DKK3 PLAYS A CRITICAL ROLE IN ISCHEMIC BRAIN DAMAGE AND PULMONARY EMBOLISM AFTER FOCAL CEREBRAL ISCHEMIA/REPERFUSION IN MICE

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Background: Reperfusion is often associated with secondary brain injury and damage to multiple peripheral organs caused by post-stroke systemic inflammation and immune dysfunction. Dickkopf-related protein 3 is a protein in the Dkk family proteins that plays key roles in many essential biological processes. In this study, we investigated the role of DKK3 in ischemic brain damage and pulmonary thrombosis after focal cerebral I/R.

Methods and results: WT and DKK3$^{-/-}$ mice were subjected to severe or mild focal cerebral ischemia induced by middle cerebral artery occlusion (MCAO) for 1 hour and 30 min, respectively, followed by reperfusion. For 1-hour ischemia/24-hour reperfusion, we found no significant differences in infarct volumes and neurologic deficits (Bederson score, grip test) between WT and DKK3$^{-/-}$ mice (Fig. 1A, B, C and D). However, DKK3$^{-/-}$ mice that underwent 1-hour MACO showed a high 24-hour mortality (55.6%, 5/9) compared with 11.1% (1/9) mortality in WT mice. Moreover, the incidence and severity of pulmonary embolism (PE) were also significantly increased in DKK3$^{-/-}$ mice compared with WT mice (Fig. 1E). To increase the survival rate, we reduced the time of MCAO to 30 min. No animals died within 24 hours after 30-min MCAO. The results showed that DKK3$^{-/-}$ mice had larger brain infarct volumes compared to WT mice at 24 hours after tMCAO, and the neurological deficits were also increased in DKK3$^{-/-}$ mice (Fig. 2A, B and C). Both the incidence and severity of pulmonary embolism were significantly increased in DKK3-/- mice compared to WT mice at 24 hours after reperfusion (Fig. 2D). DKK3-/- mice also developed more severe defects of pulmonary perfusion at 4 hours after reperfusion (Fig. 3), with markedly increased circulating platelets hyperresponsiveness (i.e., circulating platelet aggregates was significantly increased in ADP stimulation, Fig. 4) and severity of "no-reflow" phenomenon during reperfusion (Fig. 2E).

Conclusions: The present study demonstrates for the first time that DKK3 deficiency exacerbates brain tissue damage and pulmonary embolism after focal cerebral ischemia/reperfusion, which may be partially caused by the enhanced circulating platelets hyperresponsiveness. Thus, DKK3 might represent a novel and promising therapeutic target for secondary brain damage and pulmonary embolism induced by focal cerebral ischemia/reperfusion.

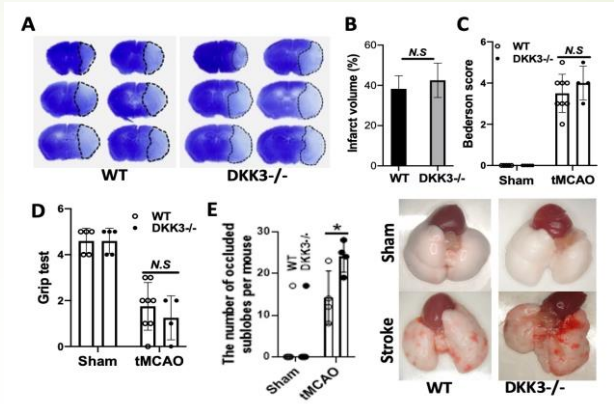

Fig. 1. DKK3 deficiency enhances mortality rate and incidence of pulmonary embolism following severe tMCAO (one hour). A, Cresyl violet-stained coronal brain sections. B, infarct volumes. C. Bederson Score. D, Grip test. E, Representative images of lungs from different groups. The incidence of PE after stroke in WT and DKK3-/- mice was 100% (4/4) and 37.5% (3/8) respectively. N=4-8/group. *N.S* means no significance. *P<0.05.

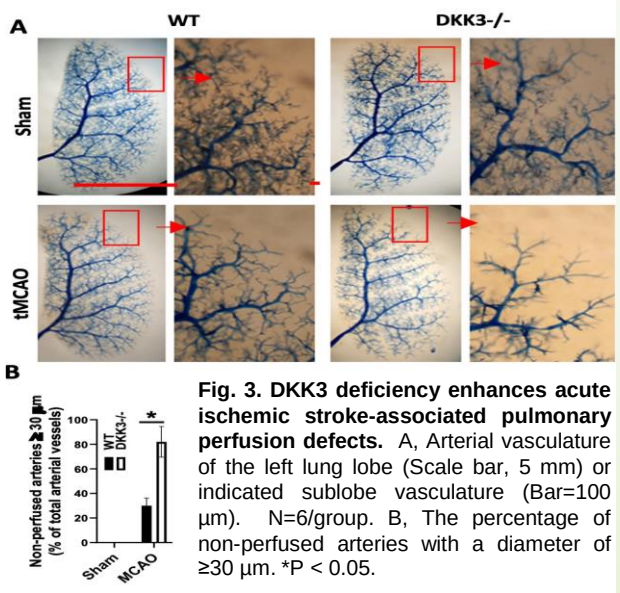

Fig. 3. DKK3 deficiency enhances acute ischemic stroke-associated pulmonary perfusion defects. A, Arterial vasculature of the left lung lobe (Scale bar, 5 mm) or indicated sublobe vasculature (Bar=100 μm). N=6/group. B, The percentage of non-perfused arteries with a diameter of ≥30 μm. *P < 0.05.

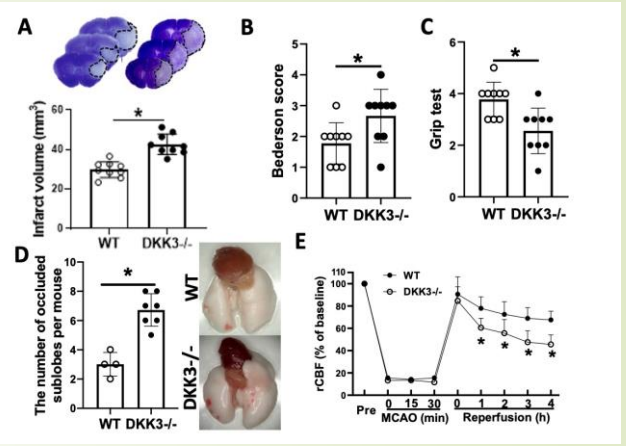

Fig. 2. DKK3 deficiency increases brain damage and pulmonary embolism in mice after mild tMCAO (30-min MCAO). A, Brain infarct. B, Bederson score. C, Grip test. D, rCBF. E, The number of occluded sublobes per mouse (left panel). Representative lung samples from WT and DKK3-/- mice 24 hours after stroke (right panel). Red spot: occluded sublobe. Error bars mean SD. N=9/group. *p<0.05.

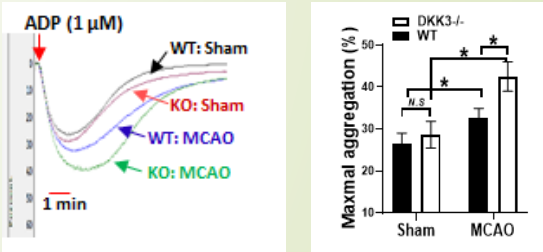

Fig. 4. DKK3 deficiency enhances platelets response to agonist after tMCAO. Platelet aggregation was measured with a platelet aggregometer. PRP was collected at 4 hours after stroke onset and stimulated with agonist (ADP, 1μm). N=6/group. Error bars mean SD. One-way ANOVA followed by the Donnett's post hoc test with selected multiple comparisons was used to assess differences between groups. *P<0.05. N.S: not significant.

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