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Experiments Reveal Ancient Blood Flow Map

Scientists studying how developing blood cells migrate to their proper destinations in fruit flies have discovered the ancestral role of a protein better known for ensuring that tumors have adequate blood supply. The protein, called vascular endothelial growth factor (VEGF), has gained notoriety for guiding the development of new blood vessels that nourish cancerous tumors. When researchers block VEGF, the tumors' blood supply is cut off because new blood vessels don't form.

Now scientists say that VEGF and its receptors also help direct individual blood cells to their destinations in developing fruit fly embryos. The researchers say that this may be VEGF's ancient function, and that only recently in evolutionary time did VEGF assume its role in blood vessel development. The results even suggest that ancestral blood cells may have evolved into blood vessels, says Howard Hughes Medical Institute investigator Mark A. Krasnow at Stanford University.

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— **Mark A. Krasnow**

"Nobody knows how blood vessels evolved," he said. "Our idea is that some population of blood cells acquired the ability to form tubular structures through which the rest of blood cells could then move. Eventually the tubules invaded the heart to form the closed circulatory system that we know today."

Nam Cho, Krasnow and colleagues at Stanford University, and Felix Karim and colleagues at the South San Francisco-based biotechnology company Exelixis Incorporated reported their findings in the March 22, 2002, issue of the journal *Cell*.

"Migration of mammalian blood cells in adult physiology has been studied intensely, but the really amazing long distance migration of blood cells during embryonic development, which appears to be a highly programmed process, is not understood at all," said Krasnow. "We believe we have discovered a molecular basis for long-range blood cell migration."

The scientists first identified four genes encoding a single VEGF receptor and three related VEGF proteins in the fruit fly *Drosophila melanogaster* by searching the recently completed *Drosophila* genome sequence for fly genes similar to their human counterparts. In mammals, VEGF has been shown to be critical for normal development and proper functioning of blood vessels. In lieu of blood vessels, fruit flies have an open circulatory system in which blood cells are moved throughout the body by the pumping of a primitive heart called the dorsal vessel. In flies, blood cells are involved in recognizing and destroying pathogens, digesting remnants of dead cells, and wound healing, among other roles.

To determine what function VEGF and its receptors played in the insects, they localized the VEGF and VEGF receptor RNA and followed where in the developing fruit fly embryo the genes were active. They found that the VEGF receptor is made almost exclusively in the developing flies' blood cells, whereas the VEGF protein lines many of the pathways that blood cells travel from their initial point of origin.

When the researchers inactivated the gene encoding the VEGF receptor and followed blood cell migration, they noticed that blood cells formed and began to migrate normally throughout the body, but never reached the posterior or tail section. Instead, blood cells clumped together at the entry point into the tail region. The researchers concluded that without a functioning VEGF receptor, the blood cells could not find their way to their destination.

"How long-range migration of blood cells is accomplished has been an open question for a long time," said Krasnow. "These results support a scenario in which signaling centers are placed at many positions along the migration pathway. It's a bit like attracting a duck by leaving a trail of breadcrumbs along the pathway."

When the researchers added VEGF to areas in the fly that normally don't make the protein, the result was "a dramatic rerouting of blood cells to totally new positions," according to Krasnow. "This provided strong support that the role of the VEGF pathway is to direct blood cells."

A natural extension of the work is to suggest that blood vessels evolved from blood cells, Krasnow said. "In mammals it has been known for more than a decade that there is an intimate developmental relationship between blood vessels and blood cells," he said. A cell called the hemangioblast, which is a type of early stem cell, gives rise to both blood cells and the endothelial cells that become blood vessels. Scientists have shown that the VEGF pathway plays a critical role in hemangioblasts, but it was not clear why these two major cell types are linked developmentally. Krasnow said his results provide an explanation. "This common precursor could be a reflection of how the cells evolved," he added.

Krasnow emphasized that this is just the first step in understanding how blood cells migrate. The studies indicate that there are additional signaling molecules guiding blood cell movement in flies and that there are most likely other pathways yet to be discovered.

“An important aspect of this research for biology and medicine is that pathways like this that are highly conserved can be dissected and understood in *Drosophila*,” he said. “Any additional components and mechanisms that we find are very likely to have mammalian cognates that could be explored. This is going to refocus research on VEGF in blood cell development and function, as well as its role in mature blood cells.”