PERSPECTIVE

Master and commander: continued expression of *Prox1* prevents the dedifferentiation of lymphatic endothelial cells

M. Gabriele Bixel and Ralf H. Adams¹

Max-Planck-Institute for Molecular Biomedicine, Department of Tissue Morphogenesis, and University of Münster, Faculty of Medicine, Münster D-48149, Germany

Cell differentiation occurs mostly during a specific developmental time window and is irreversible. The homeobox-containing transcription factor *Prox1* is a master regulator of lymphatic endothelial cell differentiation in the embryo. A study by Johnson et al. (pp 3282–3291) published in this issue of *Genes & Development* now shows that continued expression of *Prox1* is required to maintain lymphatic endothelial cell identity even in adult mice. These findings indicate that *Prox1* is essential for the differentiation and function of the lymphatic vasculature throughout life.

Endothelial cells (ECs) form two highly branched, tree-like tubular networks in the vertebrate body, the blood vasculature and the lymphatic vasculature, which have distinct functional roles and morphological features. Blood vessels carry circulating blood through arteries into extensive capillary beds, the sites where gases, nutrients, and waste products are exchanged, and back into veins. In contrast, lymphatic vessels form a blind-ended system that drains protein-rich liquid, the lymph, through a network of distal capillaries (also known as terminal or initial lymphatics), larger collecting ducts and, finally, the thoracic duct into the venous system (Fig. 1; Alitalo et al. 2005; Cueni and Detmar 2006; Adams and Alitalo 2007). While the ECs of blood vessels (BECs) are tightly sealed to each other by continuous junctions formed by VE-Cadherin and other adhesion molecules, the ECs of lymphatic capillaries have discontinuous button-like junctions surrounding small intercellular openings, which are thought to facilitate the entry of liquid and immune cells from the surrounding tissue (Dejana 2004; Baluk et al. 2007). Tightly associated pericytes form cell–cell contacts with BECs and thereby

stabilize blood vessel capillaries, reduce leakage, and prevent hemorrhaging, while terminal lymphatics are completely devoid of such supporting cells. Only lymphatic collecting ducts are sparsely covered by smooth muscle cells (SMCs), a different type of supporting cell in the vasculature, which promote slow flow with peristaltic contractions (Alitalo et al. 2005; Adams and Alitalo 2007). Numerous flap-like valves prevent the backflow of lymph toward the more distal lymphatic structures, and are essential for efficient drainage (Fig. 1). The specialization of blood vessels and lymphatic vessels and of the various subdomains within these endothelial networks are reflected by distinct gene expression profiles and signaling pathways (Fig. 2). For example, arterial ECs express a multitude of markers that are largely or entirely excluded from the venous or lymphatic endothelium (Lamont and Childs 2006; Adams and Alitalo 2007). The glycoprotein LYVE-1 is not only restricted to lymphatic ECs (LECs) but is also predominantly confined to the terminal capillaries within the lymphatic tree (Mäkinen et al. 2005; Baluk and McDonald 2008). Among the molecular markers that can be used to distinguish BECs and LECs, the homeobox-containing transcription factor *Prox1* (prospero-related homeobox 1) is of particular importance. *Prox1* is present in all nuclei throughout the lymphatic endothelium, and this pattern of expression is actually the earliest sign of lymphatic development when the first few LECs differentiate from BECs within a section of the cardinal vein (Wigle and Oliver 1999; Wigle et al. 2002).

Prox1—master regulator of LEC differentiation

Lineage tracing experiments have demonstrated that lymphatic ECs in the mouse are derived from venous ECs during embryonic development (Srinivasan et al. 2007). A cluster of ECs in the cardinal vein start to express *Prox1*, leave the vessel and migrate into the adjacent tissue and form the first primitive lymphatic structures (Wigle and Oliver 1999; Wigle et al. 2002). Signal-

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¹Corresponding author.

E-MAIL ralf.adams@mpi-muenster.mpg.de; FAX 49-251-70365-499.

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Figure 1. Morphological features of blood vessels and lymphatic vessels. (A) Schematic representation of the blood vessel network, which consists of arteries, capillaries and veins. Vascular SMCs cover larger calibre blood vessels, particularly arteries and veins, while pericytes tightly associate with the endothelium of capillary beds. (B) Blind-ended capillaries—i.e., initial lymphatics—enable the uptake of protein-rich liquid and cells into the lymphatic network. The unidirectional drainage of lymph is facilitated by flap-like valves and SMCs that are characteristic for collecting ducts. The flow of lymph is slow (dotted arrows), whereas circulation throughout blood vessel is generally fast despite significant local differences.

ing by vascular endothelial growth factor C (VEGF-C) and the receptor VEGFR3/Flt4, a receptor tyrosine kinase expressed by LECs, provides an essential chemoattractive signal for this first migration and is a critical regulator of subsequent lymphangiogenic growth (Veikkola et al. 2001; Karkkainen et al. 2004). Prox1 is not only required for VEGFR3 expression in these first lymphatic ECs but the transcription factor is a much more general master regulator of LEC differentiation. Lymphatic differentiation and markers such as LYVE-1 or the chemokine CCL21 are down-regulated and a lymphatic system fails to form in *Prox1*-deficient embryos (Wigle et al. 2002). Overexpression of Prox1 in cultured BECs up-regulates the expression of many LEC markers while many BEC-specific genes get suppressed (Hong et al. 2002; Petrova et al. 2002). Recently, the nuclear orphan receptor NR2F2 (nuclear receptor subfamily 2), also known as COUP-TFII, was identified as a coregulator of Prox1 in LEC differentiation (Lee et al. 2008). It is already known that NR2F2/COUP-TFII is an important regulator of venous BEC differentiation (Fig. 2; You et al. 2005), and its role in LECs may be a legacy of the venous origin of the lymphatic endothelium.

LECs are reprogrammed in the absence of Prox1

During organogenesis, the precursors of most cell types reach certain branch points at which their differentiation can be directed toward one or the other fate in response to changes in the gene expression profile, which are triggered in a cell-autonomous fashion by transcriptional regulators, by instructive signals from the local tissue environment, or a combination of both. A series of such fate decisions will determine the final outcome of a cell differentiation cascade and, typically, the result will be irreversible. The previously known data on Prox1 func-

tion indicated the transcription factor plays an essential but potentially transient role as a binary switch during LEC differentiation in the embryo (Wigle and Oliver 1999; Wigle et al. 2002). It was also clear that Prox1 expression in lymphatics persists into postnatal life, and a conspicuous down-regulation of Prox1 in the defective lymphatics of some mutant mouse models had been observed (Wilting et al. 2002; Odaka et al. 2006; Backhed et al. 2007; Baluk and McDonald 2008). However, the relevance of these findings remained unclear. In this issue of *Genes & Development*, a study led by Guillermo Oliver (Johnson et al. 2008) shows that the continued expression of Prox1 is required to maintain LEC differentiation throughout life. Down-regulation of Prox1 with inducible loss-of-function genetics in embryonic, postnatal, or adult mice leads to severe defects in the lymphatic vasculature and, persistently, mutant lymphatic vessels lose the expression of LEC markers and acquire ectopic markers characteristic for BECs. *Prox1* mutant lymphatic vessels also acquire morphological features characteristic for normal blood vessels but not lymphatics, such as the presence of blood cells, connection to the blood circulation, and coverage by pericyte-like cells. The typical button-like organization of junctions, visu-

Figure 2. Transcriptional program of EC differentiation. Fox and Ets family transcription factors and Erg (Ets-related gene) play major roles in the differentiation of angioblast precursors and the generation of ECs (Dejana et al. 2007). Fli-1 and members of the Hox and Sox families are other factors controlling the endothelial expression profile (Dejana et al. 2007). The Notch pathway and downstream basic helix-loop-helix (bHLH) proteins of the hairy/enhancer-of-split-related transcription factor family (named Hey and Hes in mammals) control the differentiation of arterial ECs (Fischer et al. 2004; Roca and Adams 2007). Contribution of the forkhead box transcription factors FOXC1 and FOXC2 to arterial differentiation has been reported (Hayashi and Kume 2008). Capillary beds acquire organ-specific specialization but the underlying regulatory mechanisms are largely unknown. Expression of NR2F2/COUP-TFII in the venous endothelium suppresses Notch pathway genes and arterial differentiation (You et al. 2005). COUP-TFII and Foxc2 contribute to LEC differentiation (Seo et al. 2006; Lee et al. 2008), a process that critically relies on the master regulator Prox1 (red). Without Prox1, LECs acquire BEC features (red arrow) but it is not clear whether these cells have a venous or intermediate, poorly specified identity.

alized with the junctional marker VE-Cadherin, is also lost in *Prox1* lymphatic vessels. However, a blood vessel-style zipper-like pattern is not obtained in this case, suggesting that the compromised vascular identity of mutant lymphatics is perhaps incompatible with normal EC–EC interactions. The key finding made by Johnson et al. (2008)—i.e., that the maintenance of lymphatic identity requires constant activity of *Prox1*—can be reproduced in vitro. siRNA-mediated knockdown of *Prox1* in primary human lymphatic ECs causes the loss of LEC markers and the ectopic expression of BEC markers.

LEC plasticity in health and disease

Endothelial tubules are thought to form fairly stable networks, and some larger vessels obviously stay in place for life. At the same time, ECs retain the capacity for growth and reorganization of the vessel network in response to altered tissue requirements, damage, or certain disease conditions. But this phenotypic plasticity is not known to involve the reprogramming of ECs, and therefore it is rather puzzling that LEC identity is so strictly coupled to *Prox1* expression. In the embryo, arterial and venous BEC identities appear to be interconvertible to some extent (Moyon et al. 2001; le Noble et al. 2004), which may permit the incorporation of ECs from pruned vessels into other parts of the remodeling vascular network. Whether or not reprogrammed LECs are generated under physiological conditions and incorporated into blood vessels remains open at this point and requires further investigation. Some evidence indicates that LECs can at least dedifferentiate in certain pathobiological processes. In angiosarcoma and Kaposi's sarcoma, which are malignant EC cancers, neoplastic cells express both BEC and LEC markers (Breiteneder-Geleff et al. 1999; Hong et al. 2004; Wang et al. 2004). In lymphoedema distichiasis, a hereditary syndrome in which lymphatic drainage is compromised as a consequence of mutations in the human *FOXC2* gene, lymphatic vessels in the lower limbs obtain ectopic SMCs, are filled with blood and lack valves (Fang et al. 2000; Kriederman et al. 2003; Petrova et al. 2004). Similar defects can be also seen in *Foxc2* mutant mice, and some evidence suggests that *Foxc2* may be downstream from *Prox1* in LECs (Petrova et al. 2004; Johnson et al. 2008). Expression of *Prox1* is down-regulated in the dilated and blood-filled intestinal lymphatics of mice that lack fasting-induced adipose factor (FIAF; also known as ANGPTL4) (Backhed et al. 2007). Altered expression or activity of *Prox1* may also play an important role in other mutant mouse models with lymphatic defects such as blood filling, SMC coverage, lack of valves, and shunts between LECs and BECs (Abtahian et al. 2003; Makinen et al. 2005; Foo et al. 2006), which requires further investigation. Conversely, it will be important to identify the upstream factors that control *Prox1* expression. Such a role has been attributed to interleukin 3, a chemokine that is produced by LECs but not BECs (Gröger et al. 2004), but additional inducers of lymphatic *Prox1* expression may exist. *Prox1* is a direct target of β -catenin/TCF signaling

in the intestine and promotes colorectal cancer progression (Petrova et al. 2008). The functional roles of the master regulator *Prox1* cover numerous physiological and pathological processes inside and outside the lymphatic system, which warrants further research and will surely unravel other exciting aspect of *Prox1* biology.

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