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VEGF Drives the Car toward Better Gas Exchange

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Abstract

How lung epithelium and endothelium co-develop to maintain structural integrity of alveoli remains unclear. In this issue of *Developmental Cell*, Ellis et al. define how epithelial *Vegfa* directs development of a distinct endothelial cell population that ultimately plays a critical role in ensuring appropriate alveolar septation during alveologenesis.

One of the most striking features of the complicated process of lung development is the similarity of the developing vascular and airway networks to their mature form, especially the intimate overlay of capillaries surrounding alveolar walls necessary for efficient gas exchange after birth. Interactions between the embryonic mesenchyme and airway epithelium have been shown to be critical for their temporal and spatial proximity during development (McCulley et al., 2015). Hence, production of the critical endothelial growth and survival factor VEGFA in a strict temporal and spatial organization within the embryonic lung is crucial for normal lung development (Akeson et al., 2003). While there appear to be multiple sources of VEGFA in early embryogenesis, during late development, and throughout life, the alveolar epithelium becomes a critical source of VEGFA for development of normal alveolar structure and its maintenance.

Early studies investigating the role of VEGFA in lung development were conceptually pioneering but hampered by limited tools to uncover and define subpopulations of epithelial or endothelial cells in the lung (Yamamoto et al., 2007). While it was initially thought that alveolar type II cells (AEC2s) were the main source of VEGFA, recent studies by Jichao Chen and colleagues pinpointed type I cells (AT1s) as the near-exclusive source of VEGFA during late stages of development (Yang et al., 2016). The investigators described ATI differentiation as a two-step process: first, flattening on the saccular wall, followed by a large increase in surface area leading to folding of AT1 cells at sites of secondary alveolar septation. True alveoli are formed from larger saccular structures late in gestation and continue to develop after birth by a process of infolding of the saccular epithelium thought to be initiated by a “push” from myofibroblasts expressing insoluble elastin at the infolded site (Boström et al., 1996). The authors also observed capillaries within these AT1 folds.

In this issue of *Developmental Cell*, Ellis et al. provide even greater granularity to understanding the intricate connections between maturation of the alveolar epithelium and

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its surrounding capillary networks (Ellis et al., 2020). The experimental methods in this study are state-of-the-art, and the experiments are well-controlled and beautifully illustrated. The authors' new study was powered by the observation that deletion of *Vegfa* specifically in AT1 cells (using two different AT1-specific drivers, *Aqp5* and *Hopx*) led to a striking decrease in capillaries near alveolar surfaces with modest reductions in overall lung vessels and no decrease in overall endothelial cell (EC) proliferation. This suggested to the authors that there is heterogeneity in EC responses to VEGFA withdrawal, consistent with studies in other tissues (Potente and Mäkinen, 2017), and defining this heterogeneity became the main subject of the study. Using single-cell RNA-seq analysis, Ellis et al. (2020) show that removal of AT1-derived *Vegfa* led to elimination of a small EC population (18% of total ECs) that uniquely express carbonic anhydrase 4 (*Car4*). The authors found that Car4+ ECs have a larger surface area than Car4− ECs and are enriched at capillaries near alveolar surfaces, especially at sites of thin basement membranes devoid of pericytes (Figure 1). Car4+ ECs are seen almost exclusively in close proximity to thin AT1 extensions, consistent with the concept of the lungs' function in promoting efficient gas exchange. That said, it remains to be determined whether the implied efficiency of this architecture proves important in oxygenation.

Surprisingly, deletion of all EC-derived *Vegfa* with a constitutive Shh^{Cre} driver produced essentially the same EC phenotype as AT1-specific deletion, implying that Car4+ ECs are the only ECs dependent on VEGFA production from an epithelial source. Yet, the phenotypes of AT1-specific and pan-epithelial *Vegfa* deletion are distinct because pan-epithelial disruption produced a clear emphysema-like phenotype whereas AT1-specific deletion did not. The authors attribute this difference to the delay in *Vegfa* deletion with an AT1-specific *Cre* compared with the early expression of the pan-epithelial Shh-Cre. Consistent with this interpretation, an earlier study also using a pan-epithelial approach via a promiscuous surfactant promoter to delete *Vegfa* during embryogenesis found an even stronger emphysema-like phenotype with vascular attenuation and death shortly after birth (Yamamoto et al., 2007). These findings bring up the question of whether maintenance of the Car4+ ECs with ongoing VEGFA stimulation is crucial to the integrity of adult alveolar capillary beds. The few available clues support this possibility. Reduced lung VEGFA via an Fc-anti-VEGF in adult rats, or blockade of VEGF signaling, results in an emphysema-like phenotype within weeks (Kasahara et al., 2000). In this case there is also endothelial apoptosis without an overall change in EC proliferation, findings quite consistent with the phenotype observed by the Chen group during lung development.

These results raise an important question of whether similar heterogeneous EC populations exist in the human lung. Developmental lung disorders such as bronchopulmonary dysplasia (BPD) lead to decreased alveolar capillary density and impaired septation along with reduced epithelial VEGF expression (Abman, 2010; Bhatt et al., 2001). Determining whether a VEGF-responsive Car4-like EC subpopulation exists in the developing human lung could provide new insights into BPD or other lung diseases associated with abnormal vasculature. An important element in the studies by Ellis et al. is the temporal definition of when Car4+ ECs develop, leading to a predicted lineage relationship between the earlier-born and more abundant Car4− ECs and the appearance of Car4+ ECs (Ellis et al., 2020). It is unknown whether an injured lung undergoing active regeneration elicits a similar

developmental process of Car4− to Car4+ EC differentiation and subsequent alveolar septation. If so, it is conceivable that persistent injury or diminished differentiation efficiency of Car4− ECs to give rise to Car4+ ECs could promote the failed regeneration observed in several lung dis-eases. This represents an attractive paradigm for future studies that could not only aid our understanding of these diseases but also uncover signaling cues for more effective cell transplantation strategies aimed at alveolar repair.

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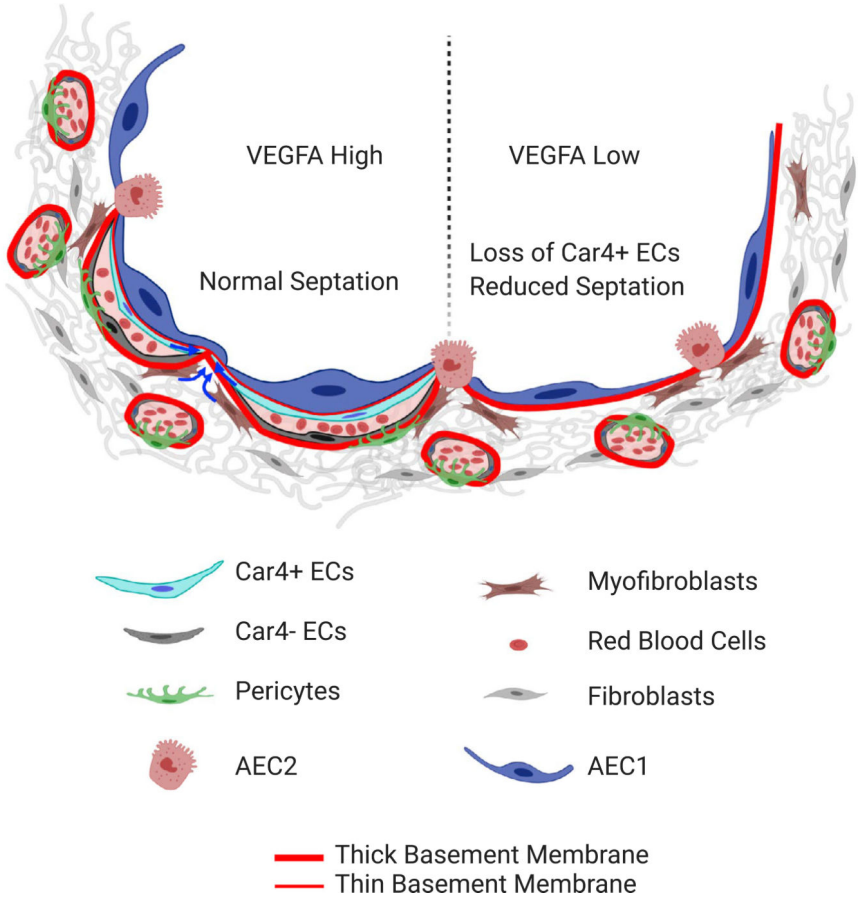

Figure 1. Epithelial VEGFA Specifies Car4+ Endothelial Cells That Drive Alveolar Septation

Car4+ endothelial cells (ECs) are separated from VEGFA-secreting AT1 cells by a thin basement membrane devoid of pericytes, while Car4− ECs line the thicker basement membrane. The Car4+ ECs, along with myofibroblasts, contribute to alveolar septation during alveologenesis (blue arrows). These vessels, unlike other vessels comprised of Car4− ECs, are lost with disruption of epithelial VEGFA, ultimately causing incomplete alveolar septation culminating in emphysematous alveoli.