Endomucin knockout mice shows delayed retinal vascular development and reduced neovascularization in oxygen-induced retinopathy

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Introduction: Endomucin (EMCN), a specific endothelial glycoprotein, has been shown to play a significant role in regulating VEGF-induced VEGFR2 internalization and downstream activities in vitro. EMCN knockdown inhibits VEGF165-induced VEGFR2 clathrin-mediated endocytosis and endothelial proliferation, migration, and tube formation. The goal of this study is to characterize the functional role of EMCN in vivo normal on retinal vascular development and pathological neovascularization in EMCN knockout mice.

Methods: Homozygous EMCN knock-out ($EMCN^{-/-}$) mice were generated by crossing EMCN-floxed mice with the ROSA26-Cre. We collected the eyes from adult EMCN-/- mice (8–16 weeks) and their EMCN+/+ control littermates. The retinas and RPE/choroids complex were dissected for RNA extraction and qPCR to measure gene expression. Isolectin-B4(IB4) was used to stain the retinal vasculature of adult mice (12–16 weeks old) and P5 mice on retinal flat mounts. In the oxygen-induced retinopathy (OIR) model, P7 mice were housed in 75 percent oxygen for five days and then switched back to room air at P12. Eyes were collected at P12 and P17, and the retinal vasculature was stained using IB4, then the avascular and neovascular areas were quantified using photoshop.

Results: The mRNA level of EMCN in both retinas and RPE/choroids from the $EMCN^{-/-}$ mice was undetectable ($n>4$, $p<0.0001$) by qPCR, compared to $EMCN^{+/+}$ mice. The retinal vascular area/ retina area ratio at P5 was significantly reduced in the $EMCN^{-/-}$ pups compared to $EMCN^{+/+}$ controls (0.14±0.01 vs 0.2±0.013, $p<0.0001$, $n>10$ for both groups). Adult (2-month-old) retinal vascular density remained lower in the $EMCN^{-/-}$ mice compared to $EMCN^{+/+}$ mice (0.135 0.015± vs 0.154±0.017, $p<0.05$, $n= 13$). In the OIR model, the avascular area P12 shows no significant difference between $EMCN^{-/-}$ and $EMCN^{+/+}$ mice (24.57±1.4% vs 23.18±1.0%, $p=0.9$, $n>6$). However, pathological neovascularization at P17 was significantly reduced in the $EMCN^{-/-}$ mice compared to $EMCN^{+/+}$ (8.98 ± 2.9% vs 11.98±1.4%, $p<0.05$, $n>10$) while the avascular area at P17 was comparable between the $EMCN^{-/-}$ and controls (9.9± 1.5% vs 8.85±1.0%, $p>0.5$, $n>10$).

Conclusion: Deletion of EMCN gene reduces retinal vascularization. As a crucial regulator of retinal angiogenesis under both normal development and pathological conditions. EMCN represents a novel therapeutic target for ocular diseased characterized by abnormal growth of blood vessels.