

Abstract No.: PO-43

Presentation Type: Poster

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Neuronal Activity–Dependent Metabolic Switch Drives Cerebrovascular Maturation in the Developing Visual Cortex

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• Reciprocal signaling between neurons and vascular cells is essential for brain development and function. Neuronal activity regulates angiogenesis, hemodynamics, and blood–brain barrier (BBB) integrity by directly influencing brain endothelial cells (BECs) or indirectly via astrocytes and pericytes. In this study, we investigated how neuronal activation affects cerebrovascular maturation. Using transcriptome analysis of BECs at postnatal day 10 (P10, before eye-opening) and postnatal day 30 (P30, after eye-opening), we identified 30 light-responsive genes, including metabolite transporters GLUT1 (glucose transporter) and MCT1 (lactate transporter), which showed opposite temporal expression patterns: GLUT1 increased, while MCT1 decreased over time. Bioinformatic analysis revealed transforming growth factor-beta (TGF- β) signaling as a key upstream regulator. In cultured BECs, inhibition of TGF- β signaling modulated the expression of both transporters, whereas TGF- β ligand specifically increased MCT1 levels. Moreover, lactate treatment upregulated TGF- β 1 expression, while MCT1 inhibition reduced it, indicating a positive feedback loop between lactate uptake and TGF- β signaling. Activation of TGF- β signaling induced BBB breakdown, associated with decreased expression of tight junction proteins ZO-1 and Claudin-5. Additionally, abnormal MCT1 expression impaired BBB maturation. Together, these findings demonstrate that a neuronal activity-driven, TGF- β -dependent shift in metabolite transporter expression is critical for cerebrovascular development and proper BBB formation during postnatal brain maturation.

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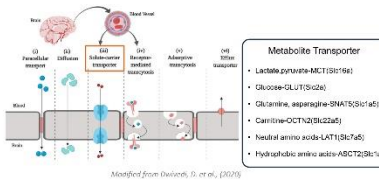
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Abstract

Reciprocal signaling between neurons and vascular cells is essential for brain development and function. Neuronal activity regulates angiogenesis, hemodynamics, and blood–brain barrier (BBB) integrity by directly influencing brain endothelial cells (BECs) or indirectly via astrocytes and pericytes. *In this study, we investigated how neuronal activation affects cerebrovascular maturation.* Using transcriptome analysis of BECs at postnatal day 10 (P10, before eye-opening) and postnatal day 30 (P30, after eye-opening), we identified 32 light-responsive genes, including metabolite transporters GLUT1 (glucose transporter) and MCT1 (lactate transporter), which showed opposite temporal expression patterns; GLUT1 increased, while MCT1 decreased over time. *Bioinformatic analysis revealed transforming growth factor- β 1 (TGF- β) signaling as a key upstream regulator.* In cultured BECs, inhibition of TGF- β signaling modulated the expression of both transporters, whereas TGF- β ligand specifically increased MCT1 levels. Moreover, lactate treatment upregulated TGF- β expression, while MCT1 inhibition reduced it, indicating a positive feedback loop between lactate uptake and TGF- β signaling. Activation of TGF- β signaling induced BBB breakdown, associated with decreased expression of tight junction proteins ZO-1 and Claudin-5. Additionally, abnormal MCT1 expression impaired BBB maturation. *Together, these findings demonstrate that a neuronal activity-driven, TGF- β -dependent shift in metabolite transporter expression is critical for cerebrovascular development and proper BBB formation during postnatal brain maturation.*

Background

Transport routes across the Blood-Brain Barrier (BBB)

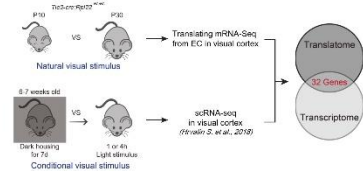


Question

To explore how neuronal activation triggered by light modulates metabolite transporter expression in developing brain endothelial cells

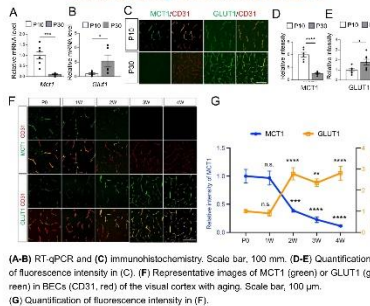
Methods

Neuronal stimulation models by light



Result I

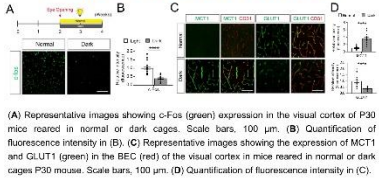
Exposure to light decreases MCT1 expression while inducing an increase in GLUT1



(A–B) RT-qPCR and (C) immunohistochemistry. Scale bar, 100 μ m. (D–E) Quantification of fluorescence intensity in (C). (F) Representative images of MCT1 (green) or GLUT1 (red) in BECs (CD31, red) of the visual cortex with aging. Scale bar, 100 μ m. (G) Quantification of fluorescence intensity in (F).

Result II

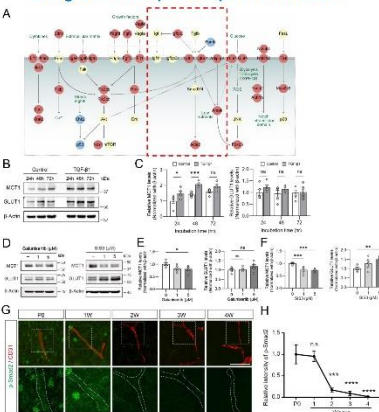
Under dark rearing conditions, the expression patterns of MCT1 and GLUT1 are reversed



(A) Representative images showing F-actin (green) expression in the visual cortex of P30 mice reared in normal or dark cages. Scale bars, 100 μ m. (B) Quantification of fluorescence intensity in (A). (C) Representative images showing the expression of MCT1 and GLUT1 (green) in the BEC (red) of the visual cortex in mice reared in normal or dark cages P30 mouse. Scale bars, 100 μ m. (D) Quantification of fluorescence intensity in (C).

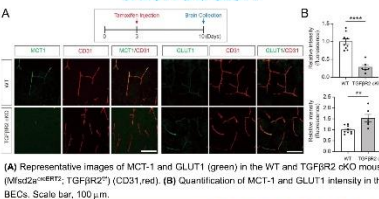
Result III

TGF- β signaling is involved in regulation of lactate and glucose transporter expression in BECs



(A) Gene expression network analysis. (B) Western blot data showing that TGF- β treatment increases MCT1 expression in hMEC/D3 cells. (C) Quantification of intensity in (B). (D) Western blot images showing MCT1 and GLUT1 expression in hMEC/D3 cells after treatment by Galunisertib (TGF- β receptor 1 inhibitor) and SIS3 (Smad3 inhibitor). (E) Quantification of intensity in (D). (F) Representative images of p-Smad2 (Ser465/Ser467) (green) in the BECs (CD31, red). (G) Quantification of p-Smad2 intensity in the BECs. Scale bar, 50 μ m.

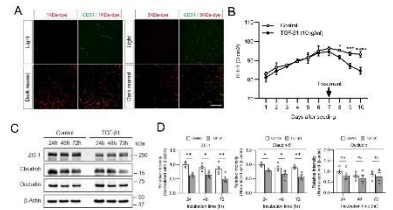
TGF- β 2R2 deletion reverses the expression patterns of MCT1 and GLUT1



(A) Representative images of MCT1 and GLUT1 (green) in the WT and TGF- β 2R2 α KO mouse (Mx22-Cre⁺/TGF- β 2R2^{fl/fl}) (CD31, red). (B) Quantification of MCT1 and GLUT1 intensity in the BECs. Scale bar, 100 μ m.

Result IV

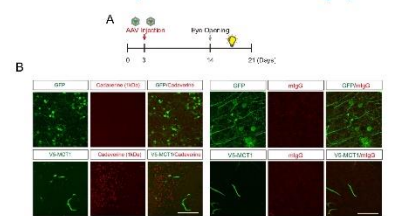
TGF- β 1 signaling negatively regulates BBB maturation



(A) Dark rearing induced the vascular leakage in the visual cortex of P30 mouse (Alexa FluorTM 555 Cadaverine, red; Dextran-Texas RedTM, 3000 MW, Lysine Fixable, red). Scale bars, 100 μ m. (B) Monolayer permeability measurement using TEER (TransEndothelial Electrical Resistance) with EVOM2. (C) Western blot analysis of p-Smad (Ser465/Ser467) and TGF- β 1 ligand following lactate treatment in hMEC/D3 cells. (D) Quantification of tight junction proteins (ZO-1, Claudin-5 and Occludin).

Result V

MCT1 overexpression weakens BBB integrity

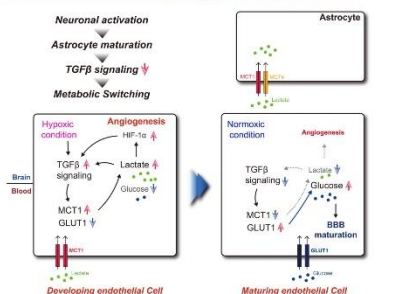


(A) Schedule of dye permeability test. (B) MCT1 overexpression induced the vascular leakage in the visual cortex. Representative images of virus and dye (Alexa FluorTM 555 Cadaverine, red) injection in WT mouse. Scale bars, 100 μ m.

Current conclusion

- ✓ Light-induced neuronal activation alters the metabolic regulation of brain endothelial cells by modulating the expression of MCT1 and GLUT1 in opposing directions
- ✓ TGF- β signaling is involved in regulation of lactate and glucose transporter expression in BECs.
- ✓ Lactate uptake via MCT1 enhances angiogenic potential activates TGF- β 1 signaling during early development.
- ✓ Light-induced neuronal activation is critical for cerebrovascular development and proper BBB formation during postnatal brain maturation.

Working Hypothesis



Acknowledgements

The research was funded by KBRI basic research program of the Korea Brain Research Institute funded by the Ministry of Science and ICT (KBRI 25-BR-01-02), Bio & Medical Technology Development Program of the NRF & funded by the Korean government (MSIT) (NRF-2022M3E5B1017701) to W.O. and Young Researcher Program of the National Research Foundation (NRF) funded by the Korean government (MSIT)(2020R1C1C1010509) to N.K.