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VEGF tips its hand in angiogenesis

In 2003, Gerhardt et al. described how vascular endothelial growth factor guides the growth of new blood vessels.

Vascular endothelial growth factor (VEGF) has many different effects on endothelial cells, stimulating, for example, their proliferation, differentiation, and migration. In the early 2000s, however, it remained unclear how all of these activities were coordinated to induce the formation of complex vascular networks. But that began to change in 2003, when Christer Betsholtz and colleagues revealed that VEGF coordinates the growth of new blood vessels by guiding the migration of specialized, filopodia-extending cells at the tips of vascular sprouts, while stimulating the proliferation of cells further back in the sprout stalk (1).

Betsholtz's lab was particularly interested in the vascularization of the mammalian retina. A small network of blood vessels initially forms in the center of the organ and then expands outwards in a stereotypic pattern guided by an underlying layer of astrocytes (2, 3). Importantly, the retina's flat structure allowed Betsholtz and colleagues, led by postdoc Holger Gerhardt, to follow the entire angiogenic process using high-resolution confocal microscopy. "When we stained mouse retinas for endothelial cells, we realized that something interesting was going on at the tips of sprouting blood vessels," recalls Betsholtz, who now works at Uppsala University and the Karolinska Institute in Sweden. "There were single cells at each tip that extended long filopodia toward the periphery in the direction of the sprouting."

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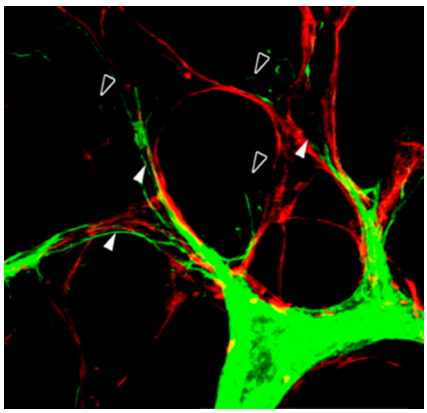
These tip cells were clearly distinct from what the researchers named stalk cells, the endothelial cells making up the bulk of the sprouting blood vessel. The tip cell's filopodia, which were sometimes more than 100 µm long, seemed to stretch out and attach to nearby astrocytes. When Gerhardt et al. perturbed the astrocytes' organization, the vascular tip cells reoriented their filopodia accordingly, suggesting that these actin-rich protrusions might guide growing sprouts along their astrocytic path. "We thought that the tip cells might be similar to axonal growth cones, which also extend filopodia," Betsholtz explains. "The tip cells might use their filopodia to sense guidance cues and lead the migration of elongating angiogenic sprouts."

VEGF-A was a good candidate to be the guidance cue sensed by tip cell filopodia. Gerhardt et al. found that the growth factor was expressed by retinal astrocytes in a graded pattern, with the highest expression occurring in the outer, hypoxic regions of the retina yet to be supplied by blood vessels. When Gerhardt and colleagues inhibited VEGF-A, or its receptor VEGFR2, tip cells retracted their filopodia and the growth of blood vessels toward the retinal periphery was slowed. When the researchers overexpressed VEGF-A, endothelial cells formed additional filopodia but, because the graded pattern of VEGF-A expression was disrupted, these protrusions were oriented randomly, and directed migration toward the periphery was also impaired.

Specialized tip cells therefore use long filopodia to guide the migration of vascular sprouts along a gradient of VEGF-A. But the stalk cells immediately behind the sprout tip respond to VEGF-A differently, Gerhardt et al. found. VEGF-A induced these cells to proliferate, a response that depended on the growth factor's absolute concentration, rather than on its graded expression pattern. The researchers therefore concluded that a balance of tip cell migration and stalk cell proliferation is required to coordinate angiogenic sprouting. "The great thing about this study was its power to explain complex pattern formation with simple principles," says *JCB* academic editor Erik Sahai.

Shortly after publishing their findings, Gerhardt left Betsholtz's lab to set up his own group, which is now based at the Max Delbrück Center for Molecular Medicine in Berlin. Gerhardt and Betsholtz continued to collaborate, however, and, in 2007, they, along with several other groups, demonstrated that Delta-Notch signaling regulates the formation of tip cells (4). Inhibiting the Notch receptor increases the number of tip cells and enhances sprouting, whereas activating the Notch pathway inhibits tip cell specification and reduces vessel branching. The role of these cells in angiogenesis has since been confirmed in a variety of organs besides the retina. "Nowadays, at every angiogenesis conference, people talk about tip cells and stalk cells, and how they are affected by all of the different growth factors that regulate angiogenesis," Betsholtz says.



During retinal angiogenesis, an endothelial tip cell (green) extends numerous filopodia towards VEGF-A–expressing astrocytes (red).

One outstanding question in the field, however, is how angiogenesis differs in different organs. "VEGF and Notch come into play in every organ," Betsholtz explains. "But, in the end, the vessels become very different in their anatomy and have distinct barrier functions. How is this brought about? What are the key molecules that determine the vasculature's organotypic features?"

1. Gerhardt, H., et al. 2003. *J. Cell Biol.* 161:1163–1177.
2. Stone, J., and Z. Dreher. 1987. *J. Comp. Neurol.* 255:35–49.
3. Fruttiger, M., et al. 1996. *Neuron*. 17:1117–1131.
4. Hellström, M., et al. 2007. *Nature*. 445:776–780.