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Comment on Ny et al, page 1740

VEGF-D: a modifier of embryonic lymphangiogenesis

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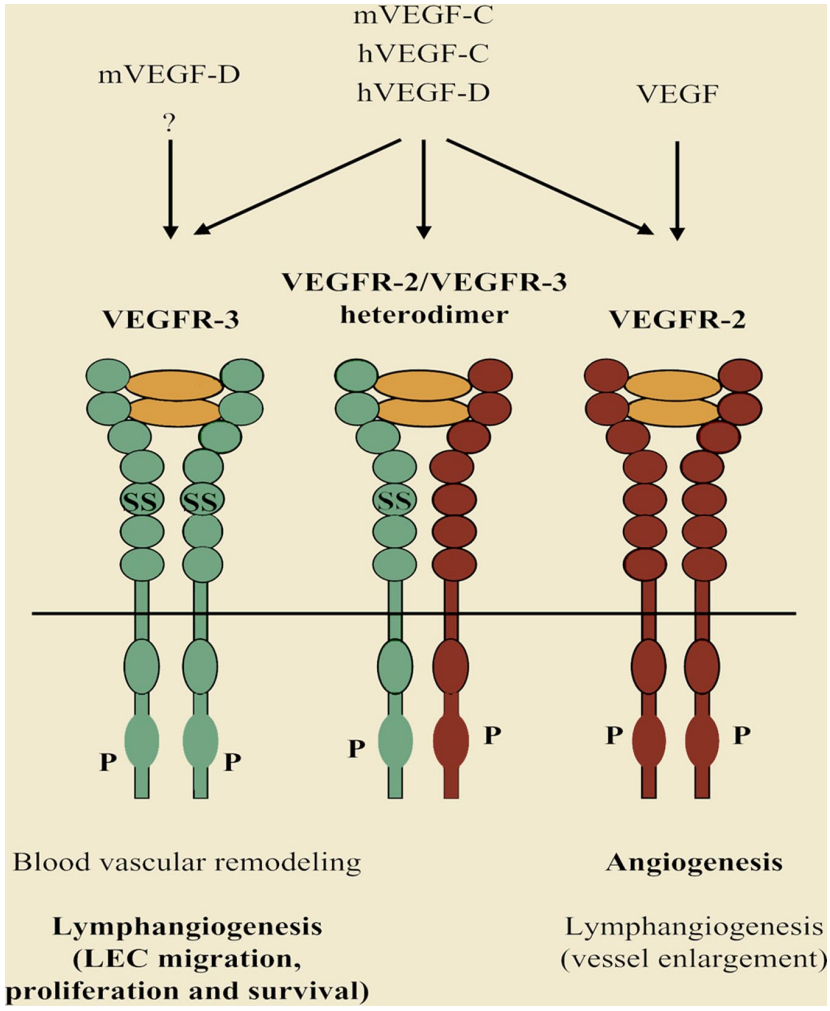
In this issue of *Blood*, Ny and colleagues disclose a modifier role for VEGF-D and a critical function for VEGFR-3 in embryonic lymphangiogenesis in *Xenopus* tadpoles.

VEGF-D potently stimulates lymphangiogenesis in adults and promotes metastasis of both human and experimental mouse tumors.[1-4] However, studies in gene-deficient mice have suggested that it is dispensable for embryonic lymphatic vascular development.[5] Ny et al now show that VEGF-D functions as a modifier of VEGF-C–driven early sprouting and migration of lymphatic endothelial cells in developing frog embryos and demonstrate the critical role of their receptor VEGFR-3 in this process.

Stimulation or inhibition of lymphangiogenesis, especially via the VEGF-C/VEGF-D/VEGFR-3 pathway, has proven beneficial in preclinical models of lymphedema and tumor metastasis, respectively, but their application to the treatment of human disease requires more detailed understanding of the molecular basis of lymphangiogenesis. Genetic studies in mice have revealed the importance of several key players in this process but many important issues remain to be resolved. Recently, the characterization of a lymphatic vascular system in zebrafish and the analysis of lymphatic vessel development in frog embryos have made these small organisms models of choice for genetic and chemical screens for regulators of lymphatic vascular development as well as for in vivo imaging of lymphangiogenic processes. Ny et al employ genetic down regulation in *Xenopus* tadpoles to demonstrate the subtle transient role of VEGF-D and its cooperation with VEGF-C in embryonic lymphangiogenesis.

VEGF-C and VEGF-D have similar domain structure, both undergo proteolytic processing in a similar manner and, in humans, both share the same receptor-binding specificity. Mouse VEGF-D differs from its human counterpart and from VEGF-C in that it does not bind to the major angiogenic



VEGF-C is essential for embryonic lymphatic vascular development whereas VEGF-D has a dispensable modifier role in this process. Nevertheless, both growth factors potently stimulate lymphangiogenesis in adults. VEGFR-3 is required for the remodeling of the primary blood vascular plexus in midgestation mouse embryos, apparently in a VEGF-C/VEGF-D independent manner.[7] Later in embryogenesis, VEGFR-3 becomes restricted to lymphatic vessels and is critical for embryonic lymphangiogenesis and postnatal lymphatic vessel survival. At least in adult lymphangiogenesis, VEGFR-3 can cooperate with the major angiogenic receptor, VEGFR-2. ? indicates possible additional ligand or interaction.

receptor VEGFR-2, which has been shown to cooperate with VEGFR-3 in lymphatic endothelial cell migration and proliferation. Whereas VEGF-C is essential in mouse lymphatic vascular development, it remains to be resolved to what extent the lack of VEGFR-2 binding accounts for the dispensable role of VEGF-D in this process.[5,6] Elucidation of the receptor-binding pattern of *Xenopus* VEGF-D and finding whether it explains the functional difference between VEGF-C and VEGF-D in this organism might bring us a step forward in solving this question. A hint to the lymphangiogenic modifier role of VEGF-D is provided by the fact that exogenous VEGF-D protein administration is able to partially rescue the impaired sprouting and migration of lymphatic endothelial cells in VEGF-C–deficient mouse embryos,[6] although loss of endogenous VEGF-D does not aggravate the VEGF-C null phenotype.[7]

Death of VEGFR-3–deficient mice due to blood vascular defects prior to lymphatic vessel development has precluded studies of the role of VEGFR-3 in mouse embryonic lymphangiogenesis.[8] The importance of VEGFR-3 signaling in embryonic lymphatic vascular development, postnatal lymphatic vessel survival, and stimulation of adult lymphangiogenesis has been directly or indirectly demonstrated in various experimental settings in several species. By using genetic down-regulation and chemical inhibition, Ny et al document the critical role of VEGFR-3 in developmental lymphangiogenesis in the *Xenopus* tadpole model and thus the functional conservation of VEGFR-3 in this species.

A profound understanding of the molecular regulation of lymphatic vascular development and the entire range of functions of its major players and modifiers should enable the development of controlled antilymphangiogenic and prolymphangiogenic therapies. Characterization of the lymphatic system in small model organisms has provided new tools to study lymphangiogenic processes and to complement the methods available in mammals. Ny et al highlight the potential of *Xenopus* tadpoles in lymphatic vascular research by demonstrating the subtle modifier role of VEGF-D in lymphatic vascular development.

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Comment on Gao et al, page 1713

Shear tango: dance of the ADAMTS13/VWF complex

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ADAMTS13, the metalloprotease that regulates the size of von Willebrand factor (VWF),[1] is critical in preventing microvascular platelet clumping, such as occurs in thrombotic thrombocytopenic purpura. The article in this issue of *Blood* by Gao et al addresses how these two proteins use multiple binding sites to facilitate proteolytic regulation of VWF.

ADAMTS13 cleaves only a single bond, Tyr^{1605}-Met^{1606}, buried within the VWF A2 domain. Shear forces unravel the normally tightly packed globular VWF, exposing this cleavage site. How does ADAMTS13 position itself on this flexible and dynamic multimeric glycoprotein, in such a way that it remains in place throughout the shear-induced folding and unfolding that occurs during transit through microcirculatory beds?

Binding is known to take place directly on the A2 domain of VWF spatially adjacent to the cleavage site,[2] but also on a more remote site of the A2 domain.[3,4] There might be additional domain interaction sites on VWF. Complementary binding sites have been identified on ADAMTS13, certainly within its Spacer[5] and Cys-rich regions,[6] but also within the TSR/CUB domains,[7] depending on shear conditions. Binding between each distinct remote site (exosite) ramps up the specificity constant of cleavage. According to Gao et al, collective exosite-driven proteolysis of the Tyr^{1605}-Met^{1606} bond accounts for approximately 2 orders of magnitude increase in cleavage of a small VWF substrate.

Remote exosite interactions are common in the hemostatic process and are exemplified by thrombin,[8] which uses exosites to direct and enhance cleavage of multiple protein and cellular substrates. In order for ADAMTS13 to utilize a similar mechanism of exosite recognition to partner its conformationally-responsive substrate during its conformational dance to shear, this enhanced proteolysis also requires that the protease can retain its binding under changing shear conditions during its transit through vessels of different sizes. Gao et al introduce an important concept of portability of exosites. This can be seen in the figure. The A2 domain model is shown folded as a compact globular molecule with the cleavage site hidden within the structure. When unfolded, it presents several distinct interaction sites near the cleavage site and extending to its C-terminus. Flexibility in spatial relationships (portability) between interacting sites is provided and/or enhanced by a redundant (dispensable) sequence. If there is no fixed distance between exosite and protease cleavage site, then the 2 partner macromolecules can be tightly bound but move together, elongating when shear stress is high in the microcirculation, and contracting in length to globular form in larger vessels when shear stress is low. Such a rhythmic dance depends on mutual and multiple exosite engagements on dynamical structures that nevertheless precisely position the active site