VASCULAR BIOLOGY

# Targeting VEGF-induced vascular permeability

Blood vascular leakage in conjunction with stroke causes edema and a worsened outcome. Through augmented and selective tyrosine phosphatase activity, endothelial junctions can be sealed, resulting in reduced stroke volume and improved survival.

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Blood vessels are in continuous exchange with their surrounding tissues. Fluids, molecules and cells pass between the endothelial cells that line the vessel lumen, at specific anatomical locations in the vascular tree. Macromolecules leak into tissues when endothelial cells in capillaries and venules, with a slow blood flow rate, are exposed to vascular endothelial growth factor (VEGF) or inflammatory cytokines[1]. Acute or persistent leakage of macromolecules in stroke, myocardial infarction, retinopathies and cancer worsen the disease process[2]. Suppressed VEGF-regulated leakage without interference with blood supply has been achieved in mouse models by genetic inactivation of key molecules in the leakage process[3]. However, therapeutic tools to specifically suppress macromolecular leakage have been missing. In this issue of *Nature Cardiovascular Research*, Corti et al. identify a strategy to specifically suppress leakage in a mouse stroke model that significantly decreases stroke volume and improves survival in mice, regardless of whether the treatment is applied to prevent or treat stroke[4].

How is macromolecular leakage established? It occurs by a transient decrease in endothelial cell–cell adhesion. Endothelial cell–cell adhesion is governed by two categories of junction complexes: adherens and tight junctions[1]. Adherens junctions consist of vascular endothelial cadherin (VE-cadherin) transmembrane molecules whose extracellular domain forms homodimeric complexes between adjacent endothelial cells while the intracellular part connects through several catenins, to the actin cytoskeleton. Tight junctions consist of a range of molecules that also connect between endothelial cells and with the actin cytoskeleton. While the role of tight junctions in leakage largely remains to be resolved[1], VE-cadherin in adherens junctions has an instrumental role in permitting macromolecular leakage. When exposed to VEGF, endothelial cell–cell junctions partially withdraw. This is accompanied by increased tyrosine phosphorylation and turnover of VE-cadherin[5]. To therapeutically prevent leakage would entail sealing the junctions by, for example, stopping the molecular signals induced by VEGF and acting on VE-cadherin.

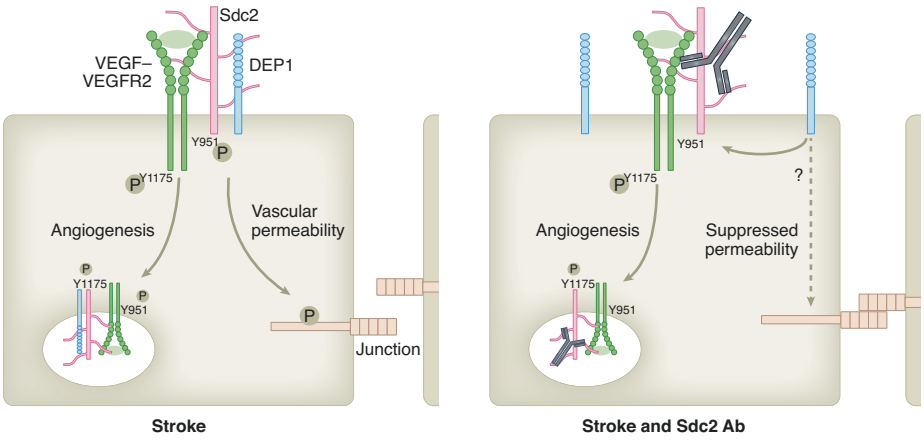


**Fig. 1 | DEP1 dephosphorylation of VEGFR2 Y951 suppresses vascular permeability in stroke.** VEGF–VEGFR2 signaling via phosphorylated tyrosine Y1175 regulates angiogenesis, while phosphorylated Y951 regulates vascular permeability. Signaling is supported by syndecan 2 (Sdc2), which in turn exists in a complex with the tyrosine phosphatase DEP1 (left). In conditions such as stroke, excess vascular permeability results in edema and poor outcome. Neutralizing antibodies (Abs) to Sdc2 releases DEP1, which specifically dephosphorylates Y951 resulting in stable junctions and suppressed permeability (right). Phosphorylation of Y1175 and downstream signaling to angiogenesis is unaffected by DEP1.

VEGF is not unique in its ability to induce leakage, but it is produced at elevated levels by many cell types in disease processes accompanied by hypoxia[6]. VEGF acts through VEGF receptor 2 (VEGFR2) tyrosine kinase on endothelial cells. Therefore, VEGFR2 or its downstream signaling pathways constitute potential targets for a permeability-suppressing therapy. Induction of macromolecular leakage in response to VEGF requires phosphorylation of tyrosine 951 in VEGFR2 (numbering according to the human VEGFR2 sequence)[3]. Through the Y951 site, Src family kinases become activated, which in turn phosphorylate VE-cadherin, causing partial junction disruption and leakage[5].

Phosphorylation at Y951 in VEGFR2 can be eliminated by suppressing VEGF's ability to activate VEGFR2. Indeed, antibody-mediated neutralization of VEGF diminishes stroke volume[2]. However, it is well known from studies on cancer that agents that completely shut down VEGF–VEGFR2 signaling eventually cause endothelial death, loss of vascular supply, and disease progression[7]. Moreover, pan-inhibition of VEGF/VEGFR2 in cancer is associated with hypertension, neutropenia, and bleeding. An added complexity is the biphasic effect of VEGF during injuries such as stroke and myocardial infarction. In the acute phase, VEGF is detrimental, causing increased permeability, while in the recovery phase the pro-angiogenic effects of VEGF promote post-stroke recovery[8]. Therefore, specifically targeting Y951 phosphorylation and suppressing pathological vessel leakage without harming the pro-angiogenic functions of VEGF is highly desirable.

Corti et al. accomplish this specific targeting of VEGF-driven hyperpermeability by increasing the actions of DEP1 (density-regulated phosphatase 1), a tyrosine phosphatase with enhanced activity towards Y951 relative to its activity towards other phosphorylated tyrosine residues in VEGFR2[4]. DEP1 exists in a complex with the heparan sulfate proteoglycan, syndecan 2 (Sdc2)[9]. However, using neutralizing antibodies to Sdc2, Corti et al. disrupt this complex with DEP1[4]. Sdc2, whose heparan sulfate side chains stabilize the VEGF–VEGFR2 complex, accompanies VEGF–VEGFR2 upon internalization, while the receptor continues to send out proliferative signals resulting in angiogenesis, the formation of new blood vessels (Fig. 1). After Sdc2 neutralization, DEP1 now remains on the endothelial surface where its increasing stoichiometric ratio enhances its ability to dephosphorylate Y951, which in turn reduces phosphorylation of VE-cadherin (Fig. 1). Thereby, junctions remain stable and macromolecular leakage is attenuated. Anti-Sdc2 antibodies delivered to the mouse circulation before or after induction of stroke significantly limit the stroke size, improving survival. This opens up new treatment options of potentially very high impact in human stroke therapy.

Interestingly, DEP1 also dephosphorylates VE-cadherin[10], thus potentially enhancing its protective effects on junctions beyond the dephosphorylation of Y951. Whether neutralization of Sdc2 and release of DEP1 would allow its direct action on junctions or have further effects, potentially arising from DEP1 targeting molecules not normally within its range, remains to be shown (Fig. 1). However, as permeability and edema need to be suppressed for a relatively limited time period during the acute stroke phase, such potential side effects may not manifest. Another perhaps more vital question is whether stabilizing junctions through DEP1-mediated dephosphorylation of VEGFR2 Y951 will also block the extravasation of inflammatory cells. Endothelial junctions are important routes of inflammatory cell diapedesis, although passage directly through endothelial cells is also possible[11], which may be unaffected by the junction-stabilizing effect of Sdc2-blocking antibodies. Inflammation is a double-edged sword that may be detrimental or beneficial in disease processes, dependent on the type and timing of inflammatory cell recruitment. Whether junctions resistant to VEGF-driven permeability affect the flavor and dynamics of extravasating inflammatory cells remains an important question. ❐

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## Competing interest

The authors declare no competing interests.