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Response to Letter regarding Article, “Norrin Protected Blood-Brain Barrier via Frizzled-4/ β -catenin Pathway after Subarachnoid Hemorrhage in Rats”

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We appreciate Dr. Zuo and Dr. Xu for their interest in our study and their concerns about the Frizzled-4 siRNA efficacy in our experiments. We concur that the magnitude of suppression of siRNA in vivo was less than that achieved in vitro, therefore we used a pool of three (3) different Frizzled-4 siRNA duplexes to improve the knockdown efficiency. As presented in Figure IV of the Online-only Data Supplement¹, Frizzled-4 siRNA pretreatment significantly inhibited Frizzled-4 receptor expression in ipsilateral hemisphere 48 hours after intraventricular siRNA injection. The average protein level of Frizzled-4 was reduced about 50 percent by siRNA than scrambled siRNA. Most importantly, the Frizzled-4 siRNA did reverse its ligand Norrin's neuroprotective effects on blood brain barrier integrity and early brain injury after subarachnoid hemorrhage.

Furthermore, we investigated the possible mechanism of how Norrin performed these protective effects on blood brain barrier and neurological functions, and found Norrin acted via Frizzled-4/ β -catenin pathway to enhance the expressions of tight junction proteins ZO-1, Occludin and adherent junction protein VE-cadherin¹. On the other hand, Dr. Zuo and Dr. Xu, with reliable speculation, proposed Norrin/Frizzled-4/ β -catenin pathway could also enhance the expression of gap junction protein Connexin-43. Although Connexin-43 is colocalized and coprecipitated with several tight junction proteins, it mainly forms ion channels and allows intercellular trafficking of ions and small signaling molecules through perivascular astrocyte networks. Nagasawa K, et al. demonstrated blocking gap junction did not influence the expression of Occludin, Claudin-5, ZO-1, or their subcellular localization in the blood brain barrier endothelial cells². With Connexin-43 knockout condition, even astrocyte end-feet enlarged, the endothelial cells were structurally normal in appearance, including their tight junctions, which was only vulnerable when subjected to elevated hydrostatic pressure and shear stress³. In addition, Claudin-5, Occludin and VE-cadherin recovered from the second day after blood nerve barrier injury, whereas Connexin-43 was

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Disclosures
None.

redetected on the fifth day⁴. These results indicate that Norrin/Frizzled-4/ β -catenin pathway could preserve tight junction proteins and adherent junction proteins in the early phase after subarachnoid hemorrhage to repair the mechanical barrier of blood brain barrier and alleviate early brain injury.

Currently, most efforts on the blood brain barrier and brain edema focus on vasogenic edema after subarachnoid hemorrhage⁵; Dr. Zuo and Dr. Xu raised the potential cytotoxic edema in their letter, which is an extremely important question, however, cytotoxic edema is mostly verified by an MRI and which is not in the scope of our published study. But we agree with Drs. Zuo and Xu that cytotoxic edema needs to be studied in similar experimental conditions after SAH.

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