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In the past, hemodynamic influences were thought to primarily control the identity of arteries and veins. Today, it is accepted that blood vessel identity is determined before the onset of blood flow because vascular precursor cells express specific genetic markers. In addition to these molecular markers interacting in complex signaling pathways, there are epigenetic mechanisms that help determine and maintain endothelial cell fate. Here, we will attempt to give the readers some insight with regard to these new concepts. We also would like to speculate as to how these new discoveries may relate to neuroradiology, and our related thoughts are found in italics.

In the embryo, the cardiovascular system forms first.[1] Formation of all other organs depends on oxygen delivery. Blood vessels form before blood starts flowing, and the circulatory system must be in place before organs develop. The oxygenated blood pumped into the arteries by the heart plays a role in the development of smooth muscle cells and an extracellular matrix capable of supporting high pressures. Once blood passes through capillaries, intraluminal pressure decreases and venous valves ensure unidirectional flow. These physiologic and mechanical changes were thought to be responsible for development of blood vessel identity—that is, flow direction, oxygen concentration, and pressure determine if vessels become arteries or veins. Recent investigations highlight the fascinating concept that endothelial cells are predestined, even before blood begins circulating, to become arteries or veins.

In embryonic life, angioblasts (endothelial precursor cells) arising mostly from mesoderm form epithelial tubes by apposition of cords.[1] Creation of these tubes is called "vasculogenesis" and is a temporary and rapidly concluding prenatal mechanism.[1-3] A second process, called "angiogenesis" is responsible for the formation of blood vessels from pre-existing ones (involving elongation, sprouting, and remodeling of those pre-existing blood vessels). Vasculogenesis leads to formation of the first blood vessels: the aorta and posterior cardinal vein. In some fish and mice, progenitor cells arising from the lateral mesodermal plates (alongside the notochord) give rise to cells that migrate to the midline under the notochord and establish the intermediate cell mass that subsequently assembles the dorsal aorta and cardinal vein.[3,4] This early process is evidence of the already defined identity of endothelial cells. The genesis of arteries and veins from closely located progenitor cells is recapitulated throughout the entire body, explaining why arteries and veins are nearly always side by side. Even before progenitor cells begin migrating ventrally, their fate as either arterial or venous has been cast. How does this happen?

The process of vascular cell differentiation has been established through animal studies. Through a variety of mechanisms, sonic hedgehog induces tubulogenesis.[3] Sonic hedgehog is expressed in the notochord and triggers endothelial cell formation but not cell-fate determination (Fig 1). When the function of hedgehog is affected, animals will not fuse their dorsal aortas; they lack differentiation of dorsal aortas from cardinal veins and have abnormal trunk circulatory systems that express only venous markers.[1-4] If the function of sonic hedgehog is completely blocked, the aorta does not form. Sonic hedgehog induces expression of vascular endothelial growth factor A (*VEGF-A*) in somites, which in turn upregulates *notch*5 signaling, leading to a specific expression of *ephrin-B2* in arteries and *EphB4* in veins.[1,3] *VEGF-A* is also involved in cell differentiation, proliferation, migration, and survival.[3,4] *VEGF-A* is critical for vasculogenesis and postnatally for angiogenesis. Animals lacking *VEGF-A* or its receptor (*VEGFR-2*) develop few or no angioblasts and die early.[3] Abnormalities of *VEGF-A* decrease arterial *ephrinB4* and upregulate venous *Flt4* (*fms-related tyrosine kinase 4*), leading to abnormal aortas.[3] *VEGF-A* is highly expressed in peripheral nerves, and if deficient, nerves and accompanying arteries are abnormal or absent.[5] It is thought that once an artery is formed, its smooth muscle cells direct the growth of adjacent sympathetic nerves. Once sympathetic neurons are established, they send axons to organs.[5] Normal blood vessel differentiation occurs only if nerves are well aligned with arteries. Similarly, there is an isomer of *VEGF* that is involved in vein and lymphatic formation. Lymphatics originate from embryonic veins. Adult lymphatic endothelial cells are different from veins and arteries because they are not covered by a continuous basal membrane and their junctions are loose, allowing exchanges of interstitial and tissue fluids. Acquisition of lymphatic phenotype is regulated by *VEGF*, *Prox1*, *cytoplasmic tyrosina kinase SYK*, and *SLP-76*.[6] *Thus, we speculate that when this process is affected, it may lead to defects occurring simultaneously in both systems, such as venolymphatic malformations.*

Let's summarize what we have learned up to this point: Arterial and venous destiny is determined by a molecular pathway involving *sonic hedgehog,* which subsequently affects *notch* and later *VEGF-A* (Fig 1). Loss of all, 2, or even 1 of these mechanisms results in lack of arterial identity. Because these 3 mechanisms are needed for arteries to develop, the default vessel identity was initially thought to be venous. However, high levels of *COUP-TFII* (*chicken ovalbumin upstream promoter transcription factor II*) and *EphB4* are found in primitive veins but not in arteries; therefore, venous identity is probably the result of a dynamic cascade of events.[4] Aberrations in these mechanisms result in abnormally large veins, malformed venous sinuses and cardinal veins, fusion of veins and arteries, and hemorrhage and edema leading to death.

Arterial progenitor cells express *ephrinB2,* whereas veins express *EphB4* and other markers such as *Flt4*. These genes are part of the larger group called "*Eph*," whose receptors modulate morphogenesis of various cell groups involved in the formation of the central nervous system. *Eph* also regulates cell migration and axonal guidance. Neuropilins are a form of *VEGF*, which is part of the gene family that controls axonal guidance in the brain.[3] Both arteries and veins express neuropilin. *Thus, one can start to understand that there is a relationship between vascular lesions (arterial and venous) and neuronal-migration abnormalities.* Neuropilins are only 1 of many substances needed for axonal guidance. These substances work on the basis of attraction/repulsion and guide axons to final destinations. Once activation of *Eph*-related proteins has taken place, a variety of mechanisms that lead to cell changes begin. The *EphB4* receptors need to be balanced with *ephrinB2* ligands in order for vessels to acquire distinct identities and for

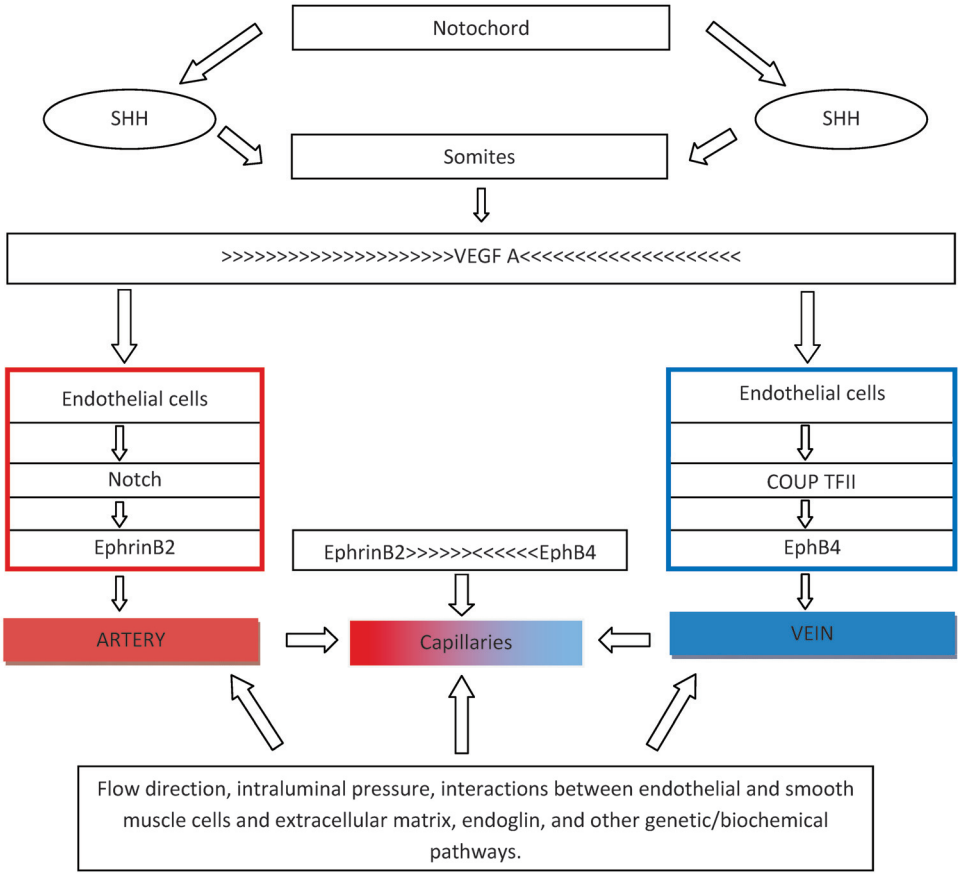

Fig 1. Sonic hedgehog (SHH) secreted by the notochord stimulates the somites to produce *VEGF-A*. Before cells form arteries or veins, their fate has been determined by a *VEGF* gradient and the presence of *ephrinB2* (arteries) or *EphB4* (veins). Balanced gradients of *ephrinB2* and *EphB4* result in capillary formation and maintenance. Once the circulatory system is established, a complex play between hemodynamics, oxygen concentration, intercellular communications, and genetic and biochemical actions aids in preserving blood vessel identity.

postnatal morphogenesis to continue. If *ephrinB2*, expressed in endothelial cells, is knocked out, normal intercalations between arteries and veins do not happen and defective artery-to-vein connections arise. Thus, a balanced development of arteries and veins occurs only if gradient-like expression of *ephrinB2* and *EphB4* is present in endothelial cells. The interaction between *ephrinB2* and *EphB4* leads to formation of a hierarchically organized system of vessels that are more artery than vein in some regions, and, in others, more vein than artery. These connections vary in size and establish capillary networks. Maintenance of the arteriovenous interfaces also depends on *ephrinB2* and *EphB4*. *It is interesting to speculate that an imbalance between these substances may be the cause of various cerebral vascular malformations.*

Notch receptors regulating early cell destiny are also involved in proliferation, apoptosis, maturation, and cell homeostasis. In humans, mutations in *NOTCH*3 or the *NOTCH* ligand (*JAGGED-1*) result in the vascular fragility seen with cerebral autosomal dominant arteriopathy (with subcortical infarcts and leukoencephalopathy) and Alagille syndrome, in which multiple arterial narrowings develop, respectively.[4] Mice lacking notch genes exhibit life-compromising vascular anomalies. There are many other mechanisms that disrupt the function of *notch* genes, resulting in arterial deficiencies. *COUP-TFII* is a type of nuclear receptor that inhibits notch activation. By doing this, *COUP-TFII* suppresses receptors for *VEGF-A*. This suppression in turn causes abnormalities in the concentration of *EphB4*, which result in conversion of arterial-destined cell lineages to veins. Overexpression of an activated NOTCH receptor called *int3* induces arteriovenous malformations (AVMs) postnatally in mice.[1] By repressing the NOTCH receptors, arteries revert to normal, implying that treatment of AVMs may be possible this way.[1]

Vessel formation is complete when endothelial cells are surrounded by pericytes (in capillaries) or smooth muscle cells, which are present to varying degrees in larger arteries and veins. These processes are influenced by *transforming growth factor β* complex, which encompasses *endoglin* and *ALK1* (*activin receptorlike kinase 1*). Endoglin is a glycoprotein found in endothelial and smooth muscle cells, which is critical for their development, morphology, and migration.[7] Mutations of *endoglin* and *ALK1* result in the loss of capillary beds and arteriovenous shunting and are thought to be the main abnormality in hereditary hemorrhagic telangiectasia types 1 and 2, respectively, syndromes characterized by AVMs in different organs and systems.

Once blood vessel identity is established, it needs to be perpetuated. Intraluminal hemodynamics and oxygen levels aid in maintaining vessel identity. However, arterial and venous identities are potentially reversible. Transplanted veins lose *EphB4* and develop intimal-medial thickening to become more arterylike. This plasticity is predominantly seen in younger endothelial cells but may be induced in older ones if

younger ones are grafted onto them. This aspect of identity maintenance is of importance for vessel transplantation.[1-3]

It is interesting to note that neo- and revascularization in postnatal life share many, if not all, of the above-described mechanisms and altering these is the basis of novel angiogenic or antiangiogenic therapies and, in the future, vessel-remodeling treatments. Blood vessels do not seem to have the luxury of asking themselves: to be or not to be? Their destiny is determined even before they are formed.

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EDITORIAL

Searching the *Journal* for Evidence-Based Radiology

In the past several years, major concern has been raised from both quality and safety advocates and third-party payers regarding medical practice patterns with excessive use of imaging. As health care reform is upon us, it has become particularly important to substantiate medical imaging for specific clinical conditions. This is coupled with the increasing public awareness and concern regarding the risks from medical imaging, especially radiation exposure. As radiologists, we are now expected to provide even more information to our patients, referring physicians, and payers. This information may be partly based on our practice experience, expert opinion, and sometimes the available published literature. However, more recently, scientific evidence is being emphasized as a major component in guiding medical decisions. Thus, the practice of evidence-based radiology has emerged as the application of the best available scientific evidence to patient care.

Evidence-based radiology is a valuable method to use when a specific clinical problem arises that initiates a literature search for additional scientific information. Critical thinking skills are necessary to appropriately gather this relevant literature and interpret its scientific merit by using established methods and criteria. Medical decisions can then be based on the most valid scientific evidence available. This process describes a bottom-up approach for problem-based learning, first developed by McMaster University and the Center for Evidence-Based Medicine, Oxford. The evidence-based radiology Web site developed by Malone et al[1] is an international effort to help radiologists who have no specific training in research to use the principles of evidence-based medicine in answering clinical questions in their practice. An established method described for practicing evidence-based radiology has been developed by Sackett et al[2] with the following 5 steps: 1) "Ask" describes how to structure a clinical question into an answerable format. 2) "Search" describes how to perform a comprehensive literature search relevant to the question. 3) "Appraise" describes how to critically evaluate the literature by assessing its validity, reliability, and usefulness. 4) "Apply" describes how to use these results in the care of patients. 5) "Evaluate" describes a self-evaluation process for improving critical thinking skills.

These 5 steps are a useful guide for radiologists in implementing evidence-based radiology in their practice. To obtain a relevant literature search for a clinical problem, one must structure the question to contain certain components by using the *P*atient, *I*ntervention, *C*omparison, and *O*utcome format. For example, a clinical problem in imaging patients with acute stroke is deciding between using CT perfusion or MR perfusion for evaluation of ischemic penumbra. The question should include the following terms: the specific *P*atient population as "patients with acute stroke" AND the *I*ntervention as "CT perfusion" AND its *C*omparison as "MR perfusion" AND the desired *O*utcome as "ischemic penumbra." The question may be phrased as the following: In patients with acute stroke, is CT perfusion better at diagnosis of ischemic penumbra than MR perfusion? The search text for this example would be "acute stroke AND CT perfusion AND MR perfusion AND ischemic penumbra." This structured question lends itself to a reproducible search of the literature that yields fewer but more relevant research articles.

Commonly, search engines such as PubMed, Ovid, Knowledge Finder, and Google Scholar are used to explore the large electronic databases of MEDLINE, EMBASE, Cochrane, and the Web of Science. Other resources include using the related articles featured in PubMed for all references as well as the reference lists of all relevant publications. Additional information may be gathered by contacting the authors or experts in the field. A librarian is an excellent resource to assist in searching your question, especially for expanding your search strategy.

Once the relevant research articles have been obtained, review and appraisal of the literature are performed. The research articles are ranked according to hierarchic scientific evidence by analyzing the Methods and Results sections. Levels of evidence have been defined on the basis of the validity of the Results and the possible sources of bias in the Methods. The base of the pyramid (level 4) is considered the lowest level of evidence and comprises the primary literature, such as original published research studies. There is wide variability in the evidence provided in original research, ranging from insufficient evidence (as seen in research with major study design flaws, case reports, observation studies, and expert reviews) to strong evidence (as seen in research with broad generalizability, prospective blinded clinical trials, and meta-analyses). The secondary literature comprises the top portion of the pyramid with evidence-based reviews, synopses, and information systems.

Level 3 contains the evidence-based reviews, such as systematic ones, which follow strict methodologic criteria in reviewing the literature for a specific clinical topic and thus provide more