ARTERIOVENOUS MALFORMATIONS

Arteriovenous Malformations in Five Dimensions

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A tightly controlled Notch4 molecular switch in blood vessel endothelial cells induces regression of arteriovenous malformations in mice.

Arteriovenous malformations (AVMs) are vascular lesions associated with abnormal arteries and veins that connect with each other directly instead of connecting through an intervening capillary network (*1*). The result is that blood at high pressure goes from the thick-walled artery through a shunt directly into the thin-walled vein, resulting in hemodynamic stress on the shunt and venous vessel. The increased blood flow in an AVM weakens the blood vessels, potentially resulting in a dangerous rupture. Several vascular diseases exhibit these abnormal AVMs, including hereditary hemorrhagic telangiectasias and aneurysms. AVMs in the brain are particularly difficult to treat, and their rupture may result in stroke or death. Although AVMs can be visualized in human patients (*1*), elucidating how and why they form has proved challenging. An exciting study by Murphy, Wang, and colleagues in this issue of *Science Translational Medicine* (*2*) describes experimentally induced regression of AVMs in mice with stunning visual and molecular clarity, with clear implications for both the clinician and the scientist.

Murphy *et al.* (*2*) describe a mouse model in which AVMs, referred to as arteriovenous (AV) shunts, are induced in the cerebral vasculature by a molecular switch that turns on activity of the signaling molecule Notch4. When they switched off Notch4 activity, the researchers were able to rapidly "normalize" the AV shunt, inducing its regression and a return to normal blood flow patterns. The authors tracked the normalization process using imaging of the AV shunt through a cranial window cut into the mouse skull, combining three-dimensional (3D) visualization of vascular topology with a fourth dimension, the time-dependent assessment of blood flow. What makes the analysis so exciting is the addition of what I will refer to as a fifth dimension, that is, the Notch4 molecular switch that induces AV shunt regression by converting enlarged vessels to capillary-like microvessels. This conversion is associated with dramatic changes in blood flow patterns, with the requisite release of hemodynamic stress on vessel walls. With this molecular switch, the authors uncover a potential mechanism of AV shunt formation with spectacular clarity. Indeed, the authors can actually see the results of their experiments in real time. It is a very satisfying analysis for the reader and overcomes the limitations of studies that simply observe static images of experimental vessels. Such snapshot images of vessels allow one to build models but require the researcher to mentally project the future events that lead to the formation or resolution of a vascular malformation. In contrast, Murphy *et al.* use actual observations of real-time events to elucidate the molecular underpinnings of AVM formation and regression.

A key component of their study is the Notch4 molecular switch, developed by the authors previously (*3*), in which a tetracycline-regulated system modulates expression of a constitutively active form of Notch4 (*4*), designated Notch4* (*3*). The authors were able to modulate Notch4 signaling in the blood vessel endothelial cells of the mouse brain vasculature by administering the drug doxycycline, which rapidly down-regulates Notch4* expression; removal of doxycycline turns Notch4 signaling back on. The consequences of this treatment were then followed using 4D two-photon imaging with cellular resolution in live mice to assess vascular topography and function.

The Notch family of signaling receptors are key regulators of vascular development, guiding processes as diverse as arterial-venous differentiation, vascular smooth muscle cell differentiation, and sprouting angiogenesis (*5*). A decade ago, endothelial cell–specific expression of a constitutively active form of Notch4 was found to induce formation of abnormally large vessels during mouse embryonic development (*6*). The authors previously demonstrated that conditional expression of Notch4* in endothelial cells resulted in the formation of AV shunts in the mouse brain vasculature (*3*) (Fig. 1). By efficiently switching off Notch4* expression, these researchers now show that the mouse brain AV shunts regress and revert to capillary-like microvessels, restoring normal blood flow patterns (*2*). Thus, the authors were able to model both pathological high-flow vessels using high Notch4* expression and normalized low-flow microvessels using low Notch4* expression. These two states can be rapidly interconverted, demonstrating that the structural features of AV shunts depend on molecular pathways and are not just secondary to high blood flow; that is, reduced Notch signaling induces the regression of AV shunts despite the fact that the vessels are still subject to high-flow conditions.

The authors proceeded to use their system to determine the ways in which AV shunts regress and to examine the mechanisms by which Notch suppression mediates this process. First, they asked whether a reduction in blood flow causes blood vessel regression. Specifically, they measured blood flow at three sites, the upstream feeding artery, the AV shunt itself, and the adjacent artery (Fig. 1). They asked whether switching off Notch4* led to shunt regression directly or indirectly through a reduction in blood flow. If Notch4* repression directly reduced the diameter of the AV shunt, then one would expect increased resistance to blood flow and hence decreased flow through the AV shunt, with total flow through the feeding artery also reduced, and that is what they observed. This observation is consistent with a direct role for Notch4* activity in vessel regression (Fig. 1).

By analyzing AV shunt regression at the cellular level, Murphy *et al.* established that vessel regression was not associated with loss of endothelial cells. Instead, they show that the vessel caliber becomes smaller and more capillary-like but the number of endothelial cells lining the vessel remains constant. In contrast, the mean area of endothelium was reduced upon AV shunt regression, with the reduced vessel caliber correlating with reduced endothelial cell size.

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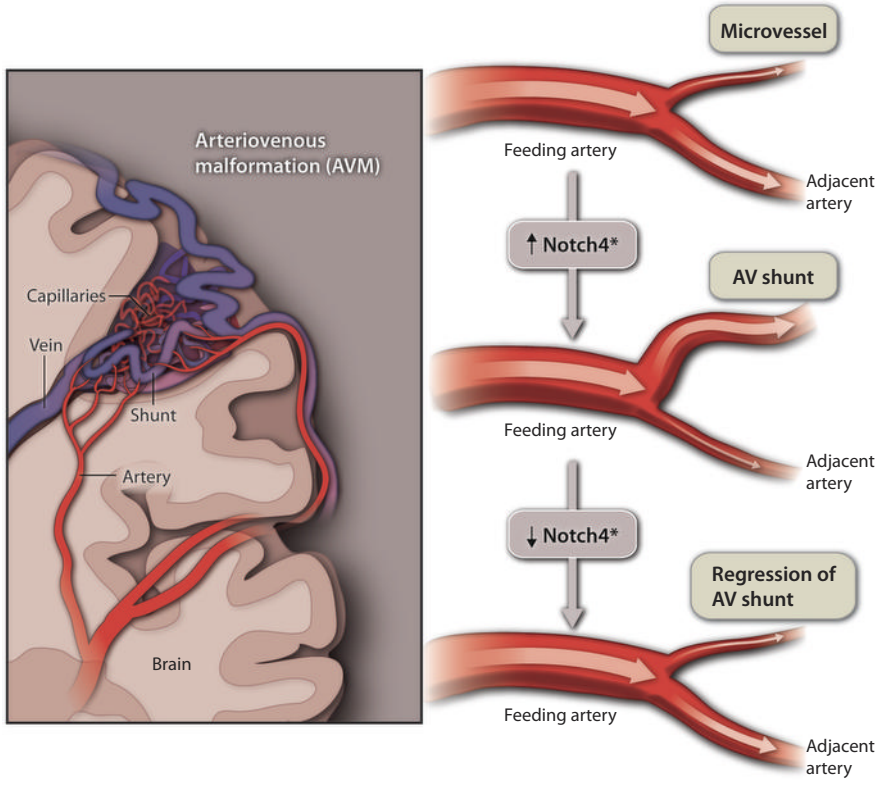

Fig. 1. Flipping the switch in AVMs. Shown is the outcome for blood vessels in the mouse brain when a genetic switch driven by Notch4 is flipped on or off. In normal blood vessels, the artery connects with capillaries (microvessels), resulting in reduced blood flow, and the capillaries then connect with the vein. In an AVM (left), the artery connects directly with the vein through a shunt, bypassing the capillaries, resulting in vein enlargement and leading to possible rupture. When constitutively active Notch4 (Notch4*) is overexpressed in a normal blood vessel (top, right), an AV shunt forms in which the blood vessel becomes enlarged, mimicking what happens during AVM formation (middle, right). This transition alters blood flow velocity and pressure, as indicated by the size of the arrow. The enlarged vessel carries blood at high velocity, which results in a concomitant reduction in blood flow in the adjacent artery (middle, right). When Notch4* expression is switched off (bottom, right), the AV shunt regresses, and the enlarged vessel shrinks in size, restoring blood flow to the normal pattern.

Notch is known to promote arterial specification during development (*7*), and it is important to know whether Notch4* directs the program of arterial-venous differentiation during AV shunt formation and regression. For instance, does an induced AV shunt result from venous endothelial cells becoming arterial-like or from expansion of arterial endothelial cells? Expression of Notch4* led not only to the misexpression of arterial markers but also to the suppression of the venous marker EphB4, demonstrating that Notch4* expression results in conversion of venous endothelial cells into arterial endothelial cells during AV shunt formation.

A careful assessment of the molecular pathways driving vessel regression highlighted the importance of EphB4, a tyrosine kinase receptor that is expressed by venous endothelium. EphB4 binds to ephrinB2, a protein downstream of Notch signaling that is expressed in arterial endothelium (*8*). Notch4* activation in venous endothelium reduces EphB4 expression, whereas down-regulation of Notch4* restores EphB4 expression, consistent with a model in which AV shunt formation results from venous endothelial cells becoming arterial-like. What is the active molecular process required to ensure vessel regression when Notch4* is switched off? The answer involves EphB4, because administration of an EphB4 antagonist markedly impaired the regression of AV shunts after down-regulation of Notch4* expression. What is the critical role for ephrinB2/EphB4 signaling during AV shunt normalization? Given that reduced endothelial cell proliferation is not the answer, the authors posit that endothelial cell migration and reorganization may be involved. They point out that the specific regression of vessels occurs at the AV interface, whereas the adjacent arteries often do not regress (Fig. 1), implying that communication between venous and arterial endothelium is involved.

It is reasonable to hope that this elegant analysis of AV shunt regression will have important implications for managing vascular lesions, such as AVMs and fistulae in human patients. Murphy *et al.* demonstrate that the conversion of abnormal high-flow large vessels to capillary-like microvessels occurs by turning off a single molecular pathway. It is encouraging that vascular normalization was not accompanied by hemorrhage or vascular damage. In addition, the authors demonstrate that AV shunt regression reversed tissue hypoxia and promoted tissue oxygenation by redirecting and slowing blood flow through brain tissue. These findings bode well for potential therapeutic targeting of the Notch signaling pathway to repair enlarged blood vessels and to prevent vessel rupture and tissue damage. Could therapeutics be developed to pharmacologically treat such "high-flow" vascular lesions? Several Notch inhibitors are in clinical trials for treating cancer patients and could be tested in the Murphy *et al.* mouse model. Gamma-secretase inhibitors designed to treat Alzheimer's disease have been extensively studied in patients but are highly toxic; however, one could explore whether nontoxic doses would be effective at inducing regression of Notch-induced AV shunts. Antibodies against Notch proteins are being developed, and it would be interesting to test whether antibodies that target endothelial Notch1 or Notch4 could induce regression of AV shunts in this mouse model. Ligands may be identified that are pathologically overexpressed in AVMs, and antibodies could be developed to target them. The elegant mouse model of Murphy *et al.* now provides a way to begin testing existing and new therapeutics that target Notch for the treatment of AVMs.

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