Could N-acetylcyesteine slow progression of idiopathic pulmonary fibrosis by inhibiting EMT?

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THE ALVEOLAR EPITHELIUM is deranged in the lungs of patients with idiopathic pulmonary fibrosis (IPF). A prominent histopathological marker of this derangement is alveolar type II cell hyperplasia (17). One hypothesis explaining the type II cell hyperplasia is that it represents the loss of lung epithelium’s attempt to cover over an injured epithelial surface that is allegedly present in IPF lungs (15). More recently, theories have emerged suggesting the hyperplastic type II epithelial cells play a direct role in the pathogenesis of lung fibrosis (16). One way they may contribute is by releasing profibrotic growth factors (e.g., PDGF or endothelin) (3, 4) or matrix metalloproteases (MMP-1 and MMP-7) that remodel the fibrotic matrix (20). In addition, alveolar type II cells in IPF lungs have increased stress of the endoplasmic reticulum (12). This “ER stress” could lead to apoptosis of the alveolar type II cells and subsequent remodeling and fibrosis of the lung as it heals. Finally, an emerging concept is that growth factor-activated type II cells transition into mesenchymal cells via a process termed epithelial-mesenchymal transition (EMT) (9, 18, 19).

EMT is the process where epithelial cells acquire characteristics of mesenchymal cells following activation by growth factors, of which transforming growth factor (TGF)-β is the prototype (7). Growth factor activation triggers upregulation of genes normally expressed in mesenchymal cells (e.g., FSP1, α-SMA) and downregulation of genes expressed in epithelial cells (e.g., E-cadherin) (7). Following this molecular reprogramming, epithelial cells lose polarity, tight junctions, and desmosomes, become mobile, and migrate into the subepithelial regions where they assume biological functions typically ascribed to mesenchymal cells (7). Normal tissue homeostasis does not require ongoing EMT (7). In contrast, EMT develops during episodes of tissue injury and remodeling, such as in kidney fibrosis, where much of the early evidence for fibrosis-associated EMT was described (7, 18).

Recently, data have emerged suggesting EMT contributes to the pathological development of lung fibrosis (18). The best evidence comes from lineage tracing studies showing that EMT of alveolar type II cells accounts for a significant proportion of the new mesenchymal cells that appear in the fibrotic lungs of mice overexpressing TGF-β (9). Additional evidence is provided by in vitro studies showing that rodent type II cells undergo morphological changes characteristic of mesenchymal cells and increase expression of mesenchymal cell proteins when cultured on fibronectin or in the presence of endothelin-1 or TGF-β (6, 9, 19). These data indicate that alveolar type II cells can undergo EMT when subjected to specific experimental conditions. However, whether or not EMT contributes to the pathogenesis of fibrotic lung diseases such as IPF has yet to be resolved. The inherent limitations of working with human tissue have limited the evidence type II cells undergo EMT in IPF patients to immunohistochemical studies colocalizing epithelial and mesenchymal cell-associated proteins within IPF lungs (9, 19), in vitro studies reporting that TGF-β mediates mesenchymal transition of transformed human lung epithelial cell lines (8), and a report that fibroblasts isolated from IPF lungs express increased levels of epithelial intermediate filament keratin-18 (13).

Establishing a dynamic process such as EMT that occurs in IPF is challenging, largely because human studies are limited to tissue samples providing information at only one time point of a complex disease process that evolves over years and ex vivo study of epithelial cells in culture conditions that likely do not mimic the lung microenvironment. Therefore, advancing the evidence that EMT plays a role in IPF is achieved via studies showing that biochemical processes promoting EMT in murine models of lung fibrosis (where the molecular mechanisms can be delineated in detail) are also found in IPF lungs. Two recent studies using this approach reported that the α2-integrin is required for formation of a pY654-β-catenin/pSmad2 complex that initiates EMT of alveolar type II cells (10) and that WISP1 promotes lung fibrosis and EMT of alveolar type II cells (11). Although these bench-to-bedside correlations don’t prove EMT occurs in IPF lungs, the demonstration of similar biochemical processes in both systems strengthen the possibility that EMT contributes to the pathogenesis of IPF.

In their recent article in AJP-Lung, Felton and colleagues (2) use a less commonly applied bedside-to-bench approach to investigate whether EMT is involved in the pathogenesis of IPF. Using this approach, the authors ask whether inhibition of EMT of alveolar type II cells by N-acetylcysteine (NAC) could contribute to the apparent clinical benefit NAC provides in slowing the loss of lung function in IPF patients (1). Importantly, they found that NAC blocks TGF-β-mediated EMT of rat type II cells in vitro. Mechanistically, it was shown that TGF-β decreased glutathione levels and significantly increased reactive oxygen species production. NAC restored both to levels similar to those found in TGF-β-treated cells. These findings are consistent with the idea that NAC acts as an antioxidant and cellular redox stabilizer, and prevents EMT of type II cells by maintaining intracellular glutathione stores. This bedside-to-bench approach is interesting because it generates new theories on how a candidate therapy could influence outcomes in IPF patients, as well as advances the understanding of potential molecular mechanisms promoting EMT of alveolar type II cells.

There are limitations to the conclusions summarized above. First, NAC has yet to be proven to treat IPF. In the study reporting its efficacy, NAC was administered in combination
with prednisone and azathioprine (1). This leaves open the possibility the reported clinical benefit was not due to NAC alone, but rather a combined treatment effect of prednisone, NAC, and azathioprine, or possibly an off-target effect of NAC such as prevention of the toxic effects of prednisone and azathioprine (5). Second, the dose of NAC required to prevent EMT in these studies (2) was relatively high, so it remains to be established whether comparable levels are achieved in human lung tissues in the doses used clinically. Third, the experiments were performed exclusively on rat alveolar type II cells. Therefore, it is important to confirm NAC similarly inhibits EMT of human type II cells. Finally, the authors conclude that NAC inhibits EMT either by blocking oxidative stress or increasing glutathione levels. Whether these are the mechanisms inhibiting EMT or whether NAC blocks EMT by directly inactivating TGF-β (14) need to be resolved. Nonetheless, despite these limitations, the study by Felton et al. (2) is important because the findings bridge current dilemmas facing clinicians treating IPF patients with novel ideas of the cell biological processes contributing to the progression of IPF.

REFERENCES


