Developmental Cell
Previews

Semaphorin Signals on the Road of Endothelial Tip Cells

Luca Tamagnone[1,*] and Massimiliano Mazzone[2,3]
[1]Institute for Cancer Research (IRCC), Laboratory of Cell Biology, University of Torino Medical School, 10060 Candiolo, Italy
[2]Laboratory of Molecular Oncology and Angiogenesis, Vesalius Research Center, VIB, B-3000 Leuven, Belgium
[3]Laboratory of Molecular Oncology and Angiogenesis, Vesalius Research Center, K.U.Leuven, B-3000 Leuven, Belgium
*Correspondence: luca.tamagnone@ircc.it
DOI 10.1016/j.devcel.2011.07.017

Blood vessels sprout toward avascular tissue in response to attractive proangiogenic factors. However, restricting signals are also required to coordinate the behavior of endothelial cells assembling the vascular network. Semaphorin cues are at the crossroad of this traffic, and they direct the behavior of endothelial tip cells leading the way.

Blood vessels and nerves share a range of common guiding signals, which include semaphorins and their receptors, plexins, and neuropilins. For instance, secreted semaphorins repel growth cones of extending axons, as well as endothelial tip cells, at the forefront of sprouting vessels (Adams and Eichmann, 2010). In the current issue of *Developmental Cell*, Zygmunt and coworkers illustrate the role of semaphorin/plexin signaling in the vascular development of zebrafish (Zygmunt et al., 2011). In this vertebrate species, endothelial cells (ECs) in the ventral side of the aortic tube are induced to sprout dorsally in response to a gradient of vascular endothelial growth factor (VEGF), and they form bilateral orthogonal vessels slithering between the somites, called intersomitic vessels (ISV). ISV elongation is thought to be guided by inhibitory cues, preventing inappropriate sprouting. A similar process has been described in mouse vascular development and has been found to depend on a graded distribution of Sema3E released by somites and on the expression of its receptor PlexinD1 in ECs (Gu et al., 2005). In zebrafish, endothelial PlexinD1 is required, too (Torres-Vázquez et al., 2004), but the homologous family member Sema3A is the actual somitic signal restricting ISV elongation (suggesting the involvement of the obligate coreceptor Neuropilin1), while Sema3E has a different distribution and regulatory function (Lamont et al., 2009). Thus, the semaphorin "code" controlling vascular development in mouse and zebrafish is not entirely overlapping.

The function of semaphorins in this process has so far been explained by a repelling activity, topographically restricting the migration of ECs (Figure 1A). However, the new study by Zygmunt and coworkers highlights a distinct mechanism by which semaphorins can determine EC behavior. In particular, strong Sema3A/PlexinD1 signaling in aortic ECs adjacent to somites results in autocrine secretion of sFlt1, a splice variant of VEGFR1 acting as a soluble trap for VEGF, with the final effect of blocking vessel sprouting (Figure 1A′). Instead, in the intersomitic regions (containing lower semaphorin levels), ECs are allowed to respond to VEGF, assuming a "tip" cell behavior at the lead of vascular sprouts. Thus, according to this study, Sema3A/PlexinD1 signaling in ECs favors the quiescent, nonmotile behavior of so called "phalanx" cells (Carmeliet et al., 2009). Upon blocking this pathway, many more ECs actively migrate as tip cells in response to VEGF and form excessive aberrant sprouts. In light of this new mechanistic interpretation, the previous study on Sema3E/PlexinD1 signaling in mouse aortic development (Gu et al., 2005) might be similarly explained. So far, Zygmunt and coworkers show that PlexinD1 signaling induces sFlt1 expression in cultured human EC; however, the potential involvement of this axis in mammals remains to be tested.

Interestingly, two independent studies have recently shown that, in a distinct model of postnatal mouse retina vascularization, VEGF signaling induces PlexinD1 expression at the tip of elongating vessels, whereas Sema3E is evenly expressed in the neuronal layer underlying the developing vasculature (Fukushima et al., 2011; Kim et al., 2011). Fukushima and coworkers found that antagonistic Sema3E and VEGF signals provide balanced control of vessel sprouting and retinal vascularization during development and ischemic retinopathy (Fukushima et al., 2011). Under normal conditions, Sema3E prevents retinal layer invasion by endothelial cells, while in ischemic retinas it counteracts VEGF-induced extraretinal vascular outgrowth. Blockade of Sema3E/PlexinD1 signaling after the retinal vascular network has been established results in endothelial tip cells with longer and more abundant filopodia. As a consequence, the vascular network gets denser, likely due to extensive fusion of tip cells within neighboring vessels; the opposite is seen upon VEGF inhibition. Intriguingly, if Sema3E signaling is blocked at the onset of retinal vascular development, the overall vascular density is instead decreased, with an uneven growing front characterized by advanced sprouting tip cells in one area and unusually short sprouts in its immediate vicinity.

These data suggest a developmental function for Sema3E beyond EC repulsion, potentially tweaking other signals controlling vessel network assembly. Kim and coworkers provided mechanistic evidence for this by showing that Sema3E/PlexinD1 signaling in the tip cells of retinal vessels not only mediates repulsion but also counteracts a VEGF-dependent pathway leading to expression of the Notch ligand, Dll4 (Figure 1B) (Kim et al., 2011). Notch activation in adjacent cells mediates "lateral inhibition" that hinders

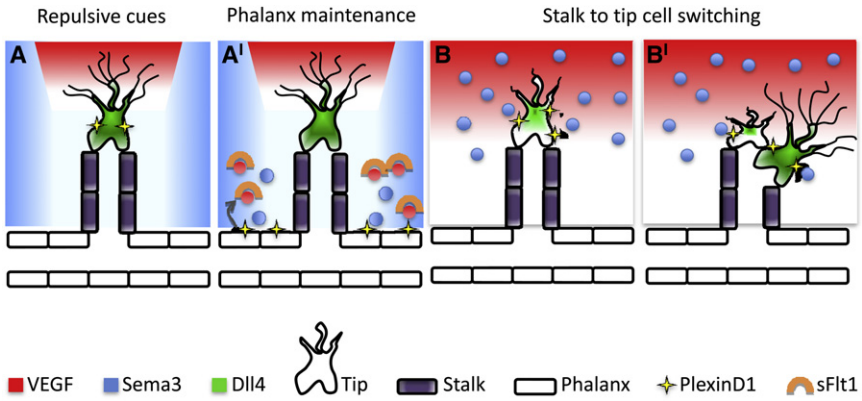

Figure 1. Mechanisms of Sema-PlexinD1 Signaling in Vascular Development
In addition to its initial role as repulsive cue restricting ISV elongation (A), Sema-PlexinD1 signaling elicits autocrine release of sFlt1 in the quiescent phalanx cells of developing aorta; by titrating free VEGF in the surroundings, sFlt1 prevents phalanx cells from sprouting (A′). In retinal vessel development, PlexinD1 expression is induced by VEGF in tip cells, where it counteracts VEGF-induced projection of endothelial filopodia and thus refines vascular pathfinding (B). In parallel, activation of PlexinD1 by Sema3E diminishes Dll4 expression in tip cells. This drop in Dll4 may cause the current tip cell to lose its competitive edge over a neighboring stalk cell, which could then become a tip cell instead (B′).

migration and determines the "stalk" cell phenotype (reviewed by Carmeliet et al., 2009). Therefore, Sema3E/PlexinD1 signaling could provide feedback control in tip cells by preventing excess inhibition of the adjacent stalk cells. In fact, when this mechanism is genetically inactivated during development, Notch-dependent lateral inhibition is further increased, and tip cell branching out of the primary sprout is abrogated, resulting in a poorly ramified retinal vessel network. Conversely, exaggerated Sema3E signaling in tip cells suppresses Notch-dependent lateral inhibition, leading to loss of hierarchy in vessel sprouts, unrestricted VEGF signaling, and vascular disorganization.

In sum, Sema3E signaling in endothelial tip cells that express PlexinD1 antagonizes VEGF-induced filopodia extension, thus providing a negative feedback loop to prevent excessive vessel sprouting. In parallel, Sema3E-PlexinD1 signaling in tip cells unleashes adjacent stalk cells from VEGF-induced lateral inhibition and thus promotes a dynamic switch between stalk and tip cell phenotypes (Figure 1B′). Notably, Zygmunt and coworkers show that the reduced sFlt1 expression in zebrafish PlexinD1 mutants leads to increased VEGF signaling and a Notch-independent phalanx-to-tip cell transition. Conversely, the release of sFlt1 by PlexinD1-competent phalanx cells might represent an indirect additional mechanism whereby Sema3E could guide ISV sprouting by blocking free VEGF in the somite (Chappell et al., 2009).

These new findings also raise new questions. Which pathways downstream of Sema-PlexinD1 signaling modulate the relative abundance of the splicing variant sFlt1? Do the mechanisms studied in the mouse retina and in zebrafish aorta hold true in additional tissue compartments? Which specific determinants account for the differential relevance of Notch signaling in PlexinD1-mediated inhibition of tip cells, seen in mouse retina but not zebrafish aorta? Given the multiplicity of pathways controlled by PlexinD1 signaling, the distribution of different ligands (including Sema3A) and coreceptors (such as Neuropilin-1) may determine the specific response of ECs to PlexinD1 signaling in different tissues. Finally, these data open up potential therapeutic perspectives based on targeting Sema-PlexinD1 signaling in pathological postnatal angiogenesis without affecting established normal vessels. Notably, we have shown that while Sema3E inhibits tumor vessel formation, it is also responsible for an independent direct activity promoting cancer cell invasiveness and metastatic spreading (Casazza et al., 2010). Thus, further studies are required to dissect these two effects and define the therapeutic potential of Sema3E in human disease.

REFERENCES

Adams, R.H., and Eichmann, A. (2010). Cold Spring Harb. Perspect. Biol. *2*, a001875.

Carmeliet, P., De Smet, F., Loges, S., and Mazzone, M. (2009). Nat Rev. Clin. Oncol. *6*, 315–326.

Casazza, A., Finisguerra, V., Capparuccia, L., Camperi, A., Swiercz, J.M., Rizzolio, S., Rolny, C., Christensen, C., Bertotti, A., Sarotto, I., et al. (2010). J. Clin. Invest. *120*, 2684–2698.

Chappell, J.C., Taylor, S.M., Ferrara, N., and Bautch, V.L. (2009). Dev. Cell *17*, 377–386.

Fukushima, Y., Okada, M., Kataoka, H., Hirashima, M., Yoshida, Y., Mann, F., Gomi, F., Nishida, K., Nishikawa, S., and Uemura, A. (2011). J. Clin. Invest. *121*, 1974–1985.

Gu, C., Yoshida, Y., Livet, J., Reimert, D.V., Mann, F., Merte, J., Henderson, C.E., Jessell, T.M., Kolodkin, A.L., and Ginty, D.D. (2005). Science *307*, 265–268.

Kim, J., Oh, W.J., Gaiano, N., Yoshida, Y., and Gu, C. (2011). Genes Dev. *25*, 1399–1411.

Lamont, R.E., Lamont, E.J., and Childs, S.J. (2009). Dev. Biol. *331*, 199–209.

Torres-Vázquez, J., Gitler, A.D., Fraser, S.D., Berk, J.D., Pham, V.N., Fishman, M.C., Childs, S., Epstein, J.A., Weinstein, B.M., and Weinstein, B.M. (2004). Dev. Cell *7*, 117–123.

Zygmunt, T., Gay, C.M., Blondelle, J., Singh, M.K., Flaherty, K.M., Means, P.C., Herwig, L., Krudewig, A., Belting, H., Affolter, M., et al. (2011). Dev. Cell *21*, this issue, 301–314.