Connecting the Cells

Vascular Differentiation via Homeobox Genes and Extracellular Matrix in the Distal Lung

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Although tremendous strides have been made in understanding how blood vessels form in the embryo, little is known about the role of individual genes during vasculogenesis or angiogenesis. Questions remain about transcriptional regulators of blood vessel formation or whether there are differences in embryonic gene expression in distinct vascular beds. An article in this issue of Circulation Research by Ihida-Stansbury et al. may provide clues to these enigmatic questions. These authors explored the relationship between the paired related homeobox gene, Prx1, one of its downstream targets, tenasin-C (TN-C), and endothelial specification during vascular development in the distal lung mesoderm.

Pulmonary vascular development represents a unique system of blood vessel formation. First, angiogenesis and vasculogenesis proceed independently in the proximal and distal portions of the growing lung buds. Proximally, the pulmonary arteries grow into the lung bud by angiogenic sprouting from the dorsal aorta or aortic sac. Distally, the foregut mesoderm organizes into a capillary bed by vasculogenesis. The distal and proximal beds join to form the complete network of arteries, veins, and capillaries. Second, interaction between the forming airways and surrounding mesenchyme is essential for inducing vasculogenesis as well as specialization of alveolar epithelial cells. Thus, in addition to angiogenic outgrowth from the aorta into the proximal lung, all vascular cell types in the distal lung are derived from foregut mesoderm through induction from the airways.

Homebox (Hox) genes encode transcription factors that are master regulators of patterning in development. These ancient genes control diversification of segments along the anteroposterior axis of all animals. Common to all of the proteins encoded by members of the Hox gene family is a 60 amino acid DNA binding homeodomain. The finding that Hox genes encoded transcription factors led to the idea that they function as activators or repressors of other genes. In 1975, Garcia-Bellido proposed that Hox genes directly activated the genes that functionally control morphogenesis. He called these downstream targets “realizator” genes, defining them as genes that determine such processes as cell shape or phenotype, adhesion, migration, and rate of mitosis or apoptosis. Many examples of such genes have been since identified as Hox targets. However, it has become clear that Hox genes primarily regulate networks of other transcription factors and signaling molecules that in turn control the “realizators.” This occurs in a tissue- and organ-specific manner such that the same Hox gene in distinct cellular contexts can regulate multiple target genes. This suggests that positional specificity of target regulation is achieved through interactions of other proteins with HOX proteins. Finally, HOX transcription factors are known to regulate several genes simultaneously in a given cell type, either directly or by activating transcriptional networks. So, in a given circumstance, multiple genes are either activated or repressed by a single Hox gene.

Prx1-null mice have skeletal abnormalities that were initially assumed to produce their respiratory distress and death in the perinatal period. However, because they also had vascular abnormalities including abnormalities of the aortic arch and ductus arteriosus, Ihida-Stansbury et al investigated the role of Prx1 on vasculogenesis in the distal lung mesoderm. They expressed Prx1 in the RFL-6 fetal lung mesodermal cell line. Remarkably, Prx1 expression in RFL-6 cells caused them to change from disorganized mesenchymal cells to an organized cobblestone monolayer typical of a vascular endothelium. This morphological change was not accompanied by an increase in expression of the endothelial specific proteins PECAM-1, von Willebrand factor (vWF), or VE-cadherin. In fact RFL-6 cells already express these markers. Furthermore, expression of Prx1 in RFL-6 cells cultured on Matrigel matrix caused them to associate into 3-dimensional networks complete with lumens. The authors confirmed that these findings were not unique to this cell line by demonstrating that Prx1 could confer a similar endothelial phenotype on another lung mesodermal cell line, MFLM-4 cells.

These intriguing observations indicate that Prx1 expression could stimulate mesenchymal to endothelial transformation. But what is the timing and mechanism of commitment of vascular precursors during vasculogenesis? It is possible that endothelial precursors are committed before they associate into networks, as suggested by the fact that the RFL-6 and MFLM-4 cells already express endothelial-specific proteins. Prx1 expression in these cells then induces expression of genes that stimulate the formation of stable adherins junctions necessary for the assembly of highly organized cell-cell contacts. It is interesting to note that in early lung buds, Prx1 expression is not observed in all mesodermal cells, but rather in clusters of aggregated cells. If lung mesoderm expresses early endothelial-specific markers, similar
to RFL-6 and MFLM-4 cells, Prx1 may be essential for the final commitment of precursors to endothelium simply by causing them to aggregate. Thus cells may become committed to the endothelial lineage by establishing junctions with other endothelial cells.

If Prx1 can confer an endothelial identity on uncommitted lung mesodermal precursors, then is this effect specific to the lung mesoderm? There are some similarities outside of the lung. Prx1 and Prx2 are both expressed in the endocardium and endocardial cushions of the developing heart as well as in the epicardium and great arteries and veins. The endocardium is the lining of the heart tube and shares characteristics with vascular endothelium, although it is derived from the precardiac mesoderm. The epicardium is also a mesodermal derivative that gives rise to a population of invasive mesenchyme that are the precursors to all of the vascular cells of the coronary arteries. However, there are many cell types that express Prx1 and do not differentiate into blood vessels. Thus Prx1 expression alone may not be sufficient to cause endothelial, vascular smooth muscle, or pericyte differentiation to occur. This indicates that other factors could exist in lung mesoderm to allow Prx1-expressing cells to aggregate into endothelial tubes. One mechanism that may facilitate this may be the necessary interaction of lung mesenchyme with airway epithelial cells. In this scenario, Prx1-expressing cells are stimulated by an airway-derived morphogen to differentiate into endothelial cells, whereas non-Prx1-expressing mesodermal cells are not. Alternatively, because HOX transcription factors form complexes with other nuclear proteins, Prx1 could simply assemble with other cell-specific expressed factors to drive the endothelial lineage.

Another compelling idea is that in lung mesoderm, Prx1-expressing cells modify their extracellular environment so that it becomes permissive for endothelial cell differentiation and vessel formation. This is consistent with a common theme for most Prx1-expressing cell types in that they are mesenchymal and form connective tissue. Prx1 has been implicated in the transcriptional regulation of extracellular matrix genes such as procollagen I and TN-C. Expression and secretion of these molecules could modify the surface of lung mesodermal cells causing them to respond by aggregating and forming endothelial tubes. This is what Ihida-Stansbury observed. In developing lungs, a TN-C–rich extracellular matrix forms around early pulmonary vessels. They also showed using reporter constructs and EMSA assays that Prx1 binds to the TN-C promoter and drives its expression in RFL-6 cells. In Matrigel assays Prx1-expressing cells expressed high levels of TN-C and a function-blocking antibody to TN-C interfered with vascular network formation. Consistent with their hypothesis that Prx1 regulates TN-C and endothelial differentiation, they found lowered levels of TN-C, Flk-1, and VCAM-1 in lungs isolated from Prx1-null mice.

Ihida-Stansbury’s studies provide important evidence for a direct role for homeobox genes in the control of pulmonary vascular development. Their findings in Prx1-null mice, which included greatly reduced numbers of vWF-positive blood vessels and hypoplastic lungs, indicate Prx1 is required for vascular network formation and normal alveolar development. Although rare, there are similar examples of disordered antenatal development of the lung in human infants. For instance, alveolar capillary dysplasia is a rare abnormality of lung development, characterized by paucity of alveolar capillaries and lack of capillary contact with alveolar epithelium. Infants develop respiratory distress and die shortly after birth despite maximal support.

Lung development can also be disrupted after birth because of lung injury. Although bronchopulmonary dysplasia has always been a clinical problem in preterm survivors of neonatal intensive care, its nature has changed as more extremely low birth weight infants survive. An arrest in lung growth associated with abnormal capillary formation is now recognized as a central hallmark of this condition, but the mechanisms that inhibit lung growth in premature infants with severe bronchopulmonary dysplasia remain poorly understood. Further studies of the functions of Prx1 and TN-C will help us answer key questions about specification of cell fate and vascular organization in the developing lung. We believe that an understanding of the role of Hox genes in early postnatal vascular development will be important in determining how to preserve and restore normal vascular growth as infants recover from their initial injury.

References

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