

Epithelial–mesenchymal transition is driven by transcriptional and post transcriptional modulations in COPD: implications for disease progression and new therapeutics

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Abstract: COPD is a common and highly destructive disease with huge impacts on people and health services throughout the world. It is mainly caused by cigarette smoking though environmental pollution is also significant. There are no current treatments that affect the overall course of COPD; current drugs focus on symptomatic relief and to some extent reducing exacerbation rates. There is an urgent need for in-depth studies of the fundamental pathogenic mechanisms that underpin COPD. This is vital, given the fact that nearly 40%–60% of the small airway and alveolar damage occurs in COPD well before the first measurable changes in lung function are detected. These individuals are also at a high risk of lung cancer. Current COPD research is mostly centered around late disease and/or innate immune activation within the airway lumen, but the actual damage to the airway wall has early onset. COPD is the end result of complex mechanisms, possibly triggered through initial epithelial activation. To change the disease trajectory, it is crucial to understand the mechanisms in the epithelium that are switched on early in smokers. One such mechanism we believe is the process of epithelial to mesenchymal transition. This article highlights the importance of this profound epithelial cell plasticity in COPD and also its regulation. We consider that understanding early changes in COPD will open new windows for therapy.

Keywords: epithelial-to-mesenchymal transition, EMT, cancer, fibrosis, inflammation, HuR, EGFR, MMP, TGFβ

Background

COPD is a devastating disease of the lung found mainly to affect cigarette smokers, though fossil-based air pollution in developing nations is now considered an equal contributor. The WHO estimates that there are over 250 million persons affected by the disease, which accounts for at least 3 million deaths annually as of 2015, which roughly averages around 5% of total worldwide reported mortality.¹ The disease is characterized clinically as a slowly progressive airway narrowing with only partial reversibility at best. In vulnerable smokers, the entire lung is affected² which is evident from the development of large airway squamous metaplasia, mucus hypersecretion and smooth muscle hyperplasia along with increases in small airway fibrosis and airway wall thickening.^{3,4} These changes affect the normal airway architecture, subsequently causing airway narrowing and obliteration, accompanied by bronchitis.⁵ It is

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also notable that patients also suffer from varying degrees of emphysematous lung destruction,^{6,7} which significantly adds to airflow limitation.⁵

The epithelial lining forms the outer layer of the lung mucosa and thus is the first line of defense for any external insults.¹⁰ These cells act as a barrier to infectious microbes and toxic particles emanating from entities such as smoke, via increased mucus secretion and ciliary clearance; however, in chronic conditions, the mucus is often thickened with phlegm and impaired ciliary function.⁵ Epithelial cells also produce various antibacterial, antioxidant, anti-protease factors and have tight epithelial junctions¹¹ and thus deny entry of foreign entities and stop them from damaging the inner lung mucosa.¹² Nevertheless, chronic exposure to these external insults causes observable changes to the normal architecture of the epithelium in both smokers and COPD patients¹³ with increases in basal cell abnormalities and decreases in epithelial cellular junction integrity.^{14,15} Ultimately, the changes associated have a direct impact on disease outcome wherein almost half of the small airways are lost at early stages of the disease. Findings by Koo et al⁸ provided evidence to this effect, demonstrating that both small airways and alveolar structures are damaged in early COPD. Terminal bronchioles number obliterated by 40% in patients with mild-to-moderate COPD patients. Interestingly, transitional bronchioles numbers reduced in both mild and mild-to-moderate patients by 55%, and alveolar surface area decreased by 40%.⁸ Further, total airway count has also been shown to decrease by 19% in both mild-to-moderate COPD compared to never smokers.⁹

This devastating effect on the lung of early COPD patients can be attributed to active remodeling, whose impact on disease progression and manifestation needs further recognition. In this commentary, we wish to highlight the factors that contribute to such changes and the role of post-transcriptional regulators such as human antigen R (HuR) as possible modulators to these responses.

In both COPD patients and active smokers, cellular reprogramming in epithelial cells¹⁴ consequently could lead to epithelial–mesenchymal transition (EMT),¹⁶ a process where epithelial cells progressively lose cellular polarity and, adhesiveness, become migratory and assume a mesenchymal phenotype.^{17–19} Previous findings have established the presence of EMT in other fibrotic diseases and cancers,^{20–22} but recent studies have increasingly considered EMT as the possible core pathological factor in the development of COPD as well.^{23–27} Indeed, studies now show that the epithelium of

COPD patients has increased cellular expression of mesenchymal markers such as S100A4 and vimentin,²⁸ which is especially higher in basal cells and is omnipresent in both large and small airways.^{26,29,30} The other characteristic EMT feature is reticular basement membrane (Rbm) fragmentation which was found to be higher in COPD patients than in nonsmokers, and this has been attributed to increase in epithelial matrix metalloprotease (MMP) activity especially of MMP-9 (collagenase/gelatinase).³¹ In the same set of patients, increased Rbm vascularity was also observed, which is another prominent feature of EMT leading to the formation of a pro-cancer stroma.³² The increased expression of mesenchymal markers S100A4 and vimentin negatively correlated with lung function, suggesting a physiological consequence.²⁶ COPD ex-smokers had significantly less Rbm S100A4 expression and a similar trend was observed for Rbm vessels.^{33,34} Appropriate smoking cessation studies are needed to further confirm these findings, but certainly, it seems to be making a difference. Initial proof of concept trial by our group revealed that inhaled corticosteroids (ICS) such as fluticasone propionate were effective in decreasing mesenchymal expression of S100A4 and vimentin, along with significant reduction in Rbm fragmentation, EGFR and MMP-9 expression in the COPD group compared to placebo.³⁵ The study provided sufficient rationale for translational strategies that can be employed with currently available therapies to overcome this pathological aspect.^{35,36} Epidemiological studies have also revealed that ICS are associated with reduced lung cancer risks in COPD patients, which might be again through the anti-EMT effects of ICS.³⁷ Milara et al³⁸ in their *in vitro* studies found that cigarette smoke extract (CSE) does induce EMT activity in normal primary bronchial epithelial cells (pHBECs) via ROS generation, which enhanced TGF β 1 expression while decreasing cyclic adenosine monophosphate (cAMP) levels. Further, the group demonstrated that EMT could be attenuated by elevating cAMP levels using a specific phosphodiesterase 4 inhibitor.³⁸ Gohy et al observed that targeting TGF- β 1 expression via specific monoclonal antibodies, during *in vitro* differentiation of pHBECs, reduced mesenchymal vimentin expression and fibronectin release.³⁹ Similar increases in mesenchymal markers N-cadherin and α -smooth muscle actin (α SMA) were reported by Wang et al, with an associated decrease in E-cadherin and α -catenin in small airway epithelial cells when stimulated *in vitro* with CSE. They further found that urokinase plasminogen activation receptor could be responsible for modulating EMT, positively correlating with an increase in vimentin expression in human small airways.⁴⁰ Recent *in vitro* epithelial–fibroblast co-culture studies also examined the

probable role of IL-1 α in promoting EMT.⁴¹ They reported that IL-1 α caused aberrant epithelial–fibroblast interactions when stimulated by CSE.⁴²

Although there are now several studies suggesting upregulation of EMT markers in COPD, there have only been few molecular signaling pathway studies undertaken to elucidate the underlying mechanisms of EMT in COPD. Recent studies suggest that EMT in smokers and early (mild to moderate) COPD patients is likely driven by the activation of canonical pathways associated with TGF β ,⁴³ which induces increases in the expression of nuclear transcription factors such as pSMAD2/3 with simultaneous reduction of inhibitory SMAD6/7 expression.^{44–46} The increased expression of pSMAD2/3 negatively correlated with lung function in patients with COPD, which indicates possible physiological consequences.⁴⁴ Although pathways triggered by TGF β and its superfamily, including bone morphogenetic proteins (BMPs) and growth differentiation factors (GDFs), play vital roles,⁴⁷ other canonical pathways such as Wingless/integrin-1 (Wnt)-mediated epithelial changes could also be crucial in COPD EMT pathology. However, there are few literature evidence in COPD. Our group observed enhanced expression of β -catenin in the large airways of both smokers and COPD, though COPD patients were observed to have increased nuclear to cytoplasmic ratio of β -catenin expression suggesting active translocation and possible transcription of genes associated with mesenchymal proteins.⁴⁸ Further, *in vitro* studies with bronchial epithelial cells showed that nicotine, which is a major component of tobacco smoke, could induce EMT in a WNT-3A/ β -catenin-dependent manner.⁴⁹ In the peripheral tissue, it was demonstrated that a decrease in Wnt/ β catenin signaling was involved in parenchymal tissue impairment and associated disruption of repair mechanism, leading to an increase in emphysema in COPD patients.⁵⁰ Again, this decrease may be further augmented by the lack of frizzled (FZD) receptors in the peripheral tissue, effectively enhancing the impairment process in alveolar cells.⁵¹ Interestingly, non-canonical pathways of Wnt signaling were found to be active in bronchial epithelial cells of COPD patients compared to smokers and nonsmokers.⁵² Wnt5B was found to modulate EMT changes independent of β -catenin possibly through the induction of TGF β /SMAD3 signaling.⁵³ Moreover, evidence also suggests cross-talk between TGF β /SMADs and Wnt/ β -catenin pathways in TGF β -induced alveolar epithelial cells, and that these interactions synergize to increase the expression of α SMA, a hallmark of EMT.^{54,55} Transcriptional factors such as SNAIL1/2, ZEB1/2 and TWIST, which are

independently activated by canonical or non-canonical pathways also show increased epithelial nuclear translocation in COPD patients.⁴⁸ Other potential interactive pathways were demonstrated to enhance EMT changes, for example, TGF- β 1 and p38 MAPK/PI3K/Akt signaling pathways complement each other and are known to activate SMAD2/3 signaling in *in vivo* models of COPD and in lung cancer.⁵⁶ Further, an increase in EGFR ligand HB-EGF in smokers and COPD was also observed to potentiate EMT-related and airway modeling changes possibly by interacting and modulating TGF β pathways.^{57,58} Considering the complexity in the number of pathways involved would indeed be hard to pinpoint one pathway which could be specifically targeted to inhibit this profound epithelial plasticity. However, given the published evidence in the literature, it is now almost certain that EMT does play an important role in COPD pathology.

In line with these findings in EMT, recent study by Sun et al⁵⁹ provided vital initial evidence of the post-transcriptional regulatory pathways that are involved in modulating EMT in COPD patients. Their study especially investigates the role of (HuR, which is an ubiquitously expressed post-transcriptional regulator of RNA synthesis and regulates several proteins including the ones involved in EMT. HuR are members of the ELAV/Hu family of RNA-binding proteins (RBP), specifically bind to AU (SRE) sites of the 3'-end of the mRNA of several growth factors, transcription factor proteins, cell cycle regulators and plays the crucial role in translocating mRNA from the nucleus to the cytoplasm for protein translation purposes.⁶⁰ Once in the cytoplasm HuR effects mRNA stability and translation of EMT-associated proteins (Figure 1). Sun et al⁵⁹ demonstrate that the levels of HuR expression are significantly increased in both smokers and COPD patients but more substantially in the latter group suggesting the possibility of a COPD effect. The cytoplasmic HuR expression was also shown to increase in human bronchial epithelial immortalized cell lines such as BEAS-2B when treated with cigarette smoke extracts (CSE). Most interestingly and relevant to EMT was their finding of a close association of cytoplasmic HuR levels and increases in the EMT trans-nuclear transcription factor ZEB1. The finding of increasingly stable mRNA levels of ZEB1 indicates that the translocated cytoplasmic HuR may play a direct role in modulation of ZEB1, which again was evidently higher in the epithelium of smokers and COPD patients compared to nonsmoking controls.⁵⁹ There are only a few other studies that demonstrate HuR having a direct role in driving

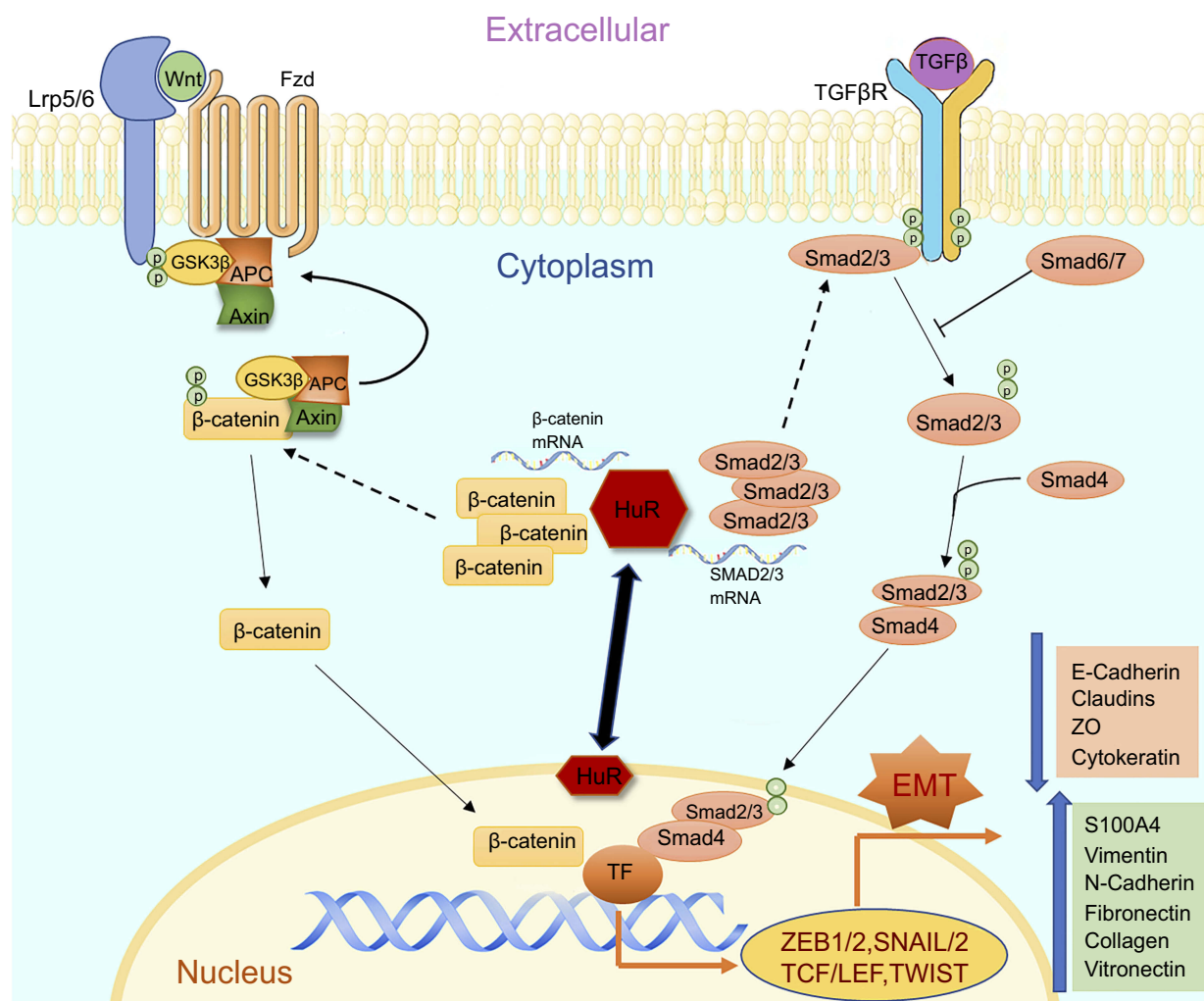


Figure 1 The figure illustrates the cellular pathways associated with EMT and HuR as a modulator regulating the changes in smokers and COPD. Both TGF β and Wnt pathways play a crucial role in inducing EMT and potentially activate their associated receptors TGF β R and Frizzled, respectively. Activation of TGF β -TGF β R results in phosphorylation of SMAD2/3 which tags with SMAD4 and translocates into the nucleus causing transcriptional changes. Activation of Wnt-Frizzled receptor signaling leads to inhibition of GSK3 β and consequential accumulation of cytoplasmic β -catenin. The free β -catenin translocates into the nucleus and regulates target gene transcription. Finally, both pathways could lead to the induction of EMT with increases in mesenchymal and corresponding decreases in epithelial proteins. HuR, under pathological conditions, translocates from peri-nuclear into the cytoplasm and enhances further synthesis and stabilization of these translocating nuclear proteins, thus increasing the potential of EMT.

Abbreviations: EMT, epithelial-mesenchymal transition; HuR, human antigen R.

EMT in COPD. A recent study by Gu et al has shown that in vitro CSE induces changes in small airway epithelial cells by increasing the expression of EMT-promoting proteins such as vimentin and SNAIL while decreasing the expression of E-cadherin in these cells. This increase in the cellular content of EMT proteins was found to be diminished in cells treated with siRNA targeting HuR.⁶¹ This inhibitory action by silencing HuR was also demonstrated by Sun et al⁵⁹ wherein siRNA against HuR reduced ZEB-1 protein expression and decreased E-cadherin while increasing the expression of mesenchymal marker such as vimentin. Previous studies in cancer showed that HuR modulates EMT regulators such as

SNAIL and Wnt/ β -Catenin by stabilizing their protein levels in the cytoplasm.^{60,62} Considering the fact that both these transcription factors and HuR are upregulated in COPD patients, investigating the possible role of HuR in their regulation is important. One of the key comorbidities of COPD patients is lung cancer. Indeed, epithelia with active EMT⁶³ are potentially vulnerable to malignant transformation, and EMT in COPD airways may well be the vital link between airway fibrosis and cancer development.⁶⁴ Recently, our group showed strong correlations between EMT activity in the leading edge of invasive cancer and EMT activity in the nonmalignant airway areas from which the tumor originated.⁶⁵ Thus,

increased expression of HuR and associated EMT in COPD patients suggest a potential therapeutic target with relevance in early COPD to cancer transition.⁶⁶

Also, EMT as a process could lead to an increase in fibroblastic cell populations that can invade the underlying mucosa, potentially causing airflow limitation in COPD, especially in the small airways wherein tissue reconfiguration and scarring occurs. Further, active fibroblasts can cause aberrant changes to the extracellular matrix (ECM) leading to an increase in airway wall thickening, stiffness and gradual obliteration of the small airways.^{26,65} Although EMT seems to be a major contributor of fibroblasts, other sources which include fibrocytes, pericytes, residential fibroblasts and mesenchymal cells could also be contributing to the overall population of fibroblasts.⁶⁷ Interestingly, endothelial cells which are the major cell type in blood vessels and responsible for structure and function possess the propensity to transit into fibroblast, through the process of endothelial-to-mesenchymal transition.^{18,34,68} During this process, endothelial cells lose endothelial adherence proteins such as VE-cadherins and gain mesenchymal markers similar to EMT.⁶⁹ Under pathological conditions such as in chronic lung disease, fibroblasts are further transformed to their more active form, myofibroblasts.³ These cells appear to have spindle-shaped morphology and are highly contractile. The migratory potential of myofibroblasts directly relates to the increased expression of α SMA myofilaments, which forms nonmuscle cells (stress fibers). Myofibroblasts are known to secrete a broad array of ECM proteins, which include fibrous proteins such as collagen and elastin, as well as glycoproteins such as fibronectin, tenascin C and various forms of proteoglycans.⁷⁰ Both canonical, Wnt/ β -catenin and TGF β /SMAD pathways are active in fibroblasts and their induction promotes myofibroblast proliferation and generation of ECM proteins.^{71,72} Thus, understanding the role of HuR in stabilizing proteins that induce these changes in fibroblast and myofibroblast populations is important and requires further investigation.⁷³

Finally, another major aspect in COPD is identifying the role played by inflammation and infections in driving epithelial and other cellular transformations.^{3,74–76} Our group recently observed an overall reduction in total airway wall cellularity that further corresponded to either decrease or nonsignificant changes in key innate immune cells such as macrophage and neutrophils in COPD patients.⁷⁷ On further evaluation of macrophage subpopulation in the bronchoalveolar lavage of COPD patients, we observed increases in M2 compared to M1 macrophage polarization

which matched the cytokine profile.⁷⁸ M2/Th2 cytokines are known to suppress M1-related inflammation, induce epithelial plasticity and promote proliferation of fibroblasts.^{79–81} Evidence also suggest dysfunctional immunity⁸² in COPD patients^{74–76} with poor phagocytic bacterial clearance by macrophage and ineffective killing of viral-infected cells by cytotoxic T cells.^{83,84} Interestingly, previous studies in colorectal cancer demonstrated that increases in cytoplasmic HuR in macrophages led to the suppression of pro-inflammatory cytokines, dysfunctional immunity and M2 polarisation.^{85,86} Thus, it would be of intrinsic value to evaluate the role of HuR in regulating inflammation in COPD and other chronic lung diseases.

Conclusions

We consider that EMT has important implications in various aspects of COPD and are crucial to large and small airway pathology, for both fibrosis and lung cancer.^{87,88} The role of TGF β and Wnt pathways is implicated in these transformations and could have a potential difference in activity in the lung based on the affected region. This is a novel field in COPD, though could have wider implications in other chronic lung diseases as well. The role of HuR in the regulation of EMT and disease manifestations makes it an attractive target for therapeutic strategies; however, further research in this area is mandated. ICS do prevent against lung cancer risk in COPD, which may be through EMT. However, a safer drug than ICS is needed for long-term use.

Disclosure

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