Imatinib may be ABL to improve anti-angiogenic therapy

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Abbreviations: ABL1, Abelson murine leukemia viral proto-oncogene homolog 1; AKT, protein kinase B, identified in the Akt retrovirus; AMD, age-related macular degeneration; EC, endothelial cell; ECM, extracellular matrix; ERK, extracellular signal-regulated kinases 1 and 2, also known as mitogen activated protein kinases 1 and 3; FDA, U.S. Food and Drugs Administration; ITGN, integrin; PXN, paxillin; NRP1, Neuropilin 1; OIR, oxygen-induced retinopathy; PDR, proliferative diabetic retinopathy; P38, P38 mitogen-activated protein kinase 14; ROP, retinopathy of prematurity; VEGF, vascular endothelial growth factor; VEGFR2, vascular endothelial growth factor receptor 2.

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We recently reported that neuropilin 1 (NRP1) drives angiogenesis by promoting extracellular matrix signaling in endothelial cells via ABL1 kinase. Imatinib targets this pathway in pathological angiogenesis and may provide a novel opportunity for anti-angiogenic therapy of age-related macular degeneration, proliferative diabetic retinopathy, or solid tumor growth.

Neuropilin 1 (NRP1) is a transmembrane protein whose function in endothelial cells (ECs) is commonly attributed to its capacity to act as a receptor for the VEGF165 isoform of vascular endothelial growth factor A (VEGF-A). However, we recently showed that the severe cardiovascular defects observed in *NRP1* knockout mouse embryos are not recapitulated in mice lacking binding of VEGF165 to NRP1.[1] The precise mechanism through which NRP1 promotes angiogenesis in a VEGF-independent fashion was not previously defined. Supporting an extracellular matrix (ECM)-related function for NRP1 in the vasculature, NRP1 has been shown to regulate endocytosis and trafficking of the fibronectin receptor α5β1 integrin in arterial endothelial cells *in vitro*.[2] We have now demonstrated that NRP1 promotes angiogenesis independently of VEGF-A and its canonical receptor, the tyrosine kinase VEGF receptor 2 (VEGFR2), by stimulating ECM-induced signaling pathways and actin remodeling through the non-receptor tyrosine kinase ABL1, originally identified as the Abelson murine leukemia viral proto-oncogene homolog 1.[3] In particular, EC stimulation with fibronectin induces paxillin phosphorylation by ABL1 in a complex with NRP1 to promote endothelial cell spreading and motility *in vitro* as well as vessel sprouting and vascular plexus formation *in vivo*.[3] We therefore propose that the current model for NRP1 function in vascular morphogenesis should be revised to include a NRP1-dependent matrix signaling pathway that regulates angiogenesis through ABL1 independently of, but synergistically with, VEGF-A (**Fig. 1**).[3]

We further showed that the angiogenic NRP1-ABL1 pathway is effectively targeted by imatinib,[3] a small molecule inhibitor of ABL1 kinase activity that is widely used to treat leukemia caused by gain-of-function *ABL1* mutations. Administration of imatinib to mice reduced physiological angiogenesis in the developing retina and pathological angiogenesis in mice with oxygen-induced retinopathy (OIR).[3] In the OIR model, sequential exposure of mouse pups to hyperoxia and then to normoxia first induces vasoobliteration of central retinal capillaries and then the formation of neovascular lesions that resemble those seen in human retinopathy of prematurity (ROP) or proliferative diabetic retinopathy (PDR). Genetic targeting of *Nrp1* in ECs or treatment with imatinib similarly reduced the formation of neovascular lesions in OIR.[3] Thus, targeting NRP1-mediated ABL1 signaling inhibits pathological angiogenesis in mice.

In PDR, VEGF-A is upregulated and stimulates angiogenesis to counter the tissue hypoxia caused by blood vessel damage. VEGF-A upregulation is also seen in the wet form of age-related macular degeneration (AMD), a condition caused by abnormal growth of choroidal vessels into the retina. In both diseases, high VEGF-A levels are associated with fluid leak from vessels, which causes edema and impairs vision. Anti-VEGF therapies such as Lucentis®, Macugen®, Avastin®, or
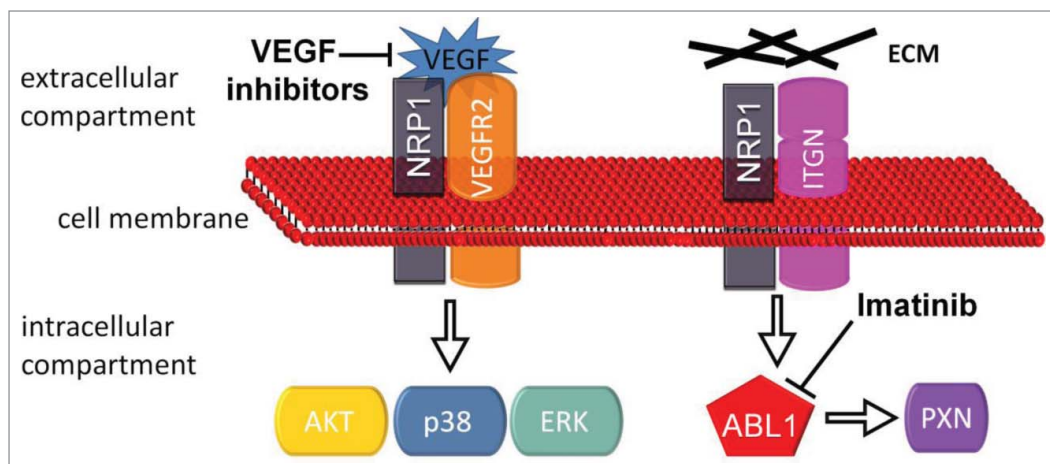
Figure 1. Inhibition of VEGFR2-dependent and ABL1-dependent NRP1 signaling in angiogenesis. Schematic representation of NRP1 function in VEGFR2-mediated and integrin-mediated signaling pathways. These pathways can be targeted independently through VEGF inhibition or imatinib. AKT, also known as protein kinase B; P38, also known as mitogen-activated protein kinase 14; ERK, also known as mitogen activated protein kinases 1 and 3; ITGN, integrin; PXN, paxillin.

Eylea® efficiently target vascular hyperpermeability and are approved treatments for edema in PDR and AMD. These drugs are administered by monthly injection into the eye.

In the case of wet AMD, anti-VEGF therapy stabilizes sight in more than 90% of patients, but only 30% show improved vision,[4] suggesting that this therapy is not sufficient for all patients. Recent evidence also suggests that anti-VEGF therapy is not curative because edema returns as soon as the treatment is discontinued.[5] Furthermore, a multicenter cohort clinical study showed that, after 7 years of treatment with anti-VEGF therapies, only one-third of patients showed good visual outcome and one-third had poor outcome.[6] Because long-term anti-VEGF monotherapy has limited efficacy, there is a need for alternative treatments. The identification of a NRP1-dependent ABL1 pathway that is central to angiogenesis and can be pharmacologically targeted with an FDA-approved drug may therefore open up new therapeutic opportunities for a wide variety of eye diseases with underlying vascular pathology. In particular, our work raises the possibility that the NRP1-ABL1 pathway may be targeted independently of, but synergistically with, VEGF-A (**Fig. 1**) to enhance the efficacy of current therapies, or might even replace anti-VEGF therapies in circumstances where they are unsuitable because the patient is refractory or shows severe side effects to anti-VEGF treatments.

Anti-VEGF was also the first anti-angiogenic therapy approved for the treatment of cancer. Even though there are currently 13 approved anticancer drugs in the US with recognized angiostatic properties (The Angiogenesis Foundation; www.angio.org), so far all have shown limited efficacy in preventing cancer progression.[7] Function blocking antibodies for VEGF-A and NRP1 have an additive effect in reducing vascular density and tumor growth in preclinical studies.[8] Our findings suggest that blocking NRP1-dependent ABL1 signaling with imatinib, either independently or in combination with anti-VEGF therapy, can provide an alternative therapeutic approach to curb tumor angiogenesis. NRP1-ABL1 signaling also promotes tumor growth by stimulating myofibroblast-mediated fibronectin fibril assembly and ECM stiffness,[9] and NRP1 expression in tumors correlates with advanced disease and increased aggressiveness in breast, colorectal, prostate, and hepatic cancers.[10] Therefore, imatinib might additionally be useful to target NRP1-dependent ABL1 signaling in the tumor microenvironment. Imatinib is already approved as a first-line treatment for chronic myelogenous leukemia caused by activating mutations in *ABL1*, and for a few other blood cancers and gastrointestinal stromal tumors that are characterized by mutations in genes encoding the platelet-derived growth factor receptor or the tyrosine kinase KIT, which are also targeted by this drug.

In summary, our findings of NRP1-ABL1–dependent ECM signaling in ECs may stimulate further investigations to extend the therapeutic use of imatinib in eye disease and cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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