

Encouragingly, compound #76 reduced *in vivo* growth of injected tumoral JAK2V617F-expressing SET2 cells in immunodeficient mice. Again, these data need confirmation in a bona fide mouse model of MPNs.

The key question raised by this study is the nature of the ERK2-D domain substrates contributing to JAK2V617F-driven MPN disease and progression. Do these findings apply to MPN cells driven by driver mutations other than JAK2V617F? For example, in mouse models expressing the active MPL W515A/K, ERK1/2 signaling promoted the MPN phenotype and MF.⁹ Finally, certain ERK2 substrates contain both D and DEF domains (such as Elk1), and the 2 substrate-binding sites communicate spatially in ERK2.¹⁰ It would be of interest to assess whether the substrates that contain both substrate domains contribute to the phenotype of MPNs.

JAK2V617F-driven MPNs remain incurable diseases. Selectively targeting the D domain of ERK2 and possibly key substrates binding to the D domain may become promising avenues of investigation for novel therapies.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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VASCULAR BIOLOGY

Comment on *Goncalves et al*, page 388

Fibrinolysis without intracranial hemorrhage

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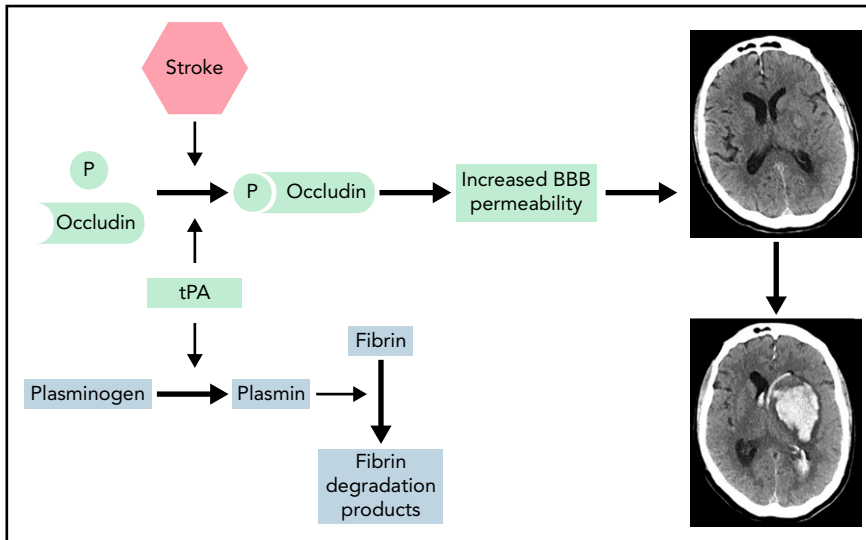
In this issue of *Blood*, Goncalves et al¹ demonstrate that targeting phosphorylation of occludin in the endothelium of the cerebrovasculature significantly reduces the risk of intracranial hemorrhage (ICH) after treatment with recombinant tissue plasminogen activator (rtPA) for acute ischemic stroke (AIS). Intravenous administration of rtPA improves the functional outcome of patients who present at any time up to 4.5 hours after suffering an AIS.^{2,3} Unfortunately, a significant number of patients who receive rtPA develop a hemorrhagic conversion of their ischemic stroke that presents as an ICH. This complication of treatment with rtPA limits its use, with less than 10% of the AIS population receiving treatment. Mitigating the risk of ICH associated with intravenous fibrinolysis can provide increased safety to patients who present with AIS. It may also expand the inclusion criteria for fibrinolysis so that more patients presenting with AIS can receive rtPA therapy.

AIS is a leading cause of the combined morbidity and mortality worldwide affecting more than 15 million people each year.⁴ The only drug approved to treat AIS is rtPA, which must be administered within 4.5 hours of the onset of symptoms to be effective.^{2,3} The main limitation of treatment with rtPA is the risk of hemorrhagic conversion, which is seen in 4% to 6.4% of patients treated with the drug.² The morbidity and mortality associated with bleeding limits the use of rtPA such that only 6% to 10% of patients who present with AIS receive rtPA.

In patients who present with large vessel occlusion (LVO) stroke (defined as whole-vessel thrombus of the intracranial portion of the internal carotid artery and proximal branches of the middle and anterior cerebral arteries), treatment

with rtPA results in recanalization only 10% of the time.⁵ For these patients, who will have significant and persistent deficits without recanalization, endovascular mechanical thrombectomy (MT) is performed. Patients can benefit from MT up to 24 hours from the onset of their stroke.^{6,7} Unfortunately, MT for LVO stroke is undertaken in only 10% of patients, which leaves ~80% of patients with no effective acute treatment options.⁸

Goncalves et al tested the hypothesis that protein kinase C beta (PKCβ) phosphorylation of occludin in endothelial cells is a downstream pathway affected by rtPA-induced platelet-derived growth factor CC (PDGF-CC) signaling in the neurovascular unit during ischemic stroke (see figure). This hypothesis is predicated on previous work that demonstrated that



tPA has a therapeutic role in AIS, but it leads to a significant rate of ICH. Administration of rtPA accelerates the conversion of plasminogen to plasmin, which in turn lyses fibrin to its degradation products. At the same time, tPA induces phosphorylation (P) of occludin, which increases BBB permeability. AIS also upregulates occludin phosphorylation and BBB permeability. Both pathways of occludin phosphorylation and increased BBB permeability increase the risk of ICH in patients presenting with AIS.

tPA induces vessel permeability by activating PDGF-CC.⁹ Rodríguez-González et al¹⁰ clinically validated this finding by reporting that patients who developed ICH after treatment with rtPA had increased plasma levels of PDGF-CC.

The authors used a murine photothrombotic middle cerebral artery occlusion (MCAO) stroke model for all of their in vivo experiments. They showed that stroke-induced occludin phosphorylation occurred on brain endothelial cell fragments at 3 and 24 hours on the affected side. It was undetectable on the non-ischemic contralateral hemisphere. They went on to demonstrate that endogenous tPA regulates phosphorylation. Wild-type mice had significantly increased occludin phosphorylation in brain endothelial cell fragments compared with a tPA^{-/-} strain 24 hours after stroke onset. Goncalves et al then verified this finding by injecting exogenous rtPA into the intraventricular (IVT) space of the brain, resulting in a significant increase of occludin phosphorylation compared with injecting a saline control.

To illustrate that occludin phosphorylation of endothelial cells was necessary to increase blood-brain barrier (BBB) permeability, they used a transgenic mouse that expressed a mutant occludin (S490A). The mutant occludin mouse strain displayed no evidence of Evans

blue dye extravasation after IVT administration of rtPA.

The importance of occludin phosphorylation on BBB permeability in stroke is central to establishing it as a target for therapeutic modulation. Comparing the BBB permeability 24 hours after MCAO, the authors showed increased permeability in control mice compared with the mutant occludin strain.

As a proof-of-concept study to illustrate the potential of phosphorylation inhibition in stroke, pretreatment with a PKC β inhibitor blocked BBB permeability. To further characterize the effect of inhibiting occludin phosphorylation on relevant end points in stroke, mice treated with PKC β demonstrated reduced infarction volume measured via magnetic resonance imaging 3 days after stroke induction. They also demonstrated improved recovery in mice treated with rtPA after stroke compared with saline-treated mice as measured by a lateralized sensory-motor assessment test 7 days after stroke induction.

The authors demonstrated a reduction in ICH 3 days after MCAO in the mutant occludin strain mice compared with the wild-type group when treated with rtPA 5 hours after stroke induction. They validated targeting of occludin phosphorylation in acute cerebral ischemia by inhibiting PKC β for 3 days starting at

either 1 or 5 hours after stroke induction. Animals that received PKC β had lower rates of ICH 3 days after being treated with rtPA at 5 hours after stroke induction.

Clinicians have known about the risk of ICH as a significant complication of rtPA since its approval by the US Food and Drug Administration in 1996 to treat patients who present with AIS.² Strategies used to mitigate rtPA-related ICH include patient selection, dose modification, advanced brain imaging, and administration of antifibrinolytics. The authors have thoughtfully addressed the issue of hemorrhage by developing mechanistic insight into the impact of both AIS and rtPA on BBB permeability, which affects development of ICH. They have also proposed a potential target that may reduce the morbidity and mortality associated with ICH, and may possibly allow more patients to receive the only drug currently approved to treat this debilitating disease.

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