Silky, Sticky Chimeras-Designer VEGFs Display Their Wares

Michael Simons

To the casual observer of the VEGF field, the panoply of players involved already looks sufficiently intimidating; 5 VEGF genes (A, B, C, D and E) with multiple splicing variants encoding proteins of varied functionality, 3 high-affinity tyrosine kinase receptors (VEGF-R1, 2 and 3), and at least 2 nontyrosine kinase receptors (neuropilin-1 and 2). One would imagine that with such a variety we would have encountered by now all possible variations on the theme of VEGF control of new vessel growth, arterial or lymphatic. But one would be wrong. Two reports in this issue of Circulation Research by Kari Alitalo and colleagues demonstrate how various VEGF domains dictate hitherto unappreciated biological nuances of vascular growth and how these can be combined into “designer” VEGFs with new activities not present in parent molecules.1,2

Principal VEGF-A domains include a signal peptide sequence, a VEGF homology domain (VHD) common to all VEGF but dictating specific VEGF receptor binding properties (VEGF-R1 and VEGF-R2 in the case of VEGF-A VHD), and a heparin binding domain (HBD) encoded by exons 6 and 7 in VEGF_A165 or a shorter HBD encoded only by exon 7 in VEGF-A189. Exon 7 also encodes a neuropilin-1 binding domain present in both VEGF-A165 and A189 (Figure). Similarly, VEGF-C principal domains include the signal peptide domain and VEGF-C VHD (in this case dictating VEGF-R2 and VEGF-R3 binding) flanked by N- and C-terminal domains that normally undergo proteolytic process to form the mature protein (Figure). The C-terminal domain of VEGF-C, because of its cysteine-rich sequences eliciting comparisons with a protein component of silk, has been termed a silk homology domain.

Although the significance of VHD in determining binding to a specific VEGF receptor and HBD in enhancing matrix-binding properties of VEGF-A have been long appreciated, the biological function of the silk homology domain of VEGF-C has not been determined. Likewise, it was also unclear how specific functions of one VEGF might be affected by introduction of non-VHD sequences from another VEGF. These questions are addressed in present studies. A chimeric protein consisting of the VEGF-A VHD and VEGF-C N-terminal and C-terminal cysteine-rich silk domain (A-VHD/CAC) induces formation of a strikingly abundant network of pericyte-covered vessels, dramatically distinct from VEGF-A-induced capillary network. At the same time, chimeras, consisting of the VEGF-C VHD and VEGF-A heparin binding domain (C-VHD/HBD) that includes exon 7 required for neuropilin binding, induce growth of lymphatics localized along tissues borders and basement membrane planes that differ equally dramatically from VEGF-C-induced lymphatics (Table).

These results bring up several interesting questions and point to a new potential in “custom” growth factor design. Clearly, we still have much to learn about VEGF domain complexity. Various VEGF family members possess, in addition to their specific receptor binding domains, other structural motif that appear to have important biological function. The silk domain of VEGF-C and VEGF-D is one such example. Unexpectedly, introduction of a VEGF-C silk domain into VEGF-A significantly altered the latter’s biological activity without apparently altering its binding to its principal receptors, VEGF-R1 and 2. At the same time, the A-VHD/CAC chimera bound with much higher affinity to neuropilins 1 and 2, an interaction that was greatly facilitated by heparin. This greater binding to neuropilins was associated with a distinctly different type of a vascular network induced by the silk chimera, a dense web of capillaries enveloped by pericytes and extracellular matrix.

This type of vasculature is in sharp contrast to the typical VEGF-A-induced vessels that tend to be larger in diameter, less packed and very permeable. In fact, increased permeability is a hallmark of VEGF-A-induced vasculature as seen, for example, in tumors, ischemic or fresh granulation tissues. That the VEGF-C silk domain should have such an effect is rather unexpected, as the lymphatic network induced by VEGF-C is typically neither dense nor impermeable.

Silk proteins (spidroins and fibroins) are assembled into well-defined insoluble nanofibrills that have a pronounced tendency to self-aggregation.3 Silk domains in nonsilk proteins are a poorly understood structure that may promote formation of silk-like disulfide-bonded multimers that may physically facilitate cell-cell association accounting for increased network density. However, the capacity of the silk domain to promote VEGF-neuropilin (Nrp) interaction is rather unexpected. Furthermore, Nrp-1 signaling is reported to regulate VEGF-induced permeability increase.4 Given then decreased permeability of the A-VHD/CAC chimera-induced vasculature, one could speculate that the chimera somehow binds to but does not activate Nrp-1, or perhaps even suppress its signaling. Alternatively, because Nrp-1 is expressed in...
smooth muscle cells and pericytes, the chimeric protein may facilitate their recruitment, thereby promoting vessel maturation.

Another interesting aspect of the A-VHD/CAC-induced network is its highly branched pattern previously observed in tissues exposed to a MMP-resistant VEGF-A mutant. The role of VEGF signaling in vascular branching has not been clearly established. In this regard, it is interesting to note that deletion of synectin, a Nrp-1 and syndecan-4 binding cytoplasmic protein, results in diminished endothelial cell VEGF responsiveness and reduced arterial branching. Neuropilin involved in vascular guidance and, potentially, regulation of arterial branching morphogenesis has long been suspected and is not altogether unexpected given that semaphorin-3A, another neuropilin-1 ligand, regulates dendritic branching via Nrp-Fyn signaling cascade.

Equally interesting, C-VHD/HBD chimera induced a distinctly different pattern of lymphatics growth compared with VEGF-C with new vessels located along heparan sulfates-rich basement membranes. This, of course, is expected given the presence of a heparin-binding domain in this chimera. At the same time, the newly formed lymphatics network was composed of rather sparse but thicker-looking vessels. The functional significance of such arterial-vessel-like lymphatics is not clear. Will they, for example, be as effective in reverse fluid and protein transport as normal lymphatics? However, this change in the lymphatic vasculature appearance is likely once again to be because of engagement of neuropilin signaling. It is also interesting to note a lack of increased branching in C-VHD/HBD-induced lymphatic vasculature. The presence of VEGF-A HBD has been associated with increased capillary and arterial branching that is thought to be mediated by an extracellular matrix gradient established by heparin-binding VEGF. Why that should not be the case in the lymphatic vasculature is somewhat puzzling.

As these two VEGF chimeras studies illustrate, meticulous analysis of protein domains can teach us much about basic biology and provide opportunities for designer growth factor that can improve on nature’s own abilities. The capacity to create such designer molecules may help address some of the key issues that have bedeviled the field of therapeutic angiogenesis: growth factor stability and half-life in the matrix, vessel stabilization and vascular guidance. The need for a prolonged exposure of tissues to an angiogenic agent for a successful generation of new and stable vasculature has long been the Achilles heel of angiogenic therapies. VEGF-A is capable of fairly rapid induction of growth of immature blood vessels, but a prolonged stimulation is required to achieve vessel maturity. Molecular details of the vessel maturation process are still poorly understood. Platelet-derived growth factors and angiopoietins are thought to play a key role, in part by recruiting pericytes to the newly formed vasculature. How prolonged versus short-term VEGF stimulation enters into this equations is at present not clear, although a recent study suggested that this may occur via recruitment of an accessory cell population from the bone marrow.

Another recently realized twist on the subject of VEGF-induced vascular growth is the need for optimal, not too high and not too low, growth factor concentration and its uniform distribution in tissues. The ability to control not only level and duration of VEGF expression but also its distribution in tissues appears to be an important factor in achieving effective therapeutic angiogenesis.

### Biological Activity of VEGF Chimeras

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Vascular Network: Capillary proliferation. No pericytes, Dense. Extensive pericyte coverage, Dense. Thin vessel, Sparse. Thick vessels

* Binding is heparin-dependent. A-VHD/CAC, VEGF-A VEGF homology domain/VEGF-C N- and C-terminal domains chimera; C-VHD/HBD, VEGF-C VEGF homology domain/VEGF-A heparin-binding domain chimera.
However, the inability to sustain newly formed vessels after a short burst of angiogenic stimulation likely explains why neither single dose protein therapies nor plasmid- or adenoviral-based gene transfer approaches have been particularly successful, although a sustained release local protein delivery was effective in one pilot trial. On the basis of these and previous studies that have investigated various VEGF modifications, one can imagine a designer “super” VEGF that would be specifically targeted to VEGF-R1 and VEGF modifications, one can imagine a designer “super” VEGF that would be specifically targeted to VEGF-R1 and R2 (A-VHD), possess enhanced ability to activate neuropilin-1 to promote pericyte coverage and maturation of the newly formed vasculature (VEGF-C silk homology domain), would accumulate in the extracellular matrix (heparin binding domain), and would be resistant to MMP proteolysis, thus greatly prolonging tissue half-life (mutations that make A-VHD MMP-resistant).

In summary, VEGF structure complexity exposed in these studies points toward still unexplored potential for both deeper insights into biological nature of VEGF signaling and provides new avenues for rationale design of effective biological therapeutic agents tailored to specific clinical needs.

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References

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