

Astrocytic neogenin regulating netrin-1, a pathway critical for astrocyte tiling and blood vessel maturation in mouse cortical brain

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Rationale

Astrocytes play multiple functions in the brain, including blood vessel (BV) and blood–brain barrier (BBB) development, homeostasis, and function. However, the detailed underlying mechanisms remain elusive. neogenin, a member of deleted in colorectal cancer (DCC) family netrin receptors, highly expressed in astrocytes. neogenin’s functions in BV/BBB development remain un-explored.

Animals and methods

Neo^{fl} mice were crossed with *GFAP-Cre* and *GFAP-CreER^{T2}* mice to generate *Neo^{GFAP-Cre}* and *Neo^{GFAP-CreER}*, respectively. For *Neo^{GFAP-CreER}* and its control mouse, tamoxifen (Sigma-Aldrich) at dose of 100 mg/kg/day was intraperitoneally injected into the mice at age of P30 (for 4 consecutive days) to induce Cre recombination.

Results

Mice with neogenin knocking out (KO) in astrocytes exhibit features of immature BVs in their cortical brain, accompanied with disrupted vascular basement membranes (vBMs), reduced pericytes, leaky BBB, and increased endothelial cell (EC) proliferation. Additionally, neogenin KO astrocytes have an altered distribution without tiling. The increased EC proliferation and

migration are detectable in HUVEC cultures exposed to the condition medium (CM) of neogenin KO astrocytes. Antibody array screening for angiogenetic factors in the CM identifies netrin-1, whose expression in astrocytes is induced by bone morphogenic protein (BMP) signaling, but reduced in neogenin KO cortical astrocytes. Adding netrin-1 into the CM of neogenin KO astrocytes attenuates EC proliferation and migration. Injection of AAV-netrin-1 (under the control of GFAP promotor) into neogenin KO cortical brain ameliorates deficits in BBBs, BVs, and astrocyte tiling.

Conclusion

Taken together, these results uncover an unrecognized pathway, astrocytic neogenin to netrin-1, in regulating astrocyte tiling and vBM, and promoting cortical BV/BBB maturation and function.