Inhibition of VEGF signaling prevents *KRAS^{G12V}*-induced brain arteriovenous malformations

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Introduction: Brain arteriovenous malformations (bAVMs) are a major risk factor of cerebral hemorrhages in young patients. Recently, somatic KRAS mutation has identified ~60% of human bAVMs, and we confirmed the causal role of endothelial *KRAS^{G12V}* mutation in bAVM development using a mouse model. Excessive uncontrolled angiogenesis is associated with the abnormal vascular outgrowth in bAVMs. While abnormal VEGF signaling is involved in pathologic angiogenesis, the role in mutant KRAS-induced bAVMs has not been studied. Here, we hypothesized that KRAS mutation induces bAVMs via activation of VEGF signaling, and tested if VEGF inhibition prevents *KRAS^{G12V}*-induced bAVM development.

Methods: We induced bAVMs in mice by systemic injection of AAV-BR1 carrying *KRAS^{G12V}* mutation (KRAS^{G12V} mice). To determine the effect of KRAS mutation on VEGF signaling, we tested the expression of VEGF-A and VEGFR2 in *KRAS^{G12V}*-induced bAVMs *in vivo* and cultured mouse endothelial cells (ECs) overexpressing *KRAS^{G12V} in vitro*. Furthermore, we performed the tube formation assay to test angiogenesis by *KRAS^{G12V}* mutation in the cultured EC. Finally, we treated VEGFR2 inhibitor (SU5416) in the KRAS^{G12V} mice and measured the bAVM size and numbers in the latex-casted brains.

Results: We found that the expression of VEGF-A, VEGFR2, and phospho(p)-VEGFR2 were significantly increased in KRAS^{G12V}-induced bAVMs in mice. We also found that KRAS^{G12V} transfection up-regulates expression of VEGF-A, VEGFR2, and p-VEGFR2, and increased tube formation in cultured ECs. Finally, we confirmed that SU5416 treatment significantly reduced the numbers and size of bAVMs in KRAS^{G12V} mice.

Conclusions: Our data show that endogenous VEGF signaling is activated by KRAS mutation. The VEGF inhibition study suggests that the endogenously activated VEGF signaling would be essential to mediate the mutant KRAS-induced bAVM development.

Key words: brain arteriovenous malformations, KRAS mutation, VEGF signaling, SU5416