Meet Me in the Middle
Dual Origins of Dermal Lymphatic Vasculature in Mammals

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Lineage-Tracing Studies Reconcile a 100-Year-Old Debate on the Centrifugal Versus Centripetal Theories on the Origin of Lymphatic Endothelial Cells

A network of thin-walled lymphatic vessels is present in virtually every tissue of the body, where it carries out several important functions, such as transport of antigen-presenting cells to lymph nodes, uptake of dietary fat, and maintenance of interstitial fluid balance. The importance of the lymphatic vasculature to human pathology is only now beginning to be appreciated, as it becomes increasingly clear that lymphatic vessels play important roles in a host of common diseases, ranging from cancer metastasis to atherosclerosis and hypertension. Research in the past 2 decades has tremendously improved our understanding of molecular mechanisms involved in the regulation of lymphatic vasculature and its function. In particular, the atypical homeobox transcription factor Prox1 plays a key role in establishing and maintaining mammalian lymphatic endothelial cell (LEC) identity, whereas the Ccebl/Adams3/Vegf-c/Vegfr-3 signaling cascade is essential for LEC proliferation, migration, and survival. Novel therapies, which are a direct result of this knowledge, are now being developed for treatments that both promote lymphatic vessel regeneration and block excessive lymphangiogenesis. However, full comprehension into the intricacies of LEC biology is still being developed. The work of Martinez-Corral et al, based on lineage-tracing analyses of genetic mouse models, now provides first insights into an unexpected complexity of LEC origins in mammals.

Historically, there have been 2 main hypotheses about the origin of the lymphatic vasculature. One idea proposed by Sabin was that lymphatic vessels arise from veins during embryogenesis. On the basis of India ink injections in pig embryos, Sabin demonstrated expansion of ink-filled lymphatic vessels from the areas of primitive lymph sacs, located near cardinal veins, toward the periphery. These data suggested that lymphatic vessels grow in a centrifugal manner by sprouting from the pre-existing venous endothelium. A second school of thought, drawing conclusions from cat embryo serial section reconstructions, suggested that the lymphatic vasculature arose from “mesenchymal clefts,” initially disconnected, but which fused to form the lymphatic vasculature in a centripetal fashion. However, it was not until the discovery of lymphatic markers, such as Vegfr-3 and Prox1 that these hypotheses could be more rigorously tested. The expression pattern of Vegfr-3 (Flt4) in early blood vessels, followed by restriction to the lymphatic vasculature, together with localized expression of the lymphangiogenic factor Vegf-c near the cardinal vein during early stages of lymphatic vascular development, favored Sabin’s hypothesis for the venous origin of lymphatics. This idea was further strengthened by the discovery of Prox1, which is expressed by a subset of cells in the embryonic cardinal vein, from which nascent lymphatic vessels were found to arise. Importantly, Prox1 was also found to be essential both for development and maintenance of the lymphatic vasculature, as Prox1-null animals failed to develop lymphatic vessels and lymphatic cell identity is lost after inducible or postnatal Prox1 deletion. Further lineage-tracing experiments, using a Prox1-CreERT2: R26R reporter mouse line showed a venous origin for LECs. In recent years, advances in both imaging and development of novel reporter mouse and zebrafish models have allowed high resolution analysis of early lymphatic vascular development, and provided further convincing evidence that during early development nascent LECs proliferate in and migrate out from veins both in mouse and in zebrafish. Thus, Sabin’s hypothesis about the origins of the lymphatic vasculature seemed to be correct, some 100 years later.

However, there was also evidence to support Huntington and McClure’s hypothesis for development of the lymphatic vasculature from lymphangioblasts, arising independently of veins in the mesenchyme. Studies using quail/chick grafts suggested that LECs could be derived from the somatic mesoderm in embryonic wings, independently from endothelial cells. Interestingly, analyses of lymphatic vessels in turtle embryos, led van der Jagt to conclude that LECs originated from 2 sources, “one from the mesenchyme and the other from the venous tributaries.” Follow-up in the quail/chick model, along with study of Xenopus tadpoles and mouse embryos also provided evidence for a dual source of embryonic LECs, as both transdifferentiation of LECs from veins and lymphangioblast structures could be observed.

New data from Martinez-Corral et al further lend important support for a hybrid mechanism of lymphatic vascular development, where both venous-derived and nonvenous-derived

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LECs contribute to formation of the skin lymphatic capillaryplexus (Figure 1). Careful examination of the embryonic dermal vasculature revealed the existence of isolated cells that expressed LEC markers, such as Prox1, Nrp2, and Vegfr-3, but were physically separated from the rest of the developing lymphatic network. Intriguingly, such isolated LECs were most numerous in the lumbar region of the embryo. To tease out the origin of these cells, Martinez-Corral et al8 analyzed crosses of endothelial/hematopoietic-specific Tie2-Cre reporter strains, which should express GFP in all cells derived from Tie2-expressing precursors. As observed before,13 LECs from the jugular region had transdifferentiated from blood endothelial cells as they were positive for GFP. Surprisingly, however, the isolated LECs from lymphatic islands were negative for GFP, suggesting they did not arise from Tie2-expressing blood endothelial cell precursors. Quantification of such cells by FACS demonstrated an impressive increase in the number of GFP+ skin LECs during the period of rapid expansion of the dermal lymphatic vascular network, from E11 to E13, while at the same time the majority of blood endothelial cells were GFP−. Furthermore, in agreement with previous results,13 targeted inactivation of Prox1 in Tie2-expressing cells resulted in severe edema and depletion of the jugular lymphatic vasculature. However, in spite of this, disconnected lymphatic islands in the lumbar region were still present and maintained Prox1 expression, further strengthening the hypothesis of a nonvenous origin. Further lineage tracing using the Prox1-CreERT2/mTmG reporter mouse showed that induction of recombination between E12.5 and E15.5 generated composite GFP+/GFPneg reporter mouse showed that induction of recombination...
should provide a wealth of new information, which will increase our understanding of the lymphatic vasculature and, hopefully, improve its targeting for clinical applications.

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None.

References


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