

## Lymphangiogenesis and its Role in Physiologic Wound Healing and the Pathogenesis of Pulmonary Fibrosis

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### Abstract

Wound healing and repair is a physiologic process that occurs following tissue injury often resulting in a controlled scar formation. However, in certain disease states, the intrinsic mechanisms that signal the completion of repair are defunct leading to continued repair, resulting in excessive fibrosis. Numerous segments of the wound healing process are known to be deregulated in idiopathic pulmonary fibrosis (IPF) including increased myofibroblast activation, excessive extracellular matrix (ECM) deposition, and decreased injury resolution. In this review we will focus on one part of the wound healing process, lymphangiogenesis and lymphatic remodeling, and its potential role in the pathogenesis of pulmonary fibrosis.

**Keywords:** Lymphangiogenesis; Lymphatic vessels; Idiopathic pulmonary fibrosis

### Introduction

Wound healing and repair is a physiologic process that occurs following tissue injury often resulting in a controlled scar formation. However, in certain disease states, the intrinsic mechanisms that signal the completion of repair are defunct leading to continued repair, resulting in excessive fibrosis. Numerous segments of the wound healing process are known to be deregulated in idiopathic pulmonary fibrosis (IPF) including increased myofibroblast activation, excessive extracellular matrix (ECM) deposition, and decreased injury resolution [1]. In this review we will focus on one part of the wound healing process, lymphangiogenesis and lymphatic remodeling, and its potential role in the pathogenesis of pulmonary fibrosis.

### Normal Wound Healing and Repair

In response to tissue injury, the wound healing process follows three tightly regulated phases: (1) injury, (2) inflammation, and (3) repair, with the goal to re-establish tissue homeostasis [1]. In the first phase following injury, damage to the endothelium results in activation of the clotting cascade, platelet activation, and the formation of a fibrin clot that is critical step for the second phase of wound healing by acting as a scaffold for infiltrating inflammatory cells and fibroblasts to accumulate at the site of injury [2].

The second phase of wound repair is marked by release of a milieu of cytokines, chemokines, and growth factors that are secreted by both resident cells and infiltrating inflammatory cells and fibroblasts. These soluble factors promote myofibroblast activation to produce collagen, direct changes in inflammatory cell phenotype, stimulate angiogenesis, and lymphangiogenesis. Vascular endothelial growth factor A (VEGF-A) and fibroblast growth factor 2 (FGF-2) are pro-angiogenic factors that are released in response to injury and initiate the growth of new blood vessels to transport nutrients and infiltrating cells to the site of injury [2]. After a temporal delay, lymphangiogenesis occurs in order to facilitate inflammatory cell trafficking to and from the site of injury. In a mouse model of chronic airway inflammation, capillary enlargement occurred by day 7 following inoculation with *Mycoplasma Pulmonis*, whereas lymphatic remodeling occurred later with lymphatic sprouting beginning on day 14 [3]. Taken together, the expansion of both blood and lymphatic vessels that occurs during the inflammatory phase of wound repair helps to regulate the influx

and phenotype of infiltrating cells in the wound through cytokine, chemokine, and growth factor signaling to ultimately facilitate re-epithelialization and wound closure.

In the final phase, inflammatory cytokines and growth factors are no longer produced and the infiltrating cells undergo apoptosis. In addition, the collagen produced by the myofibroblasts undergoes remodeling from type III to type I collagen to increase scar stability [1,2]. In IPF, there is evidence of increased type 2 inflammatory cytokine profile (IL-4, IL-13, and TGF $\beta$ 1), angiogenesis, myofibroblast activation, and importantly Lymphangiogenesis [1].

### Molecular Mechanisms of Lymphangiogenesis

Lymphatic vessels are present throughout the human body, serving as conduits for immune cell trafficking and interstitial fluid balance. Similar to the blood vasculature, new lymphatic vessels in adults are thought to arise from pre-existing lymphatics and bone marrow derived lymphatic endothelial precursor cells [4-6].

The VEGF family of receptors and ligands are important in the regulation of growth and survival of both blood and lymphatic vessels. During inflammation, leukocytes and macrophages secrete VEGF-C/D that bind to VEGFR-3 and VEGFR-2 expressed on the surface of LECs to promote Lymphangiogenesis [4,7,8]. Binding of VEGF-C to VEGFR-3 signals Akt phosphorylation and activation of p42/p44 mitogen-activated protein kinase [MAPK] resulting in LEC survival and migration [9]. Increased VEGF signaling regulates lymphatic growth and survival, whereas reduced VEGF signaling results in lymphatic regression [4]. Abnormal lymphatic growth is known to occur in many fibrotic diseases, including renal tubulointerstitial

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fibrosis and the alloimmune response to cardiac transplant [10,11]. Although lymphangiogenesis has been reported in many fibrotic diseases, the role that the remodeled lymphatic vasculature plays in the progression of fibrosis and the molecular mechanisms that drive this not well understood. It is possible that the soluble factors that are secreted by LECs during lymphangiogenesis influence both resident and circulating cells. CCL21, a cytokine known to be secreted by LECs that binds CCR7 present on activated dendritic cells, T and B cells, is being investigated as a promising soluble factor secreted by LECs to attract and alter the phenotype of circulating CCR7 positive cells [12,13].

### Idiopathic Pulmonary Fibrosis

Idiopathic pulmonary fibrosis affects 50 in 100,000 people and is a progressive fibrotic disease of the lung characterized by areas of fibroblastic foci and increased ECM deposition [14]. The pathogenesis of IPF is unknown, but is thought to involve an unknown trigger in the setting of a predisposed lung in addition to repeated micro-injury and aberrant wound repair [1,15]. What triggers the fibroblast to transform into activated myofibroblasts resulting in ECM deposition and progressive scar formation is not completely understood. Additionally, little is known about early events following the initial injury before the lung has become laden with excessive ECM. Recent evidence suggests that lymphangiogenesis may occur early after lung injury, facilitating inflammatory cell and fibroblast cell trafficking to areas of early fibrosis, and additionally may play a role in altering the phenotype of the infiltrating cells [16,17].

### The Role of Lymphatic Remodeling in Idiopathic Pulmonary Fibrosis

An expanded network of lymphatic vessels has been observed in Idiopathic Pulmonary Fibrosis (IPF) and has been implicated in the pathogenesis of pulmonary fibrosis. Morphometric abnormalities including increased lymphatic vessel length density, lymphatic vessel area can be found in IPF when compared to normal lung [16,17]. These morphometric changes correlate with degree of histopathologic fibrosis and clinical severity of disease. In addition to increased lymphangiogenesis, lymphatic vessels in IPF, in comparison to normal lung, are distributed in different areas throughout the lung. In the normal lung, most lymphatic vessels are present along large blood vessels and airways with little lymphatic vessels distant from blood vessels or airways [18]. Although it is clear that lymphangiogenesis and remodeling of the lymphatic vasculature is present in the IPF lung, there is ongoing debate regarding if lymphangiogenesis occurs de novo or is an expansion of preexisting lymphatic vessels.

Macrophages have been shown to trans-differentiate into LEC-like cells that form tubes in culture under conditions that simulate a fibrotic lung, suggesting that macrophages in IPF trans-differentiate into LECs forming the modified lymphatic vasculature [16]. Conversely, lymphatic proliferation can occur from preexisting lymphatics in response to VEGF-C/D signaling through VEGFR-3. In contrast to normal wound healing where lymphatic vessels regress as the wound is repaired, the lymphatic vessels in IPF do not appear to regress. Whether there is an absence of signaling instructing the vessels to regress or there is a persistence of pro-growth signaling is unclear. How this expanded network of lymphatic vessels contributes to the pathogenesis of IPF is unknown.

Lymphangiogenesis may contribute to the pathogenesis by attracting circulating inflammatory cells and fibrocytes; alternatively,

the persistence of lymphatic vessel growth may attenuate the degree of fibrosis that might otherwise occur by draining inflammatory cells and fibroblasts from the lung. IPF cultured fibroblasts are reported to express CCR7 and show both migratory and proliferative response when exposed to CCL21 [19]. Furthermore, CCL21 is increased in the bronchoalveolar lavage fluid (BALF) from IPF patients suggesting the lymphatic vasculature in IPF may promote the progression of fibrosis [16]. Whether CCL21-CCR7 signaling can affect the phenotype of infiltrating fibrocytes has not been elucidated.

### Conclusion

IPF is a progressive fibrotic lung disease that may partially arise from aberrant wound repair. Increased lymphangiogenesis and lymphatic remodeling is present in IPF and may contribute to disease progression by producing cytokines and growth factors that attract cells to areas of progressive fibrosis and alter the phenotype of infiltrating cells. CCL21-CCR7 signaling appears to be involved in fibrocyte trafficking to areas of progressing fibrosis. The stimulus of lymphangiogenesis in the IPF lung may remain as elusive as the trigger for fibrosis, however further insights into their role in the pathogenesis of IPF should be explored as elucidation of their role may offer novel therapeutic targets for this devastating disease.

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