



Early View

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Endothelial to mesenchymal transition (EndMT): a precursor to post-SARS-CoV-2 infection (COVID-19) interstitial pulmonary fibrosis and vascular obliteration?

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Endothelial to mesenchymal transition (EndMT): a precursor to post-SARS-CoV-2 infection (COVID-19) interstitial pulmonary fibrosis and vascular obliteration?

Mathew Suji Eapen¹, Wenying Lu¹, Archana Vijay Gaikwad¹, Prem Bhattarai¹, Collin Chia^{2,1}, Ashutosh Hardikar^{3,1}, Greg Haug^{2,1}, Sukhwinder Singh Sohal^{1*}

¹Respiratory Translational Research Group, Department of Laboratory Medicine, School of Health Sciences, College of Health and Medicine, University of Tasmania, Launceston, 7248, Australia

²Department of Respiratory Medicine, Launceston General Hospital, Launceston, Tasmania 7250, Australia.

³Department of Cardiothoracic Surgery, Royal Hobart Hospital, Hobart, Tasmania 7000, Australia.

*Corresponding Author

Dr Sukhwinder Singh Sohal

Respiratory Translational Research Group

Department of Laboratory Medicine, School of Health Sciences,

College of Health and Medicine, University of Tasmania

Locked Bag – 1322, Newnham Drive

Launceston, Tasmania 7248, Australia

Telephone number: +61 3 6324 5434

Email: sssohal@utas.edu.au

We read with interest the recent editorial by Huertas and colleagues, highlighting the importance of endothelial cell dysfunction in patients with SARS-CoV-2 (COVID-19)

infection (1). The authors have made some very fascinating insights, and we would like to take this discussion further, especially emphasizing the role of endothelial cells in initiating post-infection pulmonary fibrosis and vascular remodelling. The angiotensin-converting-enzyme-2 (ACE2) has been suggested as the primary receptor for mediating SARS-CoV-2 entry into the host cells. Apart from ACE2, the other key players that facilitate the SARS-CoV-2 entry, includes transmembrane serine protease 2 (TMPRSS2), furin, sialic acid and the extracellular matrix metalloproteinase inducer (CD147) (1). In the article, authors provide compelling comprehensions from a study comparing post-mortem lung tissues from patients who died from COVID-19 with acute respiratory distress syndrome due to influenza A (H1N1) infection and those with age-matched, uninfected control lungs (1, 2). A crucial inference was the connections between the significant increase in ACE2 positive endothelial cells and the substantial change in endothelial cell morphology, disruption of intercellular junctions, cell swelling, and the breakdown of the underlying basement membrane, all indicative of vascular structural modification in tune to the process of endothelial to mesenchymal transition (EndMT) (3). Considering the implications for post-COVID-19 pulmonary fibrosis and vascular destruction seen in this infectious pathology, we believe that the role of EndMT in disease manifestation could be consequential.

EndMT occurs when endothelial cells respond to an external insult or an internal pathological condition, transforming themselves into a more aggressive mesenchymal state, causing irreversible vascular damage or fibrosis (3). The process of EndMT has been considered as one of the major contributors to several other pathological conditions such as pulmonary arterial hypertension, arterial fibrosis, atherosclerosis, cardiac fibrosis, and malignancy (3, 4). Endothelial cells undergoing EndMT loose endothelial characteristics such as a change in morphology, loss of vascular endothelial cadherins (VE-cadherins), CD31 and Tie1/2 with subsequent increase in mesenchymal proteins such as N-cadherin, fibroblast specific protein-1 or S100A4, fibronectin, vimentin, SM22- α , calponin and alpha-smooth muscle actin (3). As a part of the process basement membrane underlying endothelial cells gets disrupted, facilitating the migration of cells. This occurs through active proteolytic degradation of basement membrane collagen by matrix metalloproteinases mediated by the transitioning cells (3, 5).

Margot Combet and colleagues in an interesting case study published in European Respiratory Journal, reported rapid onset of honeycombing fibrosis in a patient with COVID-19 (6), with typical similarity to idiopathic pulmonary fibrosis (IPF) and similar questions were raised by Paolo Spagnolo et al regarding post-COVID-19 pulmonary fibrosis (7). In histopathological micrographs from patients with IPF, we observed high ACE2 expression in the intimal, medial,

and adventitial layers of the pulmonary arteries. Same areas also had high expression of mesenchymal proteins such as S100A4 and vimentin, indicative of active EndMT. Further Movat Pentachrome Stain showed prominent intimal thickening and luminal narrowing (**Figure 1**). It is quite possible that SARS-CoV-2 may lead to such endothelial cell dysfunction. These observations also pose a concern regarding smokers and patients with chronic obstructive pulmonary disease (COPD). Indeed, this patient population is highly susceptible to SARS-CoV-2 infection, as smoking has been observed to increase ACE2 expression (8, 9). It is also essential to note in this context that these patients may be highly compromised to fibrotic conditions post-COVID-19 recovery. As similar endothelial cell dysfunction is quite possible in the vasculature of smokers and patients with COPD, and the underlying mechanism of EndMT might be central to this pathology (5). The other cell type which could also contribute to fibrosis is type-II pneumocytes. These cells are quite hyperplastic and hyperproliferative in IPF (10) and could further contribute to fibrosis in the long term after patients have recovered from the SARS-CoV-2 infection. Type-II pneumocytes could have similar implications in smokers and patients with COPD.

Like EndMT, epithelial to mesenchymal transition (EMT) is also known to play a crucial role in organ fibrosis and epithelial cell malignancy (5). We have previously reported that it is an active process in smokers and patients with COPD, both in small and large airways (5). In this process epithelial cells transform into a mesenchymal phenotype accompanied by basement membrane degradation, loss of epithelial and gain of mesenchymal proteins (5). It will be important to see if this insidious infection also leads to enhanced epithelial cell activation through EMT, contributing again to post-COVID-19 pulmonary fibrosis.

Taken together it appears, endothelial and epithelial cells may be playing a prominent role in post-COVID-19 fibrosis. Patients with other disease conditions such as COPD or IPF might be at worse after recovery. The process of EndMT and EMT might be central to post-COVID-19 pulmonary fibrosis. There is an urgent need to understand these mechanisms in the context of SARS-CoV-2 manifestations for early interventions. We should be well prepared to deal with the aftermaths of COVID-19.

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Figure 1: Histopathological micrographs of pulmonary arteries from IPF patients compared to healthy normal controls (magnification 20X). (A - B) Pulmonary arteries after ACE2 immunostaining, with high expression in intimal, medial, and adventitial layers. (C - D) Movat Pentachrome staining, note the prominent intimal thickening and luminal narrowing. (E - F) Pulmonary arteries after immunohistochemistry with S100A4 and (G - H) with vimentin antibodies. The artery wall thickening in IPF patients along with increased expression of mesenchymal biomarkers S100A4 and vimentin are suggestive of active EndMT contributing to vascular remodelling. We propose, similar pathology could be driven by SARS-CoV-2 (COVID-19) infection through ACE2 expression in the endothelium as shown in A - B. IPF tissue was collected under ethics approval from Alfred Health Ethics Committee (ethics ID:

336-13) and normal control tissue were provided by the James Hogg Lung Registry, the University of British Columbia (ethics ID: H00-50110).

