

Epithelial-mesenchymal transition: potential role in obliterative bronchiolitis?

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Lung transplantation remains the only viable option for many patients suffering from a variety of progressive or intractable end-stage lung diseases. Despite significant advances in the prevention of early graft rejection, ischaemia-reperfusion injury and acute management of lung transplant recipients, significant challenges remain in the chronic management of patients after lung transplantation.¹ In this issue of *Thorax*, Borthwick and colleagues² provide intriguing new evidence that implicates the airway epithelium directly in the pathogenesis of bronchiolitis obliterans syndrome (BOS), the most significant factor in determining long-term lung graft survival (see page 770). As discussed in the study, the pathological lesion of BOS is obliterative bronchiolitis (OB), which recently has been postulated to be at least partially a disease of aberrant epithelial repair processes.³ Borthwick and colleagues provide evidence that epithelial to mesenchymal transition (EMT), a process whereby epithelial cells undergo a complete lineage transition to become fibroblasts and/or myofibroblasts, may underlie the dysfunctional airway repair processes that lead to OB. This study, and others like it, attempt to redefine traditional paradigms regarding normal airway epithelial biology and disease pathogenesis, and have the potential to lead to entirely new therapeutic avenues for previously untreatable disease processes such as BOS.

OB is characterised by initial inflammation of the small airways followed by airway remodelling, aberrant epithelial regeneration and repair, proliferation of fibroblasts and myofibroblasts, deposition

of extracellular matrix (ECM) and eventual airway obstruction.⁴ The initial inflammatory response is the result of an allogeneic immune response initiated against donor antigens in the graft endothelial and airway epithelial cells. This response characteristically generates antigen-specific graft-infiltrating destructive lymphocytes. The lymphocytes facilitate activation of macrophages and a variety of other inflammatory cells, with resultant epithelial damage.⁵ The critical role of this allogeneic immune response as the initial trigger leading to OB is supported by the fact that the primary risk factors for the development of BOS after lung transplantation are class I and II mismatches between donor and recipient, as well as the number and severity of rejection episodes.⁴ Although it has been recognised for over a decade that the airway epithelium is a target of the initial immune response,⁶ the pathogenetic pathway that leads to disruption of normal epithelial repair processes, excessive fibroblastic responses and resultant excessive ECM deposition and eventual obliteration of small airways is still incompletely elucidated.

While bronchial epithelial cells (BECs) have been shown to directly present antigen⁷ and are potentially the primary target of immunological attack during the pathogenesis of OB,⁸ their precise link to the proliferation of fibroblasts and the propagation of fibrosis is not entirely clear. It is known that epithelial cell apoptosis and disruption of epithelial integrity probably contributes to sub-epithelial fibroblastic proliferation,⁹ but the primary source of the proliferating fibroblasts during airway fibrosis is still unknown. One possible source is direct conversion of proliferating BECs into pathogenetic fibroblasts and myofibroblasts through EMT.

EMT is a process by which epithelial cells lose fundamental epithelial characteristics such as tight junctions, apical: basolateral polarity and the expression of epithelial-specific markers and assume a mesenchymal phenotype, expressing a

variety of mesenchymal markers and acquiring functional characteristics of fibroblasts and myofibroblasts such as ECM production, motility and the ability to invade surrounding tissues.¹⁰ EMT is not a new concept, being critical during normal development and for the development of metastatic potential and increased invasiveness during cancer progression.¹¹ However, the role of EMT in the pathogenesis of adult tissue fibrosis is a relatively new area of study. EMT has been most commonly investigated as a mechanism underlying fibrosis in renal and lens epithelium. In the kidney, approximately 40% of new fibroblasts are thought to arise via EMT during injury¹² while, in the eye, EMT has been demonstrated both in vitro and in vivo.¹³ Very recently, the role of EMT in the pathogenesis of pulmonary disease has begun to be investigated. The first demonstrations of EMT in pulmonary epithelium were in alveolar epithelial cells. In response to transforming growth factor β (TGF β) in vitro, several groups have demonstrated conversion of alveolar epithelial cells to a myofibroblast-like phenotype, with a complete morphological and functional change and acquisition of mesenchymal markers concomitant with the loss of detectable epithelial characteristics.^{14–16} Furthermore, EMT has been demonstrated in alveolar epithelium in vivo in mice. In one recent study, lineage-tagged alveolar epithelial cells were shown to contribute over 30% of the alveolar fibroblastic response during experimental lung injury.¹⁷ Finally, immunohistochemical evidence from lung biopsy samples from patients with idiopathic pulmonary fibrosis (IPF) suggests that up to 80% of the epithelium in areas of active fibrosis exhibit evidence of EMT.¹⁴ Given the growing body of evidence implicating EMT of alveolar epithelial cells in the pathogenesis of pulmonary fibrosis, the possibility that BECs may undergo EMT to contribute to airway remodelling and fibrosis is intriguing.

In this issue, Borthwick *et al* cultured primary human BECs in the presence and absence of TGF β and tumour necrosis factor α (TNF α). Only TGF β alone was able to induce a fibroblastoid morphology, but the combination of both TGF β and TNF α resulted in the most robust phenotypic change together with upregulation of mesenchymal markers (vimentin and fibronectin) and downregulation of the epithelial markers cytokeratin 19 and E-cadherin. Treated cells also acquired functional characteristics of myofibroblasts,

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demonstrating an increase in the ability to invade collagen and deposit ECM. The authors extended their study to examine normal lung and pathological specimens from stable transplant patients and those with progressive BOS. They used quantitative immunohistochemistry to demonstrate a correlation between disease severity and both loss of epithelial markers and acquisition of mesenchymal markers in the bronchial epithelium. Finally, they used confocal microscopy to demonstrate co-localisation of epithelial and mesenchymal markers within the epithelium of transplant recipients with OB. Together, the findings of this study add support to the hypothesis that EMT may play a significant role in the pathogenesis of OB.

While few, there have been other suggestions of EMT as a potential pathogenetic mechanism in OB. An early report that included authors of the current study reported that 15% of the epithelium in biopsy specimens from clinically stable transplant patients co-stained for the fibroblast marker S100A4.³ Recently, mouse tracheal epithelial cells were shown to undergo at least partial EMT in response to TGF β ₁, possibly through a JNK-1 mediated mechanism.¹⁸ Finally, peribronchial fibrosis during bleomycin-induced lung injury was found to contain a subset of BECs possibly derived from EMT.¹⁹ Clearly, the possibility that BECs can contribute to the airway fibrotic response in a variety of disease processes deserves further investigation.

An intriguing aspect of the current study is the requirement for both TGF β and TNF α together to induce a robust EMT response in BECs in vitro. A potential criticism of previous studies has been that the dose of TGF β required to induce EMT in BECs has been higher than that required to induce alveolar EMT (5–50 ng/ml vs 0.5–1 ng/ml).¹⁸ Given that physiological concentrations of TGF β ₁ during lung injury only reach concentrations of approximately 1 ng/ml or less,²⁰ the possibility that bronchial EMT is a

purely in vitro phenomenon has been raised. The combination of TGF β and TNF α is intriguing in that EMT during airway fibrosis would probably occur in the presence of both of these cytokines due to the ongoing inflammation during rejection. The addition of TNF α resulted in the induction of EMT at doses of TGF β as low as 1 ng/ml, within the physiological range. This synergy between TGF β and TNF α confirms earlier observations in EMT of alveolar epithelial cells,¹⁴ and suggests an interesting cross-talk between TGF β and TNF α -induced intracellular signalling pathways.

In summary, Borthwick and colleagues² have provided additional evidence for the possibility of EMT as a pathogenetic contributor to airways fibrosis in OB and perhaps other diseases of the small airways. Studies like this force us to re-examine long-held disease paradigms and hopefully embark on new investigations to find novel therapeutics. Given the historical lack of progress in the prevention and treatment of BOS, new insights and avenues of inquiry are critical to improving the outcomes and lives of patients after lung transplantation.

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