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A "Repulsive" Protein Guides Blood Vessel Development

In a developing embryo, the growth of nerves cannot outpace the establishment of life-giving blood vessels. Now, researchers have found that a protein intimately involved in blood vessel patterning actually belongs to a family of proteins known to guide neural development.

The researchers said the studies provide more evidence of communication between developing nerves and blood vessels. Understanding how those networks talk to each other could help researchers devise methods to prevent blood vessel growth in tumors selectively - an approach to cancer treatment known as anti-angiogenesis.

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- David D. Ginty

The research team, which included Howard Hughes Medical Institute investigators David D. Ginty and Thomas M. Jessell, published its findings November 18, 2004, in *ScienceExpress*, the early online version of the journal *Science*. Co-first authors of the paper were Chenghua Gu in Ginty's laboratory at The Johns Hopkins University School of Medicine, and Yutaka Yoshida in Jessell's laboratory at Columbia University.

In their experiments, the researchers explored the roles of two proteins involved in vascular development. One of the molecules, Semaphorin 3E (Sema3E), is a member of a family of protein signals that guides the growth of nerve cells. The other protein, plexin-D1, is a receptor protein that nestles in the membranes of growing cells and responds to external signaling proteins.

Ginty said that before the current study, plexin-D1 was known to be important for vascular development, but the specific signal to which it

responded was a mystery. The molecule was also considered an important receptor in nerve cell development, and for that reason Jessell's laboratory was actively investigating plexin-D1.

Studies by Ginty and others, including former HHMI investigator Marc Tessier-Lavigne, who is now at Genentech, had shown that some of the semaphorins bind to a receptor called neuropilin, which is critical for vascular patterning in the embryo. However, in their earlier work, Gu, Ginty, and co-author Alex Kolodkin showed that semaphorins do not need to bind to neuropilin for normal patterning to occur.

“That work set us looking for other potential mechanisms by which semaphorins might control vascular pattern development,” said Ginty. The researchers found *Sema3E* in regions of the developing embryo that suggested that it should have a role in the patterning of blood vessels. They also found a strikingly similar pattern of expression of the blood vessel cell receptor plexin-D1, leading the researchers to hypothesize that *Sema3E* might be the signaling molecule that interacts with plexin-D1. If this were true, it suggested that *Sema3E* exerts a “repulsive” force, channeling the blood vessels to grow along their proper course.

Meanwhile, Yoshida discovered that unlike other members of the same protein family, *Sema3E* binds selectively to plexin-D1 - a strong hint that the two signals work together to control vascular patterning. Yoshida also found that *Sema3E* can bind to plexin-D1 whether or not it binds to neuropilin.

Researchers noted that in contrast to the careful patterning of blood vessels in normal mice, the pattern of blood vessels in mice lacking plexin-D1, produced in Jessell's laboratory, was haphazard. Furthermore, knockout mice lacking *Sema3E*, produced in the laboratory of co-author Christopher Henderson of the Developmental Biology Institute in France, showed the same defective patterning.

In additional experiments, Gu showed that overexpression of *Sema3E* protein in specific regions of chick embryos prevented vascular growth into those areas.

“*Sema3E* is a very potent chemorepellent for developing blood vessels,” Ginty noted. “So, one possibility is that drugs that mimic this function could be useful in preventing growth of the new blood vessels required by tumors.”

“One of the really interesting things about this paper is that it questions the idea that *Sema3E*'s binding to neuropilin is required for vascular patterning,” said Jessell. “This, together with the finding that *Sema3E* interacts with plexin-D1, independent of neuropilin, may turn some of the preconceptions about the role of neuropilins in vascular patterning on their head.”

Jessell and his colleagues are now exploring whether Sema3E and plexin-D1 also contribute to the development of connections in the spinal cord. Ginty and his colleagues plan to explore the role of the proteins in neural development, as well as whether the combination is involved in vascular patterning in the limbs.