Demystifying morphomolecular alterations of vasculature in interstitial lung diseases

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Focusing on remodelling-associated angiogenesis, both sprouting and intussusceptive, Ackermann and co-workers present histopathology, microvascular anatomy and gene expression in three main subtypes of interstitial lung disease: UIP, NSIP and AFE http://bit.ly/2NtmV6D

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Interstitial lung diseases (ILDs) encompass a complex group of hundreds of lung disorders that affect lung tissue with variable morphologies and clinical presentations. The most extensively studied type of ILD is idiopathic pulmonary fibrosis (IPF), which is characterised by progressive pulmonary fibrosis, a decline in lung function, and high mortality with a histological pattern of usual interstitial pneumonia (UIP). A proportion of patients with other types of ILD also develop a progressive fibrosing phenotype, including idiopathic nonspecific interstitial pneumonia (NSIP), as well as restrictive allograft syndrome (RAS) and idiopathic pleuroparenchymal fibroelastosis (iPPFE) with a histological pattern of alveolar fibroelastosis (AFE). RAS is a novel form of chronic lung allograft dysfunction first described in 2011 [1]. iPPFE was newly designated as a rare entity of idiopathic interstitial pneumonia in 2013 [2]. However, the pathogenesis of both RAS and iPPFE remains largely unknown [1, 3, 4].

These progressive fibrosing ILDs can develop vascular alterations, ultimately leading to pulmonary hypertension (PH) [5]. Microvascular alteration in IPF lungs was first described by Ebina *et al.* [6], who demonstrated an increased capillary density with increased endothelial proliferation in non-fibrotic lesions, but decreased density in remodelled fibrotic lesions. Vascular remodelling is clinically meaningful, since PH is considered the single most significant predictor of mortality in IPF patients [7–9] with limited therapeutic options [10]. Jacob *et al.* [11] also reported that pulmonary vascular volumes in computed tomography had the strongest correlation with mortality in patients with IPF. Given these facts, it is reasonable to hypothesise that microvascular alterations represent one of the steps in the pathogenesis of pulmonary fibrosis. However, the influence of pulmonary microvascular alterations on the progression of ILD remains poorly understood.

In this issue of the *European Respiratory Journal*, Ackermann *et al.* [12] present histopathology, microvascular anatomy and gene expression in three main histopathological subtypes of interstitial lung disease: UIP/IPF; NSIP/iNSIP; and AFE/RAS, iPPFE. This is a comprehensive analysis with state-of-the-art technology focusing on remodelling-associated angiogenesis (figure 1).

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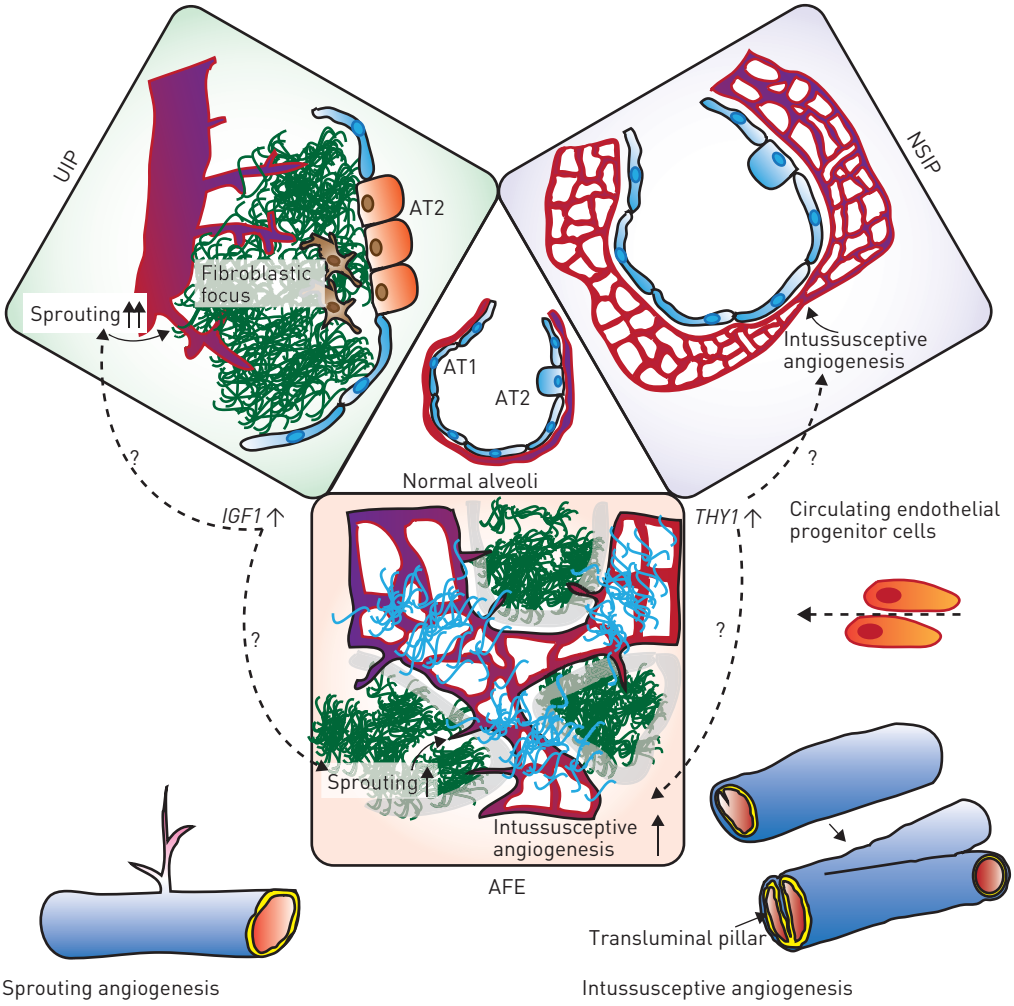

FIGURE 1 Schematic illustration of morphomolecular alterations of vasculature in histopathological subtypes of usual interstitial pneumonia (UIP), nonspecific interstitial pneumonia (NSIP) and alveolar fibroelastosis (AFE). There are two types of angiogenesis: sprouting and intussusceptive angiogenesis. Sprouting angiogenesis is characterised by sprouts composed of endothelial cells, which grow toward an angiogenic stimulus. Intussusceptive angiogenesis progresses *via* initial vasodilation, followed by the formation of a transluminal bridge, the "transluminal pillar" to split an existing blood vessel into two. The expansion of the microvascular network by intussusception is considered to be associated with the recruitment of circulating endothelial progenitor cells. Newly formed sprouting angiogenesis toward fibroblastic foci is frequently found in UIP. Thickened septa composed of dense vascular network expanded by numerous intussusceptive angiogenesis are found in NSIP. Former alveolar air spaces are filled with collagen and increased intussusceptive angiogenesis along with sprouting is found in AFE. *IGF1* is upregulated as a common dominator of AFE and UIP, while *THY1* is upregulated in NSIP and AFE. AT1: type 1 alveolar epithelial cell; AT2: type 2 alveolar epithelial cell.

The analysis of microvascular corrosion casts of UIP, NSIP and AFE lungs show different morphological patterns. Whereas UIP lungs show a higher density of upstream vascularity and lower density in perifocal blood vessels, NSIP and AFE lungs reveal densely packed alveolar septal blood vessels. Further, neoangiogenesis in UIP is characterised by sprouting of new vessels, while prominent intussusceptive angiogenesis is found in NSIP and AFE. The gene expression analysis using NanoString provides a pivotal insight for differences and similarities of angiogenesis-related gene expression: 16 genes are significantly upregulated in AFE lungs, whereas four genes are differentially expressed in UIP lungs and one gene in NSIP lungs. *IGF1* is significantly upregulated as a common denominator of AFE and UIP, whereas *MEG3* is significantly expressed in UIP and NSIP. *THY1* is highly expressed in NSIP and AFE.

As Ackermann *et al.* [12] described in the study, there are two types of angiogenesis: sprouting angiogenesis and intussusceptive angiogenesis [13]. Sprouting angiogenesis is characterised by a stereotypical series of steps including enzymatic degradation of the capillary basement membrane, endothelial cell proliferation, endothelial cell tube formation, vessel fusion, vessel pruning, and pericyte

stabilisation [13]. The initial step is driven by hypoxia, which induces angiogenic stimuli, including vascular endothelial growth factor [14]. Given that insulin-like growth factor-1 (IGF-1) is also considered as an angiogenic stimulus for sprouting [15], it is interesting to see *IGF1* was upregulated as a common dominator of AFE and UIP [12]. Indeed, Ackermann *et al*. [16] previously showed that inhibition of IGF-1 could prevent sprouting angiogenesis in a tumour model.

On the other hand, intussusceptive angiogenesis progresses *via* initial vasodilation, followed by the formation of a transluminal bridge, the "transluminal pillar". The expansion of the microvascular network by intussusception is considered to be associated with the recruitment of circulating endothelial progenitor cells [17, 18]. Given the results that a higher frequency of intussusceptive angiogenesis and *THY1* upregulation are observed in NSIP and AFE lungs, THY1 might be one of the crucial factors to attract circulating endothelial progenitor cells. Indeed, THY1 is expressed on endothelial cells at sites of chronic inflammation and is involved in the microvascular expansion by intussusceptive neoangiogenesis [19]. It might be informative to evaluate the actual levels of circulating endothelial progenitor cells in these patients in future studies. Lung tissue alteration could impact on the bone marrow, considering the recent report that pulmonary fibrosis caused significant alterations in bone marrow with expansion and activation of monocytic cells *via* soluble B7H3 and IL-33 upregulation [20].

The study of vascular alteration in interstitial lung diseases by Ackermann *et al.* [12] raises several suggestions for future investigation. First, the study assessed mRNA expression in the explant lungs. The lesion within the explant lungs used for mRNA analysis was not precisely described, but presumably included the fibrotic areas. The results, thus, mainly reflect on angiogenesis in the fibrotic lesions. Vascular degeneration occurs before fibrosis appears in IPF lungs; an analysis of the non-affected areas will be necessary to fully understand the gene expression dynamics regarding vascular alteration, as McDonough *et al.* [21] reported recently. Second, a combined analysis of mass spectrometry-based tissue imaging with vessel structure would be informative for unveiling post-translational modification and extracellular matrix shifts in sites with vascular alterations. Third, as investigators discuss in the study [12], the canonical question "Angiogenesis in pulmonary fibrosis: too much or not enough?" raised by Hanumegowda *et al.* [22] cannot be easily addressed. An analysis of endothelial cell-specific gene expression and biological analysis of isolated endothelial cells from patients in fibrogenesis is warranted to answer this question. Fourth, a preclinical study to target genes identified in the study may be useful to evaluate the therapeutic potential.

In conclusion, the outstanding work performed by Ackermann *et al.* [12] is already contributing to a better understanding of the pathogenesis of fibrosing pulmonary disease, and has opened the door for further studies that will expand our knowledge about the multiple changes in the vasculature and altered vascular cell–epithelial/mesenchymal cell interactions occurring during the progression of the disease.

Conflict of interest: T. Yanagihara has nothing to disclose. K.D. Jones has nothing to disclose.

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