PHYSIOLOGY

Outside in and brakes off for lymphatic growth

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In this issue of *Science Signaling*, Kataru *et al.* did two simple but powerful tweaks to the typical studies that aim to advance our understanding of proangiogenic interventions. They shifted the focus from the outside of the endothelial cell to the inside, and they chose not to deliver an angiogenic signal, but instead to release the brakes from an already existing signal.

Antiangiogenic treatments that target the angiogenic growth factors VEGF-A (vascular endothelial growth factor A) and VEGF-C or their receptors have become a clinical reality at the routine and trial level, respectively. Anti–VEGF-A treatment is even considered essential for the treatment of diabetic retinopathy (*1*). On the other hand, proangiogenic treatments have so far not been able to fulfill the high hopes that have been put onto them. For diseases such as coronary artery disease, even moderate effects would represent amazing progress because of the large number of affected patients (*2*). The situation is even less encouraging when we look at the prolymphangiogenic therapies that are under development. In 2018, the small-molecule protease inhibitor Ubenimex failed in phase 2 clinical trials for the treatment of lower leg edema (*3*). Earlier this year, Herantis Pharma announced that it was discontinuing the development of Lymfactin, an adenovirus-based gene therapy delivering VEGF-C, for the treatment of oncology-related lymphedema due to a refocusing on its neurodegenerative drug pipeline. Furthermore, the phase 2 clinical trials for Lymfactin were inconclusive because the baseline lymphedema status of the intervention and placebo groups were apparently not randomly distributed (*4*). This is discouraging news for patients with lymphedema, who have been almost exclusively relying on complex decongestive therapy (CDT) since it was developed about half a century ago (*5*). Despite its importance, CDT only manages the symptoms but does not cure lymphedema. Faced with the lack of pharmaceutical options to treat lymphedema, the results reported by Kataru *et al.* in this issue of *Science Signaling* (*6*) might give us some fresh ideas.

Kataru *et al.* knocked out the phosphatase PTEN (phosphatase and tensin homolog) in lymphatic endothelial cells (LECs) of adult mice. For Akt, one of the primary signaling molecules that channels lymphangiogenic signals mediated by the binding of VEGF-C to VEGFR3, phosphatidylinositol 3,4,5-trisphosphate (PIP_3) binding is a prerequisite for activity. Because PTEN removes PIP_3 from the intracellular pool by converting it into phosphatidylinositol 4,5-bisphosphate (PIP_2), the authors tipped the PIP_3/PIP_2 balance in favor of VEGFR3/Akt signaling with remarkable results (Fig. 1).

Not only did the LEC-specific PTEN-knockout mice develop a denser lymphatic network compared to control mice, but also several common off-target effects of VEGF-C application, such as the attraction of immune cells, inflammation, immaturity of the neovasculature, and spillover effects to the blood vasculature, were absent. It is not clear whether all effects of VEGF-C can be achieved by modulating PTEN activity because the extracellular signal–regulated kinase 1/2 (ERK1/2) pathway appeared unaffected. However, it is perhaps the absence of an effect on the ERK1/2 pathway that contributed to the lack of LEC leakiness that the authors observed in comparison to VEGF-C protein application. In defense of VEGF-C–induced lymphangiogenesis, it must be mentioned that the effects of PTEN deletion were compared to a deletion mutant of VEGF-C, which lacked the inhibitory propeptides that restrict the angiogenic and permeability-inducing side effects of VEGF-C by focusing the VEGF-C effect on the LEC surface (*7*). Although there are ideas on how to further improve growth factor delivery approaches, it is unclear whether the halted clinical trials will be picked up by another biotech or pharmaceutical company any time soon, if ever.

Nonetheless, the effect of PTEN deletion is systemic, whereas VEGF-C growth factor delivery, be it through gene therapy or as an exogenously applied protein, is local by nature. This distinction and the absence of exaggerated responses, which are typical for growth factors at the site of delivery due to high local concentrations, qualify the strategy used by Kataru *et al.* as molecular nudging, a concept in which the functionality of systems is maintained by constant low-level stimulation, which has been proposed to keep our immune systems healthy (*8*), but which might equally be applicable for the treatment of lymphedema (*9*).

The PTEN-deleted LECs showed a threefold increase in proliferation as measured by Ki67 positivity, which is in line with its role as a tumor suppressor, and which is of concern if similar strategies are to be therapeutically deployed. However, the authors did not see any signs of increased tumor development over the entire life of the mice. Translating these results into therapies will not be easy, but perhaps cytoplasmic and nuclear targets are worth another look for proangiogenic treatments. Antiangiogenic small-molecule drugs from the tyrosine kinase inhibitor class have been in the limelight before, but compared to the current antiangiogenic biologics, their specificity for endothelial cells is mediocre at best even for the best in class, let alone their specificity for the lymphatic subset of endothelial cells. Reminiscent of the releasing-the-brakes approach, AKB-9778 is an example of an intracellular drug that activates by inhibiting an inhibitor [the phosphatase VE-PTP (vascular endothelial protein tyrosine phosphatase), which interacts with Tie2 to determine Ang1-mediated lymphangiogenesis and downregulates VEGFR3 and VEGFR2 activity], but its specificity and clinical future are unclear (*10*). This, together with existing small-molecule

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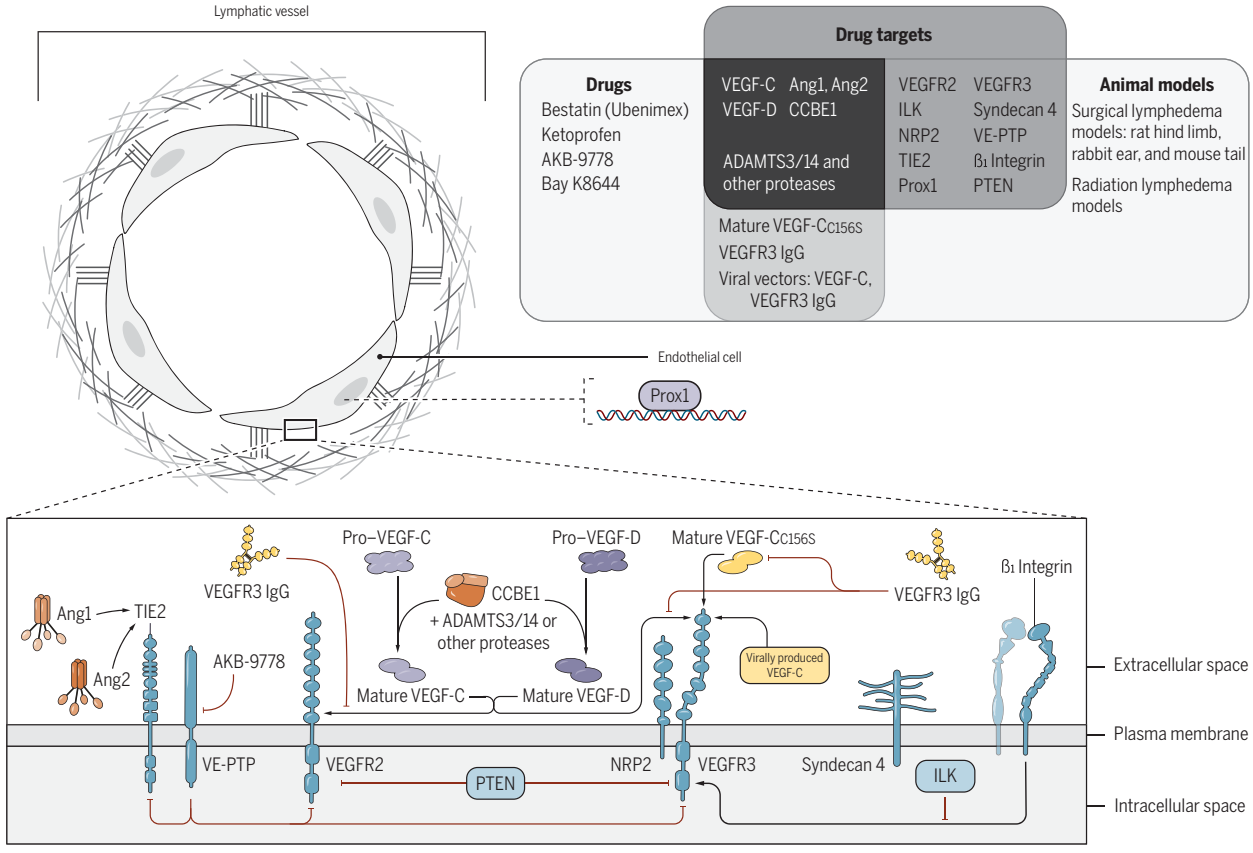

Fig. 1. (Re)search for therapies. The work by Kataru *et al.* lies at the intersection of animal models for lymphatic research and drug targets for diseases that involve the lymphatics. Although the endogenous growth factors, such as VEGF-C, VEGF-D, and the angiopoietins, firmly occupy the center stage of the research, innovative therapies for lymphatics-associated diseases, such as lymphedema, could result from approaching the topic from less conventional angles.

stimulators of lymphatic contractility, shows that small-molecule drugs for lymphedema are, in principle, possible. When used in a targeted fashion by deploying an antibody carrier or exploiting the natural propensity of lymphatics to gather particulate material from the interstitium, otherwise pharmacologically unsuitable compounds might suddenly become interesting. However, what might be lacking is the availability of more sophisticated cell-culture, organoid, or organ-on-a-chip models that make lymphangiogenic growth and functioning amenable to high-throughput screening, starting from VEGF-C expression and activation down to lymphatic vessel proliferation and functional innervation.

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