual fibrosis, scarring and tissue remodelling. Among the identified targets is the TGF-β pathway and other growth factors, like PDGF, CTGF, FGF and vascular endothelial growth factor, which are considered to trigger EMT and have a proven pathogenic role in fibrotic lung diseases.[75–78] Most of these molecular targets have been tested in animal models[77 79 80] but there has been no success so far in translating these findings to the clinic.[81] Pirfenidone is the only licensed therapy for IPF[82] but the mechanism of action is obscure and there are no in vivo data available of its effects on EMT.

As Wnt signalling pathways have been shown to be important in the pathogenesis in several lung diseases, such as fibrosis,[3] asthma,[83] COPD[84] and lung cancer,[85] they could serve as another promising therapeutic target. Currently there are multiple therapeutic approaches targeting Wnt signalling in lung

diseases which might be applied in the clinic in the near future.[86–88]

For a detailed recent review on the involvement of EMT in malignant transformation and cancer progression, see Craene and Berx.[70]

For a concise summary of recent articles on EMT in lung diseases, please see the table in the online supplement.

SUMMARY AND CONCLUSIONS

In summary, we can say that the extent and significance of EMT in lung diseases is still controversial because of the following issues:

1. A large majority of studies on EMT in lung diseases use methods which take only a 'snapshot' of this dynamic process—for example, immunohistochemistry, western blotting or flow cytometry. Although there have been moves to standardise the molecular markers of EMT,[5][33] there are doubts as to whether EMT can only be defined by the coexpression of certain epithelial and mesenchymal markers[66] since similar molecular changes occur in epithelial cells during wound repair.[11][12]

2. In the case of fibrotic changes, genetic lineage tagging of cells in animal models is thought to be a reliable method; however, the results are contradictory in different animal models for lung fibrosis and other organ fibrosis models.[33] It seems that the results in transgenic mice differ with regards to the nature of the genetic tag, the method used for its detection and also the quality of the transgenic construct used for recombination.

3. A large proportion of the in vivo data on the connection between fibrosis and EMT have been obtained from the single-dose pulmonary bleomycin challenge model. This has received criticism and clearly there is a need for the development of a more reliable murine lung fibrosis model that accurately reflects human UIP.

4. A growing body of evidence supports that EMT has a pivotal role in malignant transformation, invasion and metastatic capacity, and in the formation of cancer stem cells. In lung carcinomas, CTCs seem to have shifted towards a more mesenchymal phenotype compared with other solid tumours, suggesting EMT. Hitherto, there is no convincing direct in vivo evidence of EMT in human tumours, although suggestions were made about the detection of EMT in tumours in situ.[89]

In conclusion, much recent research has highlighted the potential of EMT to be involved in lung development and disease responses. The precise clinical importance of EMT outside of tumour biology remains to be determined, but the development of drugs that target growth factors known to promote EMT suggest that modulation of EMT processes in the clinical setting may soon be possible. Whether these are clinically effective remains to be seen.

REFERENCES
1 Xiao DK, He JX. Epithelial mesenchymal transition and lung cancer. *J Thorac Dis* 2010;2:154–9.
2 Kalluri R, Weinberg RA. The basics of epithelial–mesenchymal transition. *J Clin Invest* 2009;119:1420.
3 Königshoff M, Balsara N, Pfaff E-M, *et al*. Functional Wnt signaling is increased in idiopathic pulmonary fibrosis. *PLoS ONE* 2008;3:e2142.
4 Pongracz JE, Stockley RA. Wnt signalling in lung development and diseases. *Respir Res* 2006;7:15.
5 Zeisberg M, Neilson EG. Biomarkers for epithelial–mesenchymal transitions. *J Clin Invest* 2009;119:1429–37.
6 Kimura K, Endo Y, Yonemura Y, *et al*. Clinical significance of S100A4 and E-cadherin-related adhesion molecules in non-small cell lung cancer. *Int J Oncol* 2000;16:1125–31.
7 Günther A, Korfei M, Mahavadi P, *et al*. Unravelling the progressive pathophysiology of idiopathic pulmonary fibrosis. *Eur Respir Rev* 2012;21:152–60.
8 Kim KK, Wei Y, Szekeres C, *et al*. Epithelial cell $\alpha 3\beta 1$ integrin links β-catenin and Smad signaling to promote myofibroblast formation and pulmonary fibrosis. *J Clin Invest* 2009;119:213–24.
9 Kim KK, Kugler MC, Wolters PJ, *et al*. Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix. *Proc Natl Acad Sci USA* 2006;103:13180–5.
10 Vaughan A, Chapman HA. Regenerative activity of the lung after epithelial injury. *Biochim Biophys Acta* 2013;1832:922–30.
11 Savagner P, Kusewitt DF, Carver EA, *et al*. Developmental transcription factor slug is required for effective re-epithelialization by adult keratinocytes. *J Cell Physiol* 2005;202:858–66.
12 Leopold PL, Vincent J, Wang H. A comparison of epithelial-to-mesenchymal transition and re-epithelialization. *Semin Cancer Biol* 2012;22:471–83.
13 Brandner JM, Kief S, Grund C, *et al*. Organization and formation of the tight junction system in human epidermis and cultured keratinocytes. *Eur J Cell Biol* 2002;81:253–63.
14 Hackett T-L, Warner SM, Stefanowicz D, *et al*. Induction of epithelial–mesenchymal transition in primary airway epithelial cells from patients with asthma by transforming growth factor-β1. *Am J Respir Crit Care Med* 2009;180:122–33.
15 Hodge S, Holmes M, Banerjee B, *et al*. Posttransplant bronchiolitis obliterans syndrome is associated with bronchial epithelial to mesenchymal transition. *Am J Transplant* 2009;9:727–33.
16 Borthwick LA, Parker SM, Brougham KA, *et al*. Epithelial to mesenchymal transition (EMT) and airway remodelling after human lung transplantation. *Thorax* 2009;64:770–7.
17 Holgate ST, Davies DE, Lackie PM, *et al*. Epithelial–mesenchymal interactions in the pathogenesis of asthma. *J Allergy Clin Immunol* 2000;105:193–204.
18 Sohal S, Reid D, Soltani A, *et al*. Evaluation of epithelial mesenchymal transition in patients with chronic obstructive pulmonary disease. *Respir Res* 2011;12:130.
19 El-Gamel A, Sim E, Hasleton P, *et al*. Transforming growth factor beta (TGF-β) and obliterative bronchiolitis following pulmonary transplantation. *J Heart Lung Transpl* 1999;18:828–37.
20 Sohal SS, Reid D, Soltani A, *et al*. Reticular basement membrane fragmentation and potential epithelial mesenchymal transition is exaggerated in the airways of smokers with chronic obstructive pulmonary disease. *Respirology* 2010;15:930–8.
21 Jayachandran A, Königshoff M, Yu H, *et al*. SNAI transcription factors mediate epithelial–mesenchymal transition in lung fibrosis. *Thorax* 2009;64:1053–61.
22 Parent AE, Choi C, Caudy K, *et al*. The developmental transcription factor slug is widely expressed in tissues of adult mice. *J Histochem Citochem* 2004;52:959–65.
23 Shih J-Y, Yang P-C. The EMT regulator slug and lung carcinogenesis. *Carcinogenesis* 2011;32:1299–304.
24 Faulkner JL, Szcykalski LM, Springer F, *et al*. Origin of interstitial fibroblasts in an accelerated model of angiotensin II-induced renal fibrosis. *Am J Pathol* 2005;167:1193–205.
25 Giampieri S, Manning C, Hooper S, *et al*. Localized and reversible TGF[beta] signalling switches breast cancer cells from cohesive to single cell motility. *Nat Cell Biol* 2009;11:1287–96.
26 Tanjore H, Xu XC, Polosukhin VV, *et al*. Contribution of epithelial-derived fibroblasts to bleomycin-induced lung fibrosis. *Am J Respir Crit Care Med* 2009;180:657–65.
27 Degryse AL, Tanjore H, Xu XC, *et al*. Repetitive intratracheal bleomycin models several features of idiopathic pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2010;299:L442–L52.
28 Johnson JR, Roos A, Berg T, *et al*. Chronic respiratory aeroallergen exposure in mice induces epithelial–mesenchymal transition in the large airways. *PLoS One* 2011;6:e16175.
29 Rock JR, Barkauskas CE, Cronce MJ, *et al*. Multiple stromal populations contribute to pulmonary fibrosis without evidence for epithelial to mesenchymal transition. *Proc Natl Acad Sci USA* 2011;108:E1475–E83.
30 Perl A-K, Tichelaar J, Whitsett J. Conditional gene expression in the respiratory epithelium of the mouse. *Transgenic Res* 2002;11:21–9.
31 Hoyles RK, Derrett-Smith EC, Khan K, *et al*. An essential role for resident fibroblasts in experimental lung fibrosis is defined by lineage-specific deletion of high-affinity type II transforming growth factor β receptor. *Am J Respir Crit Care Med* 2011;183:249–61.

32 Yamada M, Kuwano K, Maeyama T, et al. Dual-immunohistochemistry provides little evidence for epithelial–mesenchymal transition in pulmonary fibrosis. Histochem Cell Biol 2008;129:453–62.

33 Quaggin SE, Kapus A. Scar wars: mapping the fate of epithelial–mesenchymal–myofibroblast transition. Kidney Int 2011;80:41–50.

34 LeBleu VS, Taduri G, O'Connell J, et al. Origin and function of myofibroblasts in kidney fibrosis. Nat Med 2013;19:1047–53.

35 Iwaisako K, Brenner DA, Kisseleva T. What's new in liver fibrosis? The origin of myofibroblasts in liver fibrosis. J Gastroenterol Hepatol 2012;27:65–8.

36 Thiery JP, Acloque H, Huang RYJ, et al. Epithelial–mesenchymal transitions in development and disease. Cell 2009;139:871–90.

37 Solnica-Krezel L. Conserved patterns of cell movements during vertebrate gastrulation. Curr Biol 2005;15:R213–R28.

38 Rorth P. Collective cell migration. Annu Rev Cell Dev Biol 2009;25:407–29.

39 Andrew DJ, Ewald AJ. Morphogenesis of epithelial tubes: insights into tube formation, elongation, and elaboration. Dev Biol 2010;341:34–55.

40 Morrisey EE, Hogan BL. Preparing for the first breath: genetic and cellular mechanisms in lung development. Dev Cell 2010;18:8–23.

41 Deng C, Wang J, Zou Y, et al. Characterization of fibroblasts recruited from bone marrow-derived precursor in neonatal bronchopulmonary dysplasia mice. J Appl Physiol 2011;111:285–94.

42 Noseda M, Fu Y, Niessen K, et al. Smooth muscle alpha-actin is a direct target of Notch/CSL. Circ Res 2006;98:1468–70.

43 Noseda M, McLean G, Niessen K, et al. Notch activation results in phenotypic and functional changes consistent with endothelial-to-mesenchymal transformation. Circ Res 2004;94:910–17.

44 Morimoto M, Liu Z, Cheng HT, et al. Canonical Notch signaling in the developing lung is required for determination of arterial smooth muscle cells and selection of Clara versus ciliated cell fate. J Cell Sci 2010;123(Pt 2):213–24.

45 Que J, Wilm B, Hasegawa H, et al. Mesothelium contributes to vascular smooth muscle and mesenchyme during lung development. Proc Natl Acad Sci USA 2008;105:16626–30.

46 Hackett T-L, de Bruin HG, Shaheen F, et al. Caveolin-1 controls airway epithelial barrier function. Implications for Asthma. Am J Resp Cell Mol Biol 2013;49:662–71.

47 Heijink IH, Postma DS, Noordhoek JA, et al. House dust mite–promoted epithelial-to-mesenchymal transition in human bronchial epithelium. Am J Resp Cell Mol Biol 2010;42:69–79.

48 Nihlberg K, Andersson-Sjöland A, Tufvesson E, et al. Altered matrix production in the distal airways of individuals with asthma. Thorax 2010;65:670–6.

49 Sheppard D. Transforming growth factor β: a central modulator of pulmonary and airway inflammation and fibrosis. Proc Am Thorac Soc 2006;3:413–17.

50 Ward C, Forrest IA, Murphy DM, et al. Phenotype of airway epithelial cells suggests epithelial to mesenchymal cell transition in clinically stable lung transplant recipients. Thorax 2005;60:865–71.

51 Borthwick LA, Sunny SS, Oliphant V, et al. Pseudomonas aeruginosa accentuates epithelial-to-mesenchymal transition in the airway. Eur Respir J 2011;37:1237–47.

52 Zhou G, Dada LA, Wu M, et al. Hypoxia-induced alveolar epithelial–mesenchymal transition requires mitochondrial ROS and hypoxia-inducible factor 1. Am J Physiol Lung Cell Mol Physiol 2009;297:L1120–L30.

53 Jiang X, Khan MA, Tian W, et al. Adenovirus-mediated HIF-1α gene transfer promotes repair of mouse airway allograft microvasculature and attenuates chronic rejection. J Clin Invest 2011;121:2336–49.

54 Felton VM, Inge LJ, Willis BC, et al. Immunosuppression-induced bronchial epithelial–mesenchymal transition: A potential contributor to obliterative bronchiolitis. J Thorac Cardiovasc Surg 2011;141:523–30.

55 Hodgson DB, Saini G, Bolton CE, et al. Thorax in focus: chronic obstructive pulmonary disease. Thorax 2012;67:171–6.

56 Young RP, Whittington CF, Hopkins RJ, et al. Chromosome 4q31 locus in COPD is also associated with lung cancer. Eur Respir J 2010;36:1375–82.

57 Zou W, Zou Y, Zhao Z, et al. Nicotine-induced epithelial–mesenchymal transition via Wnt/β-catenin signaling in human airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 2013;304:L199–209.

58 Wang Q, Wang Y, Zhang Y, et al. The role of uPAR in epithelial–mesenchymal transition in small airway epithelium of patients with chronic obstructive pulmonary disease. Respir Res 2013;14:67.

59 Churg A, Tai H, Coulthard T, et al. Cigarette smoke drives small airway remodeling by induction of growth factors in the airway wall. Am J Respir Crit Care Med 2006;174:1327–34.

60 Yang L, Lin C, Liu Z-R. P68 RNA helicase mediates PDGF-induced epithelial mesenchymal transition by displacing Axin from β-catenin. Cell 2006;127:139–55.

61 Secker GA, Shortt AJ, Sampson E, et al. TGFβ stimulated re-epithelialisation is regulated by CTGF and Ras/MEK/ERK signalling. Exp Cell Res 2008;314:131–42.

62 Kalluri R. EMT: when epithelial cells decide to become mesenchymal-like cells. J Clin Invest 2009;119:1417–9.

63 Acloque H, Adams MS, Fishwick K, et al. Epithelial–mesenchymal transitions: the importance of changing cell state in development and disease. J Clin Invest 2009;119:1438–49.

64 Milara J, Peiró T, Serrano A, et al. Epithelial to mesenchymal transition is increased in patients with COPD and induced by cigarette smoke. Thorax 2013;68:410–20.

65 Moeller A, Ask K, Warburton D, et al. The bleomycin animal model: a useful tool to investigate treatment options for idiopathic pulmonary fibrosis? Int J Biochem Cell Biol 2008;40:362–82.

66 Morbini P, Inghilleri S, Campo I, et al. Incomplete expression of epithelial–mesenchymal transition markers in idiopathic pulmonary fibrosis. Pathol Res Pract 2011;207:559–67.

67 Harada T, Nabeshima K, Hamasaki M, et al. Epithelial–mesenchymal transition in human lungs with usual interstitial pneumonia: quantitative immunohistochemistry. Pathol Int 2010;60:14–21.

68 Chilosi M, Zamo A, Doglioni C, et al. Migratory marker expression in fibroblast foci of idiopathic pulmonary fibrosis. Respir Res 2006;7:95.

69 Xiao D, He J. Epithelial mesenchymal transition and lung cancer. J Thorac Dis 2010;2:154.

70 Craene BD, Berx G. Regulatory networks defining EMT during cancer initiation and progression. Nat Rev Cancer 2013;13:97–110.

71 Byers LA, Diao L, Wang J, et al. An epithelial–mesenchymal transition gene signature predicts resistance to EGFR and PI3K inhibitors and identifies Axl as a therapeutic target for overcoming EGFR inhibitor resistance. Clin Cancer Res 2013;19:279–90.

72 Krebs MG, Sloane R, Priest L, et al. Evaluation and prognostic significance of circulating tumor cells in patients with non–small-cell lung cancer. J Clin Oncol 2011;29:1556–63.

73 Hofman V, Bonnetaud C, Ilie MI, et al. Preoperative circulating tumor cell detection using the isolation by size of epithelial tumor cell method for patients with lung cancer is a new prognostic biomarker. Clin Cancer Res 2011;17:827–35.

74 Chapman HA. Epithelial-mesenchymal interactions in pulmonary fibrosis. Annu Rev Physiol 2011;73:413–35.

75 Adamali HI, Maher TM. Current and novel drug therapies for idiopathic pulmonary fibrosis. Drug Des Dev Ther 2012;6:261.

76 Budd DC, Holmes AM. Targeting TGFβ superfamily ligand accessory proteins as novel therapeutics for chronic lung disorders. Pharm Ther 2012;135:279–91.

77 Guntur VP, Reinero CR. The potential use of tyrosine kinase inhibitors in severe asthma. Curr Opin Allergy Clin Immunol 2012;12:68–75.

78 Akhmetshina A, Palumbo K, Dees C, et al. Activation of canonical Wnt signalling is required for TGF-β-mediated fibrosis. Nat Commun 2012;3:735.

79 Nakanishi H, Sugiura T, Streisand JB, et al. TGF-β-neutralizing antibodies improve pulmonary alveologenesis and vasculogenesis in the injured newborn lung. Am J Physiol Lung Cell Mol Physiol 2007;293:L151–L61.

80 McMillan SJ, Xanthou G, Lloyd CM. Manipulation of allergen-induced airway remodeling by treatment with anti-TGF-β antibody: effect on the Smad signaling pathway. J Immunol 2005;174:5774–80.

81 Denton CP, Merkel PA, Furst DE, et al. Recombinant human anti–transforming growth factor β1 antibody therapy in systemic sclerosis: a multicenter, randomized, placebo-controlled phase I/II trial of CAT-192. Arthritis Rheum 2007;56:323–33.

82 Raghu G, Thickett DR. Pirfenidone for IPF: pro/con debate; the 'con' viewpoint. Thorax 2013;68:605–8.

83 Kumawat K, Menzen MH, Bos IST, et al. Noncanonical WNT-5A signaling regulates TGF-β-induced extracellular matrix production by airway smooth muscle cells. FASEB J 2013;27:1631–43.

84 Heijink IH, de Bruin HG, van den Berge M, et al. Role of aberrant WNT signalling in the airway epithelial response to cigarette smoke in chronic obstructive pulmonary disease. Thorax 2013;68:709–16.

85 Bartis D, Csongei V, Weich A, et al. Down-regulation of canonical and up-regulation of non-canonical Wnt signalling in the carcinogenic process of squamous cell lung. PLoS ONE 2013;8(3):e57393.

86 Kim TH, Kim S-H, Seo J-Y, et al. Blockade of the Wnt/β-catenin pathway attenuates bleomycin-induced pulmonary fibrosis. Tohoku J Exp Med 2011;223:45–54.

87 Henderson WR, Chi EY, Ye X, et al. Inhibition of Wnt/β-catenin/CREB binding protein (CBP) signaling reverses pulmonary fibrosis. Proc Natl Acad Sci USA 2010;107:14309–14.

88 Tennis M, Van Scoyk M, Heasley LE, et al. Prostacyclin inhibits non small cell lung cancer growth by a frizzled 9 dependent pathway that is blocked by secreted frizzled related protein 1. Neoplasia 2010;12:244–53.

89 Bastid J. EMT in carcinoma progression and dissemination: Facts, unanswered questions, and clinical considerations. Cancer Metastasis Rev 2012;31:277–83.

90 Fernandez IE, Eickelberg O. The impact of TGF-β on lung fibrosis: from targeting to biomarkers. Proc Am Thorac Soc 2012;9:111–16.

Disease	Reference	Model	Methods/ Treatment	Markers E-cad	Markers a-SMA	Markers Vimentin	Other markers	Main results
BPD	Deng C et al(1)	Murine model of hyperoxia-induced BPD, GFP-tagged bone marrow (BM) chimera mice	60% oxygen for 14 days, CXCL12 chemotaxis assay	N	Y	N	S100A4, TTF-1, Pro-Surfactant Protein-B, Collagen I, CXCR4	GFP-tagged BM-derived fibroblasts were engrafting the lung in active fibrotic areas; there were significantly more BM-derived CXCR4+ fibroblasts in the lungs of O2 treated animals than in controls; BM-derived TTF-1+ epithelial cells were detected in injured lungs
Asthma	Heijink I et al (2)	Bronchial epithelial cell line, Primary bronchial epithelium (hBEC), in vitro	TGF-β1, House dust mite (HDM) allergen	y	N	Y	EGFR,α-catenin activation, cytokeratin (KRT), Myosin light chain phosphorilation	HDM allergen acted synergically with TGF-β1 in inducing EMT in primary hBECs.
	Hackett T-L et al (3)	hBEC from patients with asthma (8) and healthy controls (10)	TGF-β1 SMAD3 siRNA, BMP-7	Y	N	Y	Fibronectin (FN), collagen-1, occludin-1	In vitro evidence for EMT in HBEC; basal epithelial cells are especially sensitive to EMT as in vitro data suggest; no histological evidence for EMT in sections from matched asthmatic patients
	Hackett T-L et al (4)	hBEC cell lines 16HBE and BEAS-2B; primary hBEC from healthy controls and from patients with asthma	HDM allergen; epidermal growth factor (EGF)	Y	N	N	Caveolin-1 (Cav-1), β-catenin, Thymic Stromal Lymphopoietin (TSLP),	Cav-1 levels, junctional E-cadherin and β-catenin were significantly lower, TSLP levels were higher in asthma patients compared to controls; EGF and HDM treatment induced E-cad redistribution and Cav-1 internalization in 16HBE cells. Cav-1-specific siRNA inhibited E-cad internalisation and barrier dysfunction in 16HBE cells; Cav-1 overexpression restored barrier dysfunction and reduced TSLP levels in BEAS-2B cells.
	Johnson JI et al (5)	HDM-induced asthma in ROSA-26/SPC-Cre-LacZ reporter mice	HDM for 15 weeks	Y	Y	Y	TGF-β1, occludin, pro-collagen I, SNAIL	LacZ+/α-SMA+ cells were incorporated into airway smooth muscle; LacZ+/Vim+ cells were present in the sub-epithelium; 30% of Vim+ cell were also LacZ+
BOS	Hodge et al.(6)	Bronchial brushings from lung transplant (LTx)patients with (6) or without BOS (16)	N/A	N	Y	N	Flow cytometry, S100A4 FN, HLA-DR; TGF-β1 and HGF levels in BALF-ELISA	EMT markers α-SMA, S100A4 FN and HLA-DR were increased in epithelial cells from BOS patients compared to stable LTx patients; Increased levels of HGF but not TGF-β1 in the BALF of BOS patients.
BOS	Ward et al	Endobronchial biopsy and brushings from 16 stable Ltx patients	TGF-β1	N	N	N	S100A4 IHC, MMP-2, 7 and 9 zymography, invasion assay	A median 15% of the biopsy epithelium stained for S100A4 and MMP-7 in stable lung transplant recipients; Epithelial cultures from lung allografts were positive for S100A4 and MMP-2 and 9 showed zymographic activity. MMP total protein and activity was increased after TGF-β1 treatment; Both TGF-β1 stimulated and non-stimulated epithelial cells were invasive, invasion capacity was higher in TGF-β1 stimulated cells.
BOS	Borthwick et al.(7)	In vitro culturing of hBEC form obtained via bronchial brushing from stable LTx patients, co-culture with THP-	TGF β1 with or without P.aeruginosa cell lysate (lab. strain and clinical isolates (9))	Y	N	Y	IL-8, IL-1β, TNF-α, FN, KRT-19	Supernatants, but not co-cultures of THP-1 cells treated with P.aeruginosa accentuated EMT on cultured hBECs. Clinical isolates of P.aeruginosa induced significantly higher production of inflammatory cytokines in THP-1s than the reference strain.

BOS	Borthwick et al.(8)	In vitro culturing of hBEC form obtained via bronchial brushing from stable LTx patients	TGF-β, TNF-α	Y	Y	Y	FN, KRT-19, S100A4, MMP-9 zymography, Matrigel invasion assay, collagen synthesis	Bronchial epithelium upregulating EMT and downregulating epithelial markers in BOS but not in stable transplants; epithelial cells; TNF-◻ accentuates TGF ◻◻–induced EMT and cell migration, but not ECM deposition.
BOS	Milara et al (9)	In vitro culture of hBECs isolated from small airways (>1mm) of non-smokers (5), smokers (12) and COPD patients (15); IHC of tissue samples	Cigarette Smoke Extract (CSE)	Y	Y	Y	collagen type I, NOX4, ZO-1 (IHC); TGF-β1, cAMP and MMP-9 (ELISA) ERK1/2 and Smad3 phosphorylation (WB)	hBECs from smokers and COPD patients but not from controls show EMT; CSE-induced EMT is mediated by the ROS and downregulation of cAMP;
BOS	Zou et al	HBEC cell line	Nicotine, Wnt-3a, TGF-β1 siRNA	Y	Y	Y	MMP-9, Collagen-I	Nicotine treatment leads β-catenin to nuclear translocation, E-cad downregulation, αSMA, Vim, Col-1, MMP-9 and TGF-β1 upregulation in HBECs; Knockdown of Wnt3a and TGF-β1 using specific siRNA constructs prevented these effects..

Disease	Reference	Model	Methods/ Treatment	Markers E-cad	Markers a-SMA	Markers Vimentin	Other markers	Main results
COPD	Sohal et al (10, 11)	Endobronchial biopsies from non-smokers(15); ex-smokers with COPD(15), smokers with normal lung function(16) and current smokers (17)	N/A	N	N	Y	IHC for S100A4, MMP-9, KRT and EGFR	Evidence for Rbm fragmentation / remodelling, increased EGFR expression of epithelial cells in COPD patients and smokers compared to non-smoker control(11), 7.2% of basal epithelial cells and cells in the Rbm frequently co-express KRT and S100A4 suggesting EMT in vivo(10).
COPD	Wang et al	Lung tissue samples obtained during lobectomy from 25 non-smokers, 25 smokers with and 18 smokers without COPD; Human primary small airway epithelial cells (SAEC)	CSE extract, uPAR-1 and 2-specific siRNA constructs;	Y	Y	Y	uPAR, α-catenin, N-cadherin, p-Akt, p-GSK-3β	Increased expression of EMT markers and uPAR small airway epithelium (SAE) of patients with COPD compared to controls; Vimentin and uPAR expression are correlated in SAE; CSE-induced EMT in SAEC was associated with high uPAR expression; Targeted silencing of uPAR inhibited CSE-induced EMT; uPAR mediates EMT in SAEC through the PI3K/Akt signaling pathway.
COPD	Churg et al.	C57Bl/6 mice were exposed to cigarette smoke for up to 6 months	Smoke of 2R1 Kentucky research cigarettes in various doses	N	N	N	Procollagen, TGF-β1, platelet-derived growth factor (PDGF)-A, PDGF-B, connective tissue growth factor (CTGF), p-SMAD2	With a single smoke exposure, increases in procollagen, CTGF, TGF-β1, PDGF-A and -B expression were seen after 2 h of smoke exposure; With chronic smoke exposures increases in procollagen, CTGF, PDGF-B expression persisted through 1 wk, 1 mo, and 6 mo; Increased p-Smad2 was present at all time points, indicating constitutively active TGF-β signalling; Small airways in smoke-exposed mice had more collagen at 6 mo.
COPD	Milara et al.	Air-liquid interface (ALI) cultures were set up from HBECs from non-smokers (n=5), smokers (n=12) and patients with COPD (n=15)	CSE exposure	Y	Y	Y	collagen-I, NOX4, ZO-1, MMP-9	EMT markers were upregulated and epithelial markers were downregulated in HBECs of smokers and patients with COPD compared with non-smokers; CSE exposure caused EMT within 72 h in in vitro differentiated HBECs; CSE effects were mediated by intracellular reactive O2 species, autocrine action of TGF-β1, activation of ERK1/2 and Smad3 and by the downregulation of cAMP
IPF	Kim et al (12)	ROSA-26/SPC-Cre-LacZ reporter mice, in vitro culture of transgenic ATIIs	Intranasal Adeno-TGF-β1, culturing ATIIs on Matrigel or FN, TGFR kinase inhibitor SB431542	N	Y	N	Pro-SP-C, FN, N-cad, p-SMAD2, TUNEL	Lung injury induced by TGF-β1 expressing adenoviri causes massive EMT AECs; genetic fate tracking proves epithelial origin of myofibroblasts. Culturing genetically tagged AECs on FN but not on Matrigel induces EMT by activating intracellular TGF-⬚/SMAD signalling through αvβ6 integrin.

IPF	Tanjore et al (13)	ROSA-26/SPC-Cre-LacZ reporter mice, in vitro culture of transgenic ATIIs	IT bleomycin (single 0.08U dose	N	Y	N	S100A4, pro–SP-C	Approximately one-third of S100A4+ fibroblasts are derived from tagged epithelial cells; α-SMA+ myofibroblasts are a distinct population from EMT-derived S100A4+ Fibroblasts; some S100A4+ Fibroblasts derive from bone marrow
IPF	Deagryse et al. (14)	ROSA-26/SPC-Cre-LacZ reporter mice	IT bleomycin (0.04U given biweekly 8 times)	N	Y	N	pro-SP-C, Clara cell 10 (CC-10), β-galactosidase, S100A4, TUNEL	Repetitive bleomycin dosing results in greater lung fibrosis, less neutrophilic inflammation, greater cell death, and more prominent EMT compared with the single-dose model; one-half of the S100A4+ fibroblasts were of epithelial lineage. The authors suggest this recapitulates better the features of IPF than the single-dose bleomycin model.
IPF	Harada et al (15)	13 patients with UIP histology; 11 IPF, 2 autoimmune; 10 control patients w/ normal lung function	N/A	N	Y	Y	Pro-SP-B, TTF-1, KRT7/8 (CAM5.2)	-SMA were detectable in some epithelial cells covering the fibroblastic foci in UIP but not in healthy control lungs. Spindle-shaped cells positive for TTF-1, ProSP-B, and KRT7/8 were detectable in the fibroblastic foci of UIP lungs.
IPF	Rock et al (16)	ROSA-26 / Sftpctm1-Cre-Tomato transgenic reporter mice	IT bleomycin (1.25 U/kg – 5 U/kg body weight dose) in a single injection	N	Y	Y	SP-C; AQP-5; S100A4; NG2; Desmin; CC-10; PECAM	Proliferating fibroblasts were derived from NG2+ and/or PDGFRB+ stromal populations but not from SP-C+ or CC-10+ epithelial cells using genetically tagged murine models.

Disease	Reference	Model	Methods/ Treatment	Markers			Other markers	Main results
				E-cad	a-SMA	Vimentin		
Lung Cancer	Prudkin et al (17)	Tissue from human lung cancers: squamous metaplasia (13), squamous dysplasia (34) & carcinoma in situ (20), Brain metastases: adenocarcinoma (37), squamous cell (11).	N/A	Y	N	Y	N-Cadherin, Integrin αvβ6, MMP-9, and phosphorylated EGFR. Methods: Tissue microarray construction, IHC, EGFR mutation analysis.	EMT phenotype was commonly expressed in dysplastic lesions, lung squamous cell carcinoma and adenocarcinoma. Brain metastases from these tumours expressed higher levels of E-cadherin than primary tumours implicating the occurrence of MET after dissemination.
Lung Cancer	Pirozzi et al (18)	A549s (control) and LC31 (lung cancer primary cell line). LC31 cells grown as pneumospheres were subcutaneously injected in NOD/SCID mice.	TGF-β1 2ng/ml: A549s for 30 days, LC31s for 80 days	Y	N	Y	KRT, N-cad, CD90, SLUG, TWIST, β-catenin. Stem markers: Oct4, Nanog, Sox2, c-kit, CD133.	TGF-β1 lung cancer cells underwent EMT showing mesenchymal phenotype and LC31s also showed over-expression of stemness markers. TGF-β1treatmentincreased the pneumoshere-forming capacity and invasiveness of LC31s in NOD/SCID mice.
	Ren et al (19)	Cell lines: Doxetacel (DTX) sensitive and resistant human NSCLC line SPC-A1. Animal model: SPC-A1/DTX cells injected into nude mice.	ZEB1 siRNA knockdown. DTX treatment.	Y	N	Y	N-cad, KRT-19, TWIST. Methods: apoptosis assay, colony formation assay, wound healing assay, migration/invasion assays.	Acquisition of DTX resistance coincides with EMT/mesenchymal phenotype, an increased migratory and invasive capacity, increased ZEB1 expression. ZEB1 knockdown was found to reverse the EMT phenotype and inhibit the migratory ability of SPC-A1/DTXs, enhanced the chemo-sensitivity.
	Tominaga et al (20)	Human NSCLC lines: A549, PC-9, RERF-LC-KJ, and LC-2/ad	Cell lines were transfected to over-express miR-1.	Y	N	Y	Vinculin, occludin, SNAIL, SLUG, ZEB1	miR-1 expression was down-regulated in lung cancer cell lines. Overexpression of miR-1 endowed A549 cells with epithelial features upregulated E-cad by inhibiting SLUG. miR-1 suppressed the migratory & invasive capacity of A549s.
	Witta et al (21)	21 NSCLC cell lines were used: squamous (4), large cell (5), adeno-carcinoma (10), & bronchio-alveolar (2).	Gefitinib, HDAC inhibitor (MS-275). E-cad transfection.	Y	N	N	EGFR, ZEB1	Gefetinib sensitivity correlated with E-cadand ZEB1 expression. E-cad overexpression restored gefetinib sensitivity. HDAC inhibitor (MS-275) treatment induced E-cadherin & EGFR, and led to a growth-inhibitory & apoptotic effect with gefitinib treatment, even in resistant cell lines harbouring EGFR mutations.

1.   Deng C, Wang J, Zou Y, et al. Characterization of fibroblasts recruited from bone marrow-derived precursor in neonatal bronchopulmonary dysplasia mice. J Appl Physiol2011;**111**(1):285-94.
2.   Heijink IH, Postma DS, Noordhoek JA, et al. House Dust Mite–Promoted Epithelial-to-Mesenchymal Transition in Human Bronchial Epithelium. Am J Resp Cell Mol Biol 2010;**42**(1):69-79.
3.   Hackett T-L, Warner SM, Stefanowicz D, et al. Induction of Epithelial–Mesenchymal Transition in Primary Airway Epithelial Cells from Patients with Asthma by Transforming Growth Factor-β1. Am J Respir Crit Care Med 2009;**180**(2):122-33.
4.   Hackett T-L, de Bruin HG, Shaheen F, et al. Caveolin-1 Controls Airway Epithelial Barrier Function. Implications for Asthma. Am J Respir Cell Mol Biol 2013;**49**(4):662-71.
5.   Johnson JR, Roos A, Berg T, et al. Chronic Respiratory Aeroallergen Exposure in Mice Induces Epithelial-Mesenchymal Transition in the Large Airways. PLoS ONE. 2011;**6**(1):e16175.
6.   Hodge S, Holmes M, Banerjee B, et al. Posttransplant Bronchiolitis Obliterans Syndrome Is Associated with Bronchial Epithelial to Mesenchymal Transition. Am J Transpl 2009;**9**(4):727-33.
7.   Borthwick LA, Sunny SS, Oliphant V, et al. Pseudomonas aeruginosa accentuates epithelial-to-mesenchymal transition in the airway. Eur Respir J 2011;**37**(5):1237-47.
8.   Borthwick LA, Parker SM, Brougham KA, et al. Epithelial to mesenchymal transition (EMT) and airway remodelling after human lung transplantation. Thorax. 2009;**64**(9):770-7.
9.   Milara J, Peiró T, Serrano A, et al. Epithelial to mesenchymal transition is increased in patients with COPD and induced by cigarette smoke. Thorax 2013;**68**(5):410-20
10.   Sohal S, Reid D, Soltani A, et al. Evaluation of epithelial mesenchymal transition in patients with chronic obstructive pulmonary disease. Respir Res 2011;**12**(1):130.
11.   Sohal SS, Reid D, Soltani A, et al. Reticular basement membrane fragmentation and potential epithelial mesenchymal transition is exaggerated in the airways of smokers with chronic obstructive pulmonary disease. Respirology. 2010;**15**(6):930-8.
12.   Kim KK, Kugler MC, Wolters PJ, et al. Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix. Proc Natl Acad Sci U S A 2006;**103**(35):13180-5.
13.   Tanjore H, Xu XC, Polosukhin VV, et al. Contribution of Epithelial-derived Fibroblasts to Bleomycin-induced Lung Fibrosis. Am J Respir Crit Care Med 2009;**180**(7):657-65.
14.   Degryse AL, Tanjore H, Xu XC, et al. Repetitive intratracheal bleomycin models several features of idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 2010;**299**(4):L442-L52.
15.   Harada T, Nabeshima K, Hamasaki M, et al. Epithelial–mesenchymal transition in human lungs with usual interstitial pneumonia: Quantitative immunohistochemistry. Pathol Int 2010;**60**(1):14-21.
16.   Rock JR, Barkauskas CE, Cronce MJ, et al. Multiple stromal populations contribute to pulmonary fibrosis without evidence for epithelial to mesenchymal transition. Proc Naltl Acad Sci U S A 2011;**108**(52):E1475–E83.
17.   Prudkin L, Liu DD, Ozburn NC, et al. Epithelial-to-mesenchymal transition in the development and progression of adenocarcinoma and squamous cell carcinoma of the lung. Mod Pathol. 2009;**22**(5):668-78.
18.   Pirozzi G, Tirino V, Camerlingo R, et al. Epithelial to mesenchymal transition by TGFbeta-1 induction increases stemness characteristics in primary non small cell lung cancer cell line. PLoS ONE. 2011;**6**(6):e21548.
19.   Ren J, Chen Y, Song H, et al. Inhibition of ZEB1 reverses the phenotype of epithelial-mesenchymal transition and chemoresistance in docetaxel-resistant human lung adenocarcinoma cell line SPC-A1/DTX. J Cel Biochem 2012;**114**(6):1395-1403
20.   Tominaga E, Yuasa K, Shimazaki S, et al. MicroRNA-1 targets Slug and endows lung cancer A549 cells with epithelial and anti-tumorigenic properties. Exp Cell Res 2013;**319**(3):77-88.
21.   Witta SE, Gemmill RM, Hirsch FR, et al. Restoring E-Cadherin Expression Increases Sensitivity to Epidermal Growth Factor Receptor Inhibitors in Lung Cancer Cell Lines. Cancer Res 2006;**66**(2):944-50.