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Review

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Vascular remodelling in the pathogenesis of idiopathic pulmonary fibrosis

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Summary

Idiopathic pulmonary fibrosis (IPF) is a progressive and irreversible fibrosing interstitial pneumonia of unknown aetiology that usually leads to respiratory failure and death within 5 years of diagnosis. Alveolar epithelial cell injury, disruption of alveolar capillary membrane integrity and abnormal vascular repair and remodelling have all been proposed as possible pathogenic mechanisms. This review summarizes our current knowledge of the abnormalities in vascular remodelling observed in IPF and highlights several of the cytokines thought to play a pathogenic role, which may ultimately prove to be future therapeutic targets.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive fibrosing disease of the lungs of unknown cause, associated with a median survival of only 3–5 years following diagnosis.[1] The pathogenesis of the disease remains poorly understood, although current paradigms focus on the importance of alveolar epithelial cell injury as a critical initiating event with subsequent dysregulated wound healing and fibrosis, resulting in distortion of the lung architecture. IPF not only destroys the normal lung parenchyma but also affects the pulmonary vasculature with aberrant microvascular and macrovascular remodelling. A better understanding of the basic biology of IPF is crucial to enable the development of novel therapeutic agents for this disease. This review summarizes the abnormalities in vascular remodelling observed in IPF and discusses the potential role of several key regulatory factors implicated in the current literature; promising targets for the development of novel therapies are thus highlighted.

Microvascular remodelling in IPF

Angiogenesis is the physiological or pathological process of new capillary blood vessel sprouting and growth from existing vasculature and is distinct from vasculogenesis; the *de novo* formation of blood vessels from precursor cells that predominantly occurs during embryogenesis.[2]

A report by Turner-Warwick[3] first implicated aberrant vascular remodelling in the pathogenesis of IPF, demonstrating anastomoses between the systemic and pulmonary microvasculature and extensive neovascularization within areas of fibrosis. Conflicting reports were subsequently published, however, suggesting an overall reduction in vessel density in the IPF lung.[4]

Animal models supported the concept not only of abnormal vascular remodelling as a pathogenic mechanism in pulmonary fibrosis (PF), with reports of newly formed vascular networks within the fibrotic lung,[5] but also of increased capillary irregularity and dilatation.[6]

The demonstration that both regions of increased and reduced vascularity could be found in the same IPF lung provided an explanation for the apparently conflicting early studies of vascular density.[7,8] In these studies, vessel redistribution was demonstrated; regions of the IPF lung at the interface to normal parenchyma displayed increased vascularization, whereas minimal vascularity was noted in the most extensively affected areas. The fibrotic focus (FF) was almost completely devoid of vessels. Interestingly, large, abnormally dilated vessels were also noted in areas of severe architectural distortion (honeycomb lung),[7] corroborating with evidence from animal models that the vascular phenotype of these newly formed vessels is abnormal.[6]

Although it is now clear that vascular heterogeneity exists in the IPF lung, whether the primary vascular abnormality is a lack or excess of neovascularization has yet to be determined. Furthermore, is angiogenesis a protective antifibrotic strategy or harmful? Is it central to disease pathogenesis or a peripheral consequence?[9] It has been proposed that angiogenesis may be compensatory mechanism in response to the vascular regression observed in severely fibrotic areas to support alveolar epithelial regeneration[7] although further evidence is required to confirm this hypothesis.

Angiogenic and angiostatic factors in IPF

As with many biological processes, homeostatic control of angiogenesis depends on the simultaneous regulation of stimulatory (angiogenic) and inhibitory (angiostatic) factors. It appears likely that in IPF this balance of angiogenesis vs. angiostasis is disrupted. Several factors thought to play a prominent role in the regulation of vascular remodelling in IPF are discussed below.

Keane *et al.*[10] were the first group to attempt to determine which factors dominated in the fibrotic milieu and thus address whether the primary abnormality in IPF was an excess or lack of neovascularization. They proposed that in IPF, an imbalance in the expression of angiogenic chemokines (CXCL5, CXCL8) vs. angiostatic factors (CXCL10) existed, favouring net angiogenesis. Interestingly, fibroblasts appeared to be the predominant source of CXCL8 in the interstitium of IPF, supporting a role for fibroblasts in mediating angiogenic activity in this disease.[10] Consistent with these findings and in one of the first studies connecting vascular remodelling, fibrogenesis and extracellular matrix (ECM) deposition, inhibition of CXCR2, a chemokine receptor for CXCL5 and CXCL8, attenuated bleomycin-induced PF, mediated through an inhibitory effect on angiogenesis.[11]

Subsequent work investigating into balance of angiogenic and angiostatic factors, described temporal and spatial variation in the expression of these mediators, in keeping with the vascular heterogeneity observed in this disease. One such study by Cosgrove *et al.*[8] sought to determine the relative expression of the angiogenic factor, VEGF (vascular endothelial growth factor) and the angiostatic factor, PEDF (pigment epithelium-derived factor) in IPF; factors expressed reciprocally in the regulation of several angioproliferative diseases. They demonstrated marked over-expression of PEDF, but minimal VEGF expression within the FF. In contrast, augmented expression of the angiogenic factors, VEGF and IL-8 was observed in the capillary endothelial cells (ECs) and Alveolar Type II epithelial cells of the highly vascularized alveolar septa in relatively preserved areas of the IPF lung.[7] It is tempting to speculate that in these areas, angiogenic factors such as VEGF may contribute to alveolar wall protection and attempted regeneration, with locally increased vascularity occurring as part of this attempted repair process.

Distinct from its traditional role as an angiogenic factor, evidence also exists linking VEGF signalling directly to fibrogenesis; highlighting the potential pleiotropic effects of many growth factors implicated in IPF disease pathogenesis. Transfection of the naturally occurring VEGF inhibitor, sFlt[12] and VEGF receptor (VEGFR) blockade[13] both resulted in the attenuation of bleomycin-induced PF. Indeed, inhibition of the VEGF/VEGFR signalling pathway, using a novel tyrosine kinase inhibitor (nintedanib), is currently the subject of Phase 3 clinical trials (ClinicalTrials.gov Identifier NCT01335464).

The role of VEGF in IPF pathogenesis is controversial however. Several contrasting reports exist suggesting that VEGF may be protective against the formation of PF,[14,15] with studies consistently describing a reduction in VEGF levels in the bronchoalveolar fluid (BALF) of IPF patients.[16]

Endostatin is a naturally occurring proteolytic cleavage product of type XVIII collagen and a potent inhibitor of angiogenesis. It has been shown to interfere with both VEGF/VEGFR and TGF-β1 (transforming growth factor-β1) signalling (reviewed by Ref. 17). The finding of elevated serum levels of endostatin in IPF led to the speculation that endostatin may be released by activated tissue fibroblasts

and contribute directly to fibrotic process.[18] Endostatin treatment has recently been shown to ameliorate bleomycin-induced PF through the inhibition of angiogenesis, inflammation, early collagen deposition and epithelial cell apoptosis.[19] Further studies of endostatin in existing fibrosis models are therefore warranted.

The angiopoietin biological axis has also been implicated in the regulation of vascular remodelling in IPF.[20] Angiopoietin-1 (Ang-1) is thought to assist in blood vessel stabilization during angiogenesis by promoting interactions between the ECM and ECs. Distinct angiogenic profiles of Ang-1 and its naturally occurring inhibitor Ang-2 have been demonstrated in the BALF of IPF patients compared with both controls and interstitial pneumonias associated with collagen tissue disorders. Morphological studies of angiopoietin expression profiles are required to develop understanding of this possible novel pathogenic pathway.

TGF-β1 is a pleiotropic cytokine considered to have a major role in fibrogenesis, promoting the activation, proliferation and differentiation of epithelial cells and collagen-producing myofibroblasts (reviewed by Ref. 21). Several studies provide evidence that TGF-β1 also mediates vascular remodelling; promoting angiogenesis through VEGF stimulation but on the other hand, inhibiting the proliferation and migration of ECs.[22]

Several transcription factors are appreciated as 'master regulators' of the aberrant angiogenic processes in IPF. Nuclear factor $_{K}$B is an essential transactivator of angiogenic chemokines,[23] whereas hypoxia inducible factor-1α (HIF-1α) is a master transcriptional regulator of the cellular adaptive response to hypoxia. VEGF is considered a major target gene of HIF-1α, although it induces the expression of virtually all pro-angiogenic growth factors. Whilst almost absent from normal lung tissue, HIF-1α has been shown to co-localize with VEGF expression and appear in histologically normal areas of the IPF lung.[24] The authors hypothesize that HIF-1α induction may be an early event in disease pathogenesis but further work is required to validate this concept.

Pulmonary hypertension in IPF

Vascular remodelling of macrovascular structures also occurs in IPF. The development of pulmonary hypertension (PH) as a secondary complication of IPF is well documented, occurring in as many as 32–85% of patients (reviewed by Ref. 25), although despite this, the pathobiology is not completely understood.

Histopathologically, destruction of the capillary bed in addition to numerous structural alterations affecting arteries, arterioles and venules have been described in IPF (reviewed by Ref. 25). Historically, changes were attributed to hypoxic vasoconstriction. It was subsequently recognized that ablation of vessels in areas of severe fibrosis may also contribute to elevated pulmonary vascular resistance, thus linking vascular pathologies at the micro- and macrovascular level. Furthermore, newly formed vessels in IPF lack an elastin layer and as such, may demonstrate reduced vascular compliance.[7] These hypotheses do not, however, account for all the pathological changes observed in the development of IPF-PH, nor explain the development of PH in IPF patients with only mild to moderate interstitial disease and an absence of hypoxaemia.

It appears increasingly likely that the cytokine milieu underpinning fibrogenesis also plays a significant role in vascular remodelling and in the development of PH. Farkas *et al.*[26] illustrate that these cytokines may have opposing roles in different lung compartments; in an experimental model of PF, concomitant delivery of TGF-β1 and VEGF resulted in attenuation of features of PH compared with TGF-β1 alone, but concurrently aggravated fibrogenesis. It highlights the potential pitfalls of developing anti-angiogenic therapies for the treatment of IPF-PH.

It is also unlikely that factors governing microvascular and macrovascular remodelling in IPF are mutually exclusive, although the recognition of a cohort of patients with PH disproportionate to the degree of fibrosis implies variable expression of angiogenic factors or possibly, alternative mechanisms. EC dysfunction, with the reduced release of vasodilatory factors such as nitric oxide and prostacyclins, together with the release of vasoactive mediators from fibroblasts and apoptotic ECs, appears to be important mechanisms mediating vascular remodelling and PA muscularization.[25] Endothelin-1 (ET-1) is one such factor initially considered a strong candidate in the pathogenesis of IPF-PH, promoting pulmonary arterial vasoconstriction and smooth muscle cell growth, but clinical trials of ET-receptor antagonists have proved disappointing.[27]

TGF-β1, fibroblast growth factor (FGF) and platelet derived growth factor (PDGF) are additional growth factors that have also been implicated in both fibrogenesis and the development of PH (reaviewed by Ref. 28) and may provide potential targets for future therapies. Indeed, it is the dual mitogenic and fibrogenic effects of PDGF, FGF and VEGF that provides the scientific rationale behind the clinical trial of tyrosine kinase inhibition

in IPF (as nintedanib) (ClinicalTrials.gov Identifier NCT01335464).

Conclusion

Multiple pathogenic mechanisms contribute to the development of IPF. This review has highlighted that although progress has been made into defining the vascular abnormalities that are present in IPF, many unanswered questions still exist as to its role in the fibrotic process. Given the potentially reciprocal effects of mediators on different compartments of the lung and the inherent heterogeneity of the disease, a better understanding of vascular remodelling in the context of IPF is required before targeted therapies can be trialled. One of the greatest challenges in developing any future modulatory therapy will be how to deliver it in a highly directed manner.

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