Endothelial Cell Differentiation by SOX17
Promoting the Tip Cell or Stalking Its Neighbor Instead?

Jermaine Goveia, Annalisa Zecchin, Francisco Morales Rodriguez, Stijn Moens, Peter Stapor, Peter Carmeliet

Vessel sprouting relies on the differentiation of endothelial cells (ECs) into a migratory tip cell leading at the forefront, proliferating stalk cells elongating the vessel stalk, and quiescent phalanx cells lining the perfused vessel. The tip versus stalk cell balance is under the control of vascular endothelial growth factor (VEGF) and Notch signaling, respectively. During recent years, the transcription factor SRY-related HMG box 17 (SOX17) has emerged as a regulator of arterial (at the expense of venous) EC specification, but its role in inducing the tip versus stalk EC behavior remained incompletely defined. In this issue of Circulation Research, Lee et al identified SOX17 as an inducer of the tip cell phenotype and showed that Notch signaling suppresses SOX17 levels to promote a stalk cell phenotype (Figure). However, using similar genetic mouse models, another recent study reported noncongruent findings. Can we explain these divergent interpretations and what are the possible implications of these results?

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Except for a brief period of embryonic vasculogenesis during which the primitive vascular plexus is established, tissues are vascularized by angiogenesis via formation of new vessel sprouts. VEGF and Notch are key orchestrators of the specification of ECs into migratory, sprout-guiding tip cells and proliferating, sprout-elongating stalk cells, respectively (Figure). VEGF, secreted by cells in response to hypoxia, induces tip cell formation and Delta-like 4 expression in ECs. Delta-like 4, a ligand of the Notch receptor, activates stalk cell–promoting Notch signaling in neighboring ECs to ensure that there is only a single tip cell followed by stalk cell neighbors. Intriguingly, tip and stalk cell phenotypes are fluidly interchangeable, and competition for the tip ensures that the most competitive EC leads the vessel sprout. Apart from VEGF and Notch, other genetic and even metabolic signals determine the tip versus stalk cell phenotype, but the nature of many of those signals still remains elusive. In this respect, the finding that SOX17 is a new signal orchestrating tip versus stalk cell behavior is exciting.

Lee et al provide several lines of evidence that SOX17 induces tip cell function. First, they show SOX17 expression in ECs at the vascular front of angiogenic capillary plexuses, a finding that hints at a role in tip cell formation. Second, Sox17-silenced ECs have decreased expression of Delta-like 4, VEGF receptor 2, angiopoietin-2, Platelet derived growth factor B-B, and other genes associated with the tip cell phenotype. Third, silencing of Sox17 impairs EC migration, formation of lamellipodia, and other characteristic features of endothelial tip cells. Fourth, Sox17 deletion in ECs from embryonic day 8.5 in Tie2-Cre×Sox17GFP/fl mice results in lethal vessel defects. Furthermore, tamoxifen-induced deletion of Sox17 in ECs after birth in VE-cadherin-CreER2×Sox17GFP/fl mice reduced vascular plexus outgrowth, vessel branching, and tip cell formation. And finally, EC-specific Sox17 overexpression induced vascular hypersprouting in both embryonic and postnatal angiogenesis. Thus, Sox17 overexpression promotes ECs to adopt a tip cell phenotype, whereas conversely a lack of Sox17 promotes stalk cell differentiation.

The SOX (SRY-related HMG box) family of proteins constitutes a group of 20 highly conserved transcription factors playing a pivotal role in the regulation of gene expression in various developmental processes. The group of SOX group F (SOXF) proteins, namely SOX7, SOX17 and SOX18, act in an overlapping manner to support the formation and integrity of the vascular system, as demonstrated by the severe cardiovascular defects displayed by knockout mouse embryos lacking either 1 (Sox7, Sox17) or 2 (Sox17 and Sox18) of these genes. The importance of SOX17 in inducing angiogenesis has also been highlighted in retinal and tumor angiogenesis. Interestingly, SOX transcription factors, including SOX17, interact with Notch signals to determine hemogenic and arterial specific expression of ECs. By using a combination of genetic and pharmacological loss- and gain-of-function approaches, Lee et al demonstrate that Notch suppresses SOX17 levels in ECs to promote a stalk cell phenotype.

Although these exciting insights advance our understanding of the fundamental mechanisms of vessel sprouting, they also introduce another level of complexity in the proposed model of SOX17 vascular regulation. Another recent study by Corada et al reported that SOX17 is an upstream, not downstream, regulator of Notch signaling in arterial differentiation. Furthermore, this group observed a vascular hypersprouting, not hyposprouting, phenotype upon Sox17 deletion in ECs. How can these apparently contradictory findings be reconciled? Although the precise underlying causes remain to be identified, some hypothetical reasons are discussed. First,
The findings by Lee et al and Corada et al also raise several outstanding questions. For instance, if SOX17 is a bona fide tip cell signal, is it then also capable of ensuring the competitiveness of ECs to reach the tip position in mosaic cell–cell competition assays in vivo and more importantly in vitro, as used in previous studies? SOX17 is preferentially expressed in arterial ECs, and silencing of SOX17 not only favors venous at the expense of arterial EC specification but also stimulates increased tip cell formation. Given that ECs are generally thought to sprout from veins, does this imply that deficiency of SOX17 then promote formation of tip cells after prior differentiation to venous subtypes? How are SOX17’s context-dependent functions regulated, such as arterial differentiation and tip–stalk cell differentiation? Which other transcriptional cofactors are involved and how are they regulated? Which signals upregulate SOX17 levels in tip cells? How does the interplay between Notch and SOX17 affect the dynamic process of tip and stalk cell differentiation?

Another outstanding question is whether SOX17 can become a target for angiogenic therapy and, if so, whether SOX17 should be blocked or activated to inhibit pathological angiogenesis. A previous study reported that EC-specific deficiency of SOX17 reduces vessel density while inducing vessel normalization in models of melanoma and lung cancer. These findings would lend support for the strategy to block SOX17 for inhibiting tumor angiogenesis. However, answering more conclusively the question whether SOX17 should be inhibited or stimulated to block pathological angiogenesis will require a better understanding of the contextual role of SOX17 in tip versus stalk cell–driven angiogenesis.

Sources of Funding

J. Goveia is a PhD student supported by a Bijzonder onderzoeksfonds (BOF) fellowship from the University of Leuven. S. Moens is supported by an Emmanuel Vanderschueren fellowship from the Flemish Association against Cancer (VLK). The work of P. Carmeliet is supported by a Federal Government Belgium grant (IUAP P7/03), long-term structural Methusalem funding by the Flemish Government, grants from the Research Foundation Flanders (FWO), the Foundation of Leducq Transatlantic Network (ARTEMIS), Foundation against cancer, an European Research Council (ERC) Advanced Research Grant (EU-ERC269073), and the AXA Research Fund.
Disclosures

None.

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doi: 10.1161/CIRCRESAHA.114.304234

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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