# 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.[1] The tip versus stalk cell balance is under the control of vascular endothelial growth factor (VEGF) and Notch signaling, respectively.[1] 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[2] 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.[3] Can we explain these divergent interpretations and what are the possible implications of these results?

**Article, see p 215**

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.[4] 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.[1] 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,[1,5] 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[2] 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*×*Sox17*$^{GFP/fl}$ mice results in lethal vessel defects. Furthermore, tamoxifen-induced deletion of Sox17 in ECs after birth in *VE-cadherin-CreER*$^{T2}$×*Sox17*$^{fl/fl}$ 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.[6,7] The importance of SOX17 in inducing angiogenesis has also been highlighted in retinal and tumor angiogenesis.[8,9] Interestingly, SOX transcription factors, including SOX17, interact with Notch signals to determine hemogenic and arterial specification of ECs.[3,10] By using a combination of genetic and pharmacological loss- and gain-of-function approaches, Lee et al[2] 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[3] 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,



From the Laboratory of Angiogenesis and Neurovascular link, Department of Oncology, University of Leuven, Leuven, Belgium (J.G., A.Z., F.M.R., S.M., P.S., P.C.); and Laboratory of Angiogenesis and Neurovascular link, Vesalius Research Center, VIB, Leuven, Belgium (J.G., A.Z., F.M., S.M., P.S., P.C.).

Correspondence to Peter Carmeliet, MD, PhD, Vesalius Research Center, VIB, KU Leuven, Campus Gasthuisberg, Herestraat 49-B912, Leuven B-3000, Belgium. E-mail peter.carmeliet@vib-kuleuven.be

**(*Circ Res*. 2014;115:205-207.)**



***Circulation Research*** **is available at http://circres.ahajournals.org**
**DOI: 10.1161/CIRCRESAHA.114.304234**

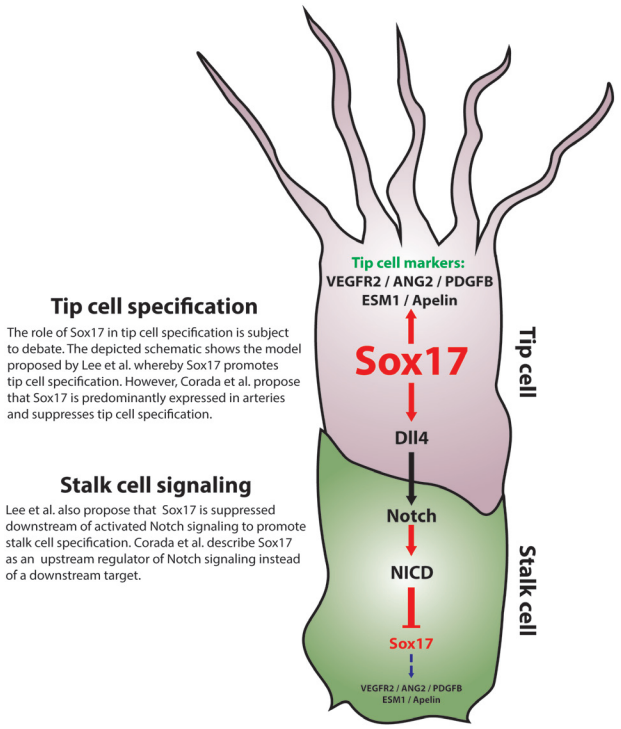


**Figure.** Scheme illustrating the proposed models of the mechanism of Sox17 in vessel sprouting according to Lee et al.[2] Sox17 plays a central role in the induction of tip cell differentiation. Expression of Sox17 in tip cells induces tip cell behavior, whereas Notch signaling downregulates Sox17 in endothelial cells to induce stalk cell specification. These results contradict with previous observations from Corada et al[3] (discussed in insets) who report that Sox17 is a tip cell suppressor, upstream, not downstream, of Notch signaling. Red arrows indicate novel regulatory pathways dissected by Lee et al.[2] Ang2 indicates angiopoietin-2; Dll4, Delta-like 4; ESM1, endothelial cell-specific molecule 1; NICD, Notch intracellular domain; and VEGFR2, vascular endothelial growth factor receptor 2.

both groups used exactly the same genetically engineered knockout mouse models, thus excluding the possibility that a different sort of genetic manipulation caused the divergence. Nonetheless, slight alterations in genetic backgrounds of the transgenic models used could affect the phenotype.

Second, the postnatal mouse retina model of angiogenesis is a dynamic model, and EC branching, plexus outgrowth, and arteriovenous differentiation occur in a short timeframe. Therefore, differences in tamoxifen administration schemes used by both groups (administration route, dose, and duration) possibly evoked differences in recombination efficiency and vessel remodeling dynamics. At least from a theoretical perspective, a deletion of a proangiogenic gene in only a fraction of ECs might initially impair vessel outgrowth, but resultant ischemia because of hypoperfusion might lead to vessel overgrowth by nonrecombined wild-type ECs in an effort to compensate for the vascular defect. Compensation by nontransgenic ECs has been previously shown to rescue vascular defects by mutant ECs with a competitive angiogenic disadvantage.[11] Documenting the in situ recombination efficiency of ECs in a particular vascular bed might thus aid to understand the phenotype under investigation.

Another discrepancy is the relationship between Notch and SOX17. Lee et al[2] provide evidence that Notch signaling downregulates SOX17 expression at the post-transcriptional level to promote a stalk cell phenotype, whereas Corada et al[3] report that Notch signaling is regulated by SOX17 (Figure). Although these divergent findings require further reconciliation, it is noteworthy that SOX transcription factor function is dependent on the formation of complexes with interacting partners. Thus, the type and level of activity of SOX factors might theoretically vary depending on the availability and the sort of its binding partners in distinct EC subtypes. Also, the levels of SOX17 might influence its association with additional or other types of partners. Nevertheless, beyond the apparent contradictions and contextual effects of SOX17, the findings by Lee et al[2] and Corada et al[3] seem to direct toward an intriguing model that defines SOX17 as an integral part of Notch signaling in the vascular biology governing EC specification.

The findings by Lee et al[2] and Corada et al[3] 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 vitro and more importantly in vivo, as used in previous studies?[12] 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.[3] 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.[8] 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 onderzoeksfond (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.

## References


1. Potente M, Gerhardt H, Carmeliet P. Basic and therapeutic aspects of angiogenesis. *Cell*. 2011;146:873–887.
2. Lee SH, Lee S, Yang H, Song S, Kim K, Saunders TL, Yoon JK, Koh GY, Kim I. Notch pathway targets proangiogenic regulator Sox17 to restrict angiogenesis. *Circ Res*. 2014;115:215–226.
3. Corada M, Orsenigo F, Morini MF, Pitulescu ME, Bhat G, Nyqvist D, Breviario F, Conti V, Briot A, Iruela-Arispe ML, Adams RH, Dejana E. Sox17 is indispensable for acquisition and maintenance of arterial identity. *Nat Commun*. 2013;4:2609.
4. Eichmann A, Yuan L, Moyon D, Lenoble F, Pardanaud L, Breant C. Vascular development: from precursor cells to branched arterial and venous networks. *Int J Dev Biol*. 2005;49:259–267.
5. De Bock K, Georgiadou M, Carmeliet P. Role of endothelial cell metabolism in vessel sprouting. *Cell Metab*. 2013;18:634–647.
6. Wat MJ, Beck TF, Hernández-García A, et al. Mouse model reveals the role of SOX7 in the development of congenital diaphragmatic hernia associated with recurrent deletions of 8p23.1. *Hum Mol Genet*. 2012;21:4115–4125.
7. Sakamoto Y, Hara K, Kanai-Azuma M, Matsui T, Miura Y, Tsunekawa N, Kurohmaru M, Saijoh Y, Koopman P, Kanai Y. Redundant roles of Sox17 and Sox18 in early cardiovascular development of mouse embryos. *Biochem Biophys Res Commun*. 2007;360:539–544.
8. Yang H, Lee S, Lee S, Kim K, Yang Y, Kim JH, Adams RH, Wells JM, Morrison SJ, Koh GY, Kim I. Sox17 promotes tumor angiogenesis and destabilizes tumor vessels in mice. *J Clin Invest*. 2013;123:418–431.
9. Ye X, Wang Y, Cahill H, Yu M, Badea TC, Smallwood PM, Peachey NS, Nathans J. Norrin, frizzled-4, and Lrp5 signaling in endothelial cells controls a genetic program for retinal vascularization. *Cell*. 2009;139:285–298.
10. Clarke RL, Yzaguirre AD, Yashiro-Ohtani Y, Bondue A, Blanpain C, Pear WS, Speck NA, Keller G. The expression of Sox17 identifies and regulates haemogenic endothelium. *Nat Cell Biol*. 2013;15:502–510.
11. Schoors S, Cantelmo AR, Georgiadou M, Stapor P, Wang X, Quaegebeur A, Cauwenberghs S, Wong BW, Bifari F, Decimo I, Schoonjans L, De Bock K, Dewerchin M, Carmeliet P. Incomplete and transitory decrease of glycolysis: a new paradigm for anti-angiogenic therapy? *Cell Cycle*. 2014;13:16–22.
12. De Bock K, Georgiadou M, Schoors S, et al. Role of PFKFB3-driven glycolysis in vessel sprouting. *Cell*. 2013;154:651–663.


KEY WORDS: Editorials ■ receptors, Notch