The Unexpected Brains Behind Blood Vessel Growth

Two of the hottest fields in developmental biology—neural guidance and angiogenesis—are beginning to merge as scientists find that similar proteins control both processes

Whether in San Francisco or Singapore, almost everyone knows what the colors on a traffic light mean. But how did red, green, and yellow get chosen? It turns out railroad signals were already using these colors to guide trains. And the railroad industry may have gotten the idea from the electrical industry, which apparently used red to show that a motor was stopped and green to signal that it was running. When something works, why not use it more than once?

Evolution follows that principle too, as researchers studying the growth of blood vessels and nervous systems are beginning to appreciate. Scientists probing the development of the veins, arteries, and capillaries that guide nutrients and oxygen to cells are finding more and more evidence that the genes and proteins that were first discovered to guide growing nerve cells also direct blood vessels.

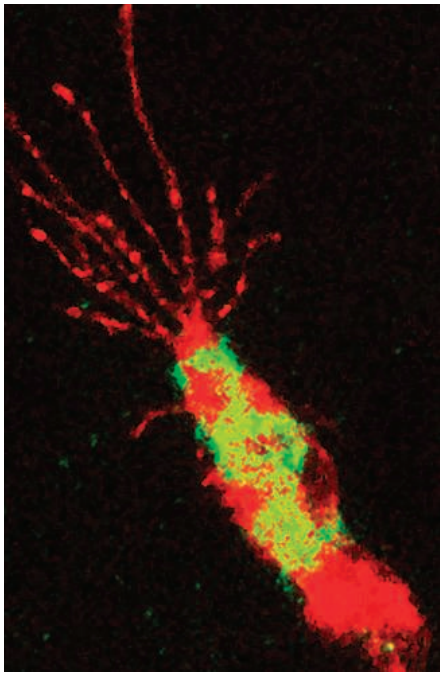
Decades of work by neuroscientists detailing the complex interactions of those cues is now giving researchers who study angiogenesis—the growth of blood vessels—a boost in their understanding of the vascular system. "Neurobiology has made an immeasurable contribution to angiogenesis," says David Anderson of the California Institute of Technology in Pasadena, who noticed some of the first overlaps. "All the insights we've gained from studying these signaling systems in the nervous system have put us in a much better position to understand how they work in the vascular system."

At the same time, one of the most powerful triggers of blood vessel growth, a protein called vascular endothelial growth factor (VEGF), is turning up in nerve cells and may play a key role in keeping them healthy and alive. "There is remarkable overlap in the use of these [signaling] systems," says David Ginty of Johns Hopkins University in Baltimore, Maryland.

These insights not only are inspiring a new respect for the complexity and precision of growing blood vessels, but they also have potential medical implications. Animal trials suggest that VEGF is a potential weapon against amyotrophic lateral sclerosis (ALS), an incurable disease that attacks nerves and gradually paralyzes its victims. And for those trying to control the growth of blood vessels—either to stop them from supporting cancerous tumors or to help them regrow after illness or injury—the nerve proteins offer a wealth of new targets to manipulate.

The tipping cell

One of the first signs of flirtation between the two fields came in 1998: Michael Klagsbrun of Children's Hospital in Boston and his colleagues reported in *Cell* that neuropilin, a cell surface protein originally identified as a receptor for a signal that guides growing nerves, also responds to VEGF (*Science*, 27 March 1998, p. 2042). Klagsbrun's observation "was an amazing discovery," Ginty says, although in hindsight it makes perfect sense, because both the blood vessel and nervous systems are "vast networks of complicated connections." Later that year, Anderson and his colleagues reported that another set of neuronal guidance molecules, cell surface proteins called Ephrin B2 and EphB4, were also present in the developing vascular system.



Looking for direction. The end of a developing blood vessel sends out sensory tentacles that resemble the growth cones of axons.

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Before these new observations, blood vessels were largely thought to form along a path of least resistance, without much active guidance. But the recent work paints a subtler picture, in which guidance molecules provide precise attractive and repulsive cues to specific growing vessels, notes Christer Betsholtz of the Karolinska Institute in Stockholm, Sweden. Work by Betsholtz and his colleagues revealed some of the first evidence for that precision. They showed in 2003 that specialized cells at the tip of developing blood vessels are attracted by slight changes in the concentration of VEGF. That reminded many biologists of what they see at the front of extending axons: the long extensions of a nerve cell that reach out and connect with other cells. "There are certainly some differences," says Ruediger Klein of the Max Planck Institute of Neurobiology in Martinsried, Germany, "but if you look at the pictures [from Betsholtz], the tip cells look very much like an axon's growth cone, extending and sensing the environment and responding to cues."

And the tip cells seem to respond to at least some of the same cues as growth cones. Last November in *Nature*, a group led by Anne Eichmann of the College of France in Paris described how those tips respond to netrins, a family of secreted proteins that help attract some axons and repel others during the formation of the spinal cord. How nerve cells react to netrins depends on which receptors they express, and, the new work shows, blood vessels can also react in different ways to the chemicals. Eichmann, with Peter Carmeliet of the University of Leuven in Belgium and Mark Tessier-Lavigne of Stanford University in California and their colleagues, reported that the gene for one of the previously identified netrin receptors, called *Unc5b*, is expressed in the tip cells of developing blood vessels. When the team created mice and zebrafish that made a faulty version of UNC5B, the vascular system of the mutant animals had far more sprouts and branches than normal—suggesting that the tip cells were impervious to a "stay away" signal from netrins. Indeed, the team subsequently showed that Netrin 1 causes the sprouts of rat blood vessels growing in culture to retract.

But that is not the whole story. In a paper published nearly simultaneously in the *Proceedings of the National Academy of Sciences*, Dean Li and his colleagues at the University of Utah, Salt Lake City, showed that Netrin 1 can also encourage the growth of new blood vessels, suggesting that the molecule may reprise its sometimes attractive, sometimes repellent role in the vascular system.

Eichmann and her colleagues have come across hints of other roles for netrins and their receptors in blood vessel development. They found that the UNC5B receptor is

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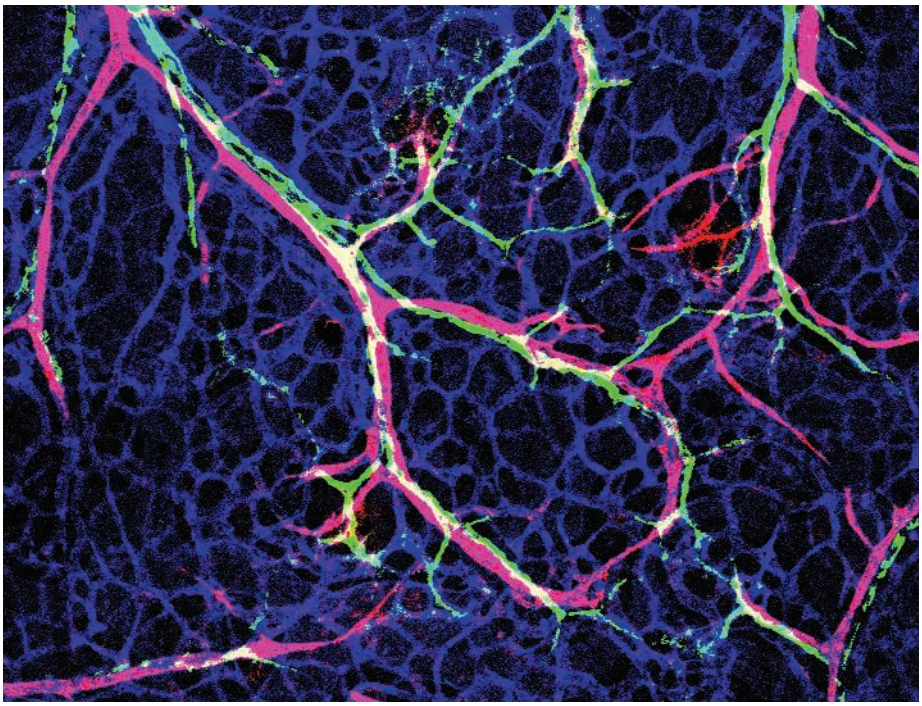
widely expressed in arteries, which deliver oxygen-rich blood to tissues, but it is apparently absent in veins, which return oxygen-depleted blood to the heart.

The early work on ephrin and Eph molecules from Anderson and his colleagues had showed a similar pattern. In their 1998 paper that established some of the first links between neuronal guidance and angiogenesis, the team showed that Ephrin B2 is expressed in arteries but not veins. Conversely, the ephrin receptor called EphB4 is expressed in veins but not arteries. These data were the first sign that arteries and veins are molecularly distinct at the earliest stages of development. Klein and his group confirmed that finding several months later and showed that the proteins could prompt the growth of new capillaries.



Follow me. In developing chick skin, arteries (red) align closely with nerves (green).

CREDIT: Y. MUKOUYAMA AND D. ANDERSON/CALIFORNIA INSTITUTE OF TECHNOLOGY

Anderson and his colleagues found another bond between developing nerves and blood vessels. They showed that in the skin of developing chicks, arteries are guided in part by the development of nerves, whereas veins are not. They also studied mice lacking Semaphorin3A, one of the proteins that neuropilins recognize. These animals develop badly misdirected nerves, and their developing arteries followed the deviant paths of the nerves, providing more evidence that the systems are closely intertwined.

That observation is consistent with work on semaphorins by two other groups, one led by Ginty and the other by Brant Weinstein of the National Institute of Child Health and Human Development in Bethesda, Maryland. Each showed last year that semaphorins keep developing blood vessels on the straight and narrow. According to their research, zebrafish and mice lacking semaphorins or their receptors develop strikingly disorganized vessels.

A role in blood vessel growth for a fourth category of neuronal guidance molecules—the Slit proteins and their Robo receptors—may be emerging as well. In 2003, Jian-Guo Geng of the Shanghai Institutes for Biological Sciences at the Chinese Academy of Sciences in Shanghai and his colleagues reported that a wide variety of tumor cells produce a protein called Slit2, and that endothelial cells, the precursors of blood vessels, express the receptor Robo1. They suspect that the tumor cells might be using Slit proteins to attract new blood vessels to the growing tumor tissue. And in October, Roy Bicknell of Oxford University and his colleagues reported evidence in the *FASEB Journal* that a newly identified Robo receptor, which they call Robo4, is present in areas where new blood vessels are forming.

Receptive nerves

Neuroscientists are also learning from angiogenesis researchers. VEGF, the classic trigger of blood vessel growth, is showing up more and more in studies of nerve growth and development. The first clues to VEGF's neuronal role came from experiments in Carmeliet's lab at the University of Leuven in Belgium. To sort out some of the multiple roles VEGF plays in vascular development, Carmeliet and his colleagues created several strains of mutant mice that carried slightly altered versions of the protein. The mice seemed to develop normally but became ill as adults. "To our surprise, we found that they had motor neuron degeneration similar to that seen in ALS," says Carmeliet.

Normally, VEGF is expressed in response to low oxygen levels—it attracts new blood vessels to tissues that are short of oxygen. Carmeliet's mice carry a mutation that prevents that oxygen-dependent increase in expression, suggesting that perhaps a lack of VEGF leaves nerves vulnerable to hypoxia. Indeed, in studies of nerves in culture, introducing VEGF seemed to help the cells survive stressful conditions such as low oxygen or serum deprivations.

There are early hints that VEGF might play a role in some human ALS cases as well. In a study of 2000 people in England and Sweden, Carmeliet and his colleagues found that those carrying a certain version of the VEGF gene, one which seems to lower its overall production, were 1.8 times more likely to develop ALS than the general population.

Carmeliet and his colleagues have tested in animal models of ALS whether increasing production of VEGF combats the condition. In one rodent trial, they injected into muscles a rabies virus, which homes in on and infects nerve cells, modified to churn out VEGF. The mice that received the virus took longer to develop ALS-like symptoms and survived longer than their untreated counterparts. Working with a rat model of ALS, the team has also injected the VEGF protein directly into the cerebral fluid and documented similar benefits. The team is now preparing human trials, Carmeliet says, which could be under way within 2 years.

Angiogenesis researchers are hoping that the molecules that originally held the promise of regrowing severed or damaged nerves may pay off in another clinical area as well: the fight against cancer. These researchers have been attempting to fight tumors by cutting off their blood supply—essentially starving them to death. The finding that neural guidance molecules influence normal blood vessel growth has suggested a wealth of potential new targets, says Tessier-Lavigne: "There is every reason to believe [these molecules] will regulate pathological angiogenesis as well."

The discoveries in both fields may have even wider impact. Eichmann and her colleagues have shown that mice lacking neuropilin-2 have defects in their lymph systems. Similarly, in the 1 February issue of *Genes and Development*, Klein and his colleagues describe how mice lacking Ephrin B2 develop major defects in their lymphatic systems as well. Nature, it seems, has made the most of a good idea.

–GRETCHEN VOGEL

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