



Abstract No.: 0498

Presentation Type: Poster

Primary Topic: Physiology

Secondary Topic: Developing Brain

Neuronal Activity-Driven Shift From Angiogenesis to Vascular Maturation through Metabolic Changes

Namsuk Kim¹, Sehyun Chae^{1,2}, Eun-Young Choi¹, Yan Li¹, Won-Jong Oh^{1*}

¹ Neurovascular unit research group, Korea Brain Research Institute, Republic of Korea

² Department of Biotechnology and Bioengineering, Kangwon National University, Republic of Korea

Background

Brain endothelial cells (BECs) play a crucial role in maintaining the brain's metabolic state by forming the blood-brain barrier (BBB), which regulates the selective exchange of metabolites, nutrients, waste products, and other molecules between the bloodstream and the brain parenchyma. Notably, neuronal activation also influences the brain's metabolic environment by regulating the expression and localization of metabolite transporters in BECs. For a long time, glucose has been considered the brain's primary source, while lactate was viewed merely as a byproduct. However, recent studies have shown that lactate can also serve as fuel and, according to the lactate shuttle hypothesis, acts as a key signaling molecule.

Aim

In this study, we investigated metabolic changes and their functional implications during visual cortex development, driven by neuronal activation.

Method

We employed transcriptome analysis using the RiboTag method to compare BECs at postnatal day 10 (P10) and P30, before and after eye opening, and performed bioinformatic analysis.

Result

We identified 32 genes regulated by neuronal activation. We confirmed that light exposure induces developmental changes in gene expression, which were abolished in dark-reared mice. Among these genes, we focused on two metabolite

transporters: glucose transporter GLUT1, which is involved in vascular maturation, and lactate transporter MCT1, which plays a role in angiogenesis. Interestingly, our translome analysis revealed a significant increase in GLUT1 expression and a marked decrease in MCT1 expression, with these changes reversed in the absence of light. Furthermore, vascular leakage and immature astrocytes were observed in the visual cortex in dark-reared mice. We also found that TGF- β signaling plays a key role in the reciprocal switch of MCT1 and GLUT1 in BECs.

Conclusions

In conclusion, neuronal activity shifts the balance from lactate-driven angiogenesis to glucose-mediated vascular maturation by modulating MCT1 and GLUT1 expression in BECs through the suppression of TGF- β signaling via astrocytes, thereby promoting vascular maturation and BBB formation.

Figure/Table:



Neuronal Activity-Driven Shift from Angiogenesis to Vascular Maturation through Metabolic Changes

Namsuk Kim¹, Sehyun Chae^{1,2}, Eun-Young Choi¹, Yan Li¹, and Won-Jong Oh^{1*}

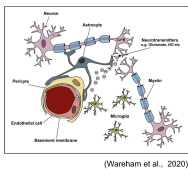
¹ Neurovascular Unit Research Group, Korea Brain Research Institute, Daegu 41062, Republic of Korea.

² Department of Biotechnology and Bioengineering, Kangwon National University, Chuncheon, Gangwon state 24341, Republic of Korea

Abstract

Among Brain endothelial cells (BECs) play a crucial role in maintaining the brain's metabolic state by forming the blood-brain barrier (BBB), which regulates the selective exchange of metabolites, nutrients, waste products, and other molecules between the bloodstream and the brain parenchyma. Notably, neuronal activation also influences the brain's metabolic environment by regulating the expression and localization of metabolite transporters in BECs. For a long time, glucose has been considered the brain's primary source, while lactate was viewed merely as a byproduct. However, recent studies have shown that lactate can also serve as fuel and, according to the lactate shuttle hypothesis, acts as a key signaling molecule. In this study, we investigated metabolic changes and their functional implications during visual cortex development, driven by neuronal activation. We performed transcriptome analysis using the RiboTag method to compare BECs at postnatal day 10 (P10) and P30, before and after eye-opening, identifying 32 genes regulated by neuronal activation. We confirmed that light exposure induces developmental changes in gene expression, which were abolished in dark-reared mice. Among these genes, we focused on two metabolite transporters: glucose transporter GLUT1, which is involved in vascular maturation, and lactate transporter MCT1, which plays a role in angiogenesis. Interestingly, our transcriptome analysis revealed a significant increase in GLUT1 expression and a marked decrease in MCT1 expression, with these changes reversed in the absence of light. Furthermore, vascular leakage and immature astrocytes were observed in the visual cortex in dark-reared mice. We also found that TGF- β signaling plays a key role in the reciprocal switch of MCT1 and GLUT1 in BECs. In conclusion, neuronal activity shifts the balance from lactate-driven angiogenesis to glucose-mediated vascular maturation by modulating MCT1 and GLUT1 expression in BECs through the suppression of TGF- β signaling via astrocytes, thereby promoting vascular maturation and BBB formation.

Background

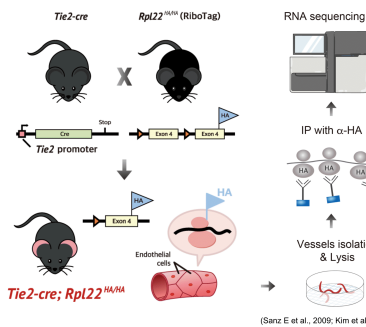


The **neurovascular unit (NVU)** is a complex functional structure composed of neurons, astrocytes, endothelial cells, basal lamina covered with pericytes or smooth muscle cells, and extracellular matrix

The appropriate **communication between components of the NVU** is essential for normal brain functions. In particular, the reciprocal interaction between blood vessels and their neighboring neurons is associated with **major neurodegenerative diseases** including Alzheimer's disease (AD), Parkinson's disease (PD), Frontotemporal dementia (FTD), and Amyotrophic Lateral Sclerosis (ALS)

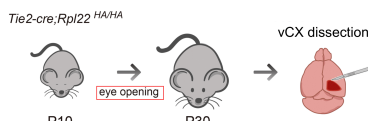
Methods

RiboTag *Translatome* Method



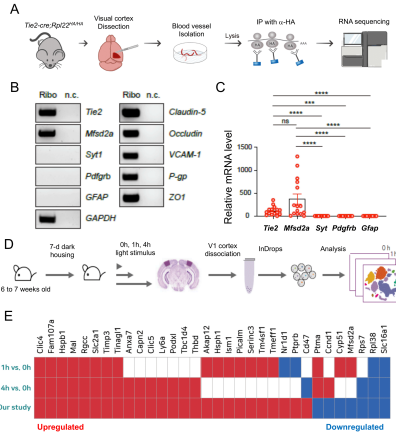
Neuronal stimulation type

- Natural stimuli by light



Result I

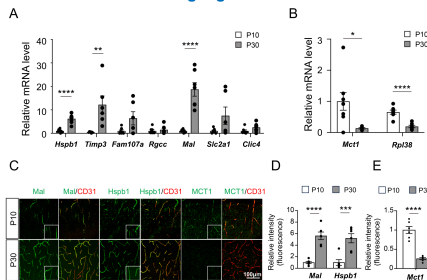
Identification of light-responsive genes through RiboTag Method and Bioinformatic analysis



(A) Workflow diagram and validation of the RiboTag method. (B) RT-PCR to verify the BEC specificity of mRNA pools isolated by the RiboTag protocol. Brain endothelial cell markers: *Tie2*, *Mfsd2a*, *Claudin-5*, *Occludin*, *VCAM-1*, *P-gp*, neuronal cell marker: *Syt1*, astrocyte marker: *Gfap*, and pericyte marker: *Pdgfra*. (C) The specificity of the cDNA library from the cortex of *Tie2-Cre*; *Alb*; *Rpl22^{HAHA}* mice was verified by using RT-qPCR. Mean $\pm$ SEM; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ by the Kruskal-Wallis test, $n = 15$. Bioinformatic approach to identify the light-responsive genes in the visual cortex BECs during development. (D) Schematic Diagram for Natural Light Stimulation in previously published datasets (Havtin et al., 2018, *Nat Neurosci*). (E) Selected 32 genes that were overlapped with the previously published datasets.

Result II

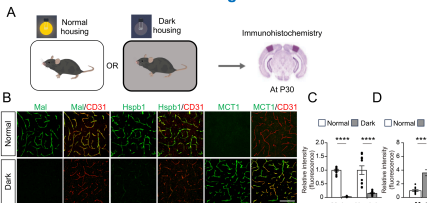
Verification of the target genes in the visual cortex



Validation of upregulated genes (A) and downregulated genes (B) by RT-qPCR. Mean $\pm$ SEM; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ by two-way ANOVA with Tukey's post hoc correction for multiple comparisons, $n = 8$. (C-D) Immunohistochemistry to validate expression of *Mal*, *Hspb1*, and *Slc16a1* (green), in endothelial cell (CD31, red) of the visual cortex at P10 and P30. (*Hspb1*, *Mal*: upregulated genes, *Slc16a1*: downregulated gene). Scale bar, 100 μ m. (D-E) Quantification of fluorescence intensity in (C). Mean $\pm$ SEM; *** $P < 0.001$, **** $P < 0.0001$ by two-way ANOVA with Tukey's post hoc correction for multiple comparisons (D), $n = 6$ or Student's *t* test (E), $n = 6$.

Result III

Changes in expression of target genes were completely abolished in the absence of light



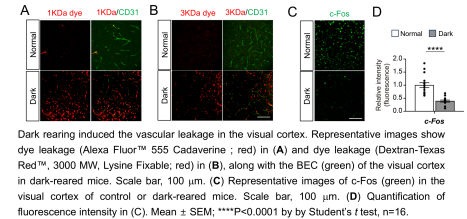
Acknowledgement

The research was funded by KBRI basic research program of the Korea Brain Research Institute funded by the Ministry of Science and ICT (KBRI 24-BR-01-02), Bio & Medical Technology Development Program of the NRF & funded by the Korean government (MSIT) (NRF-2022M3E5E8017701) to WO, and Young Researcher Program of the National Research Foundation (NRF) funded by the Korean government (MSIT) (2020R1C1C1010509) to NK.

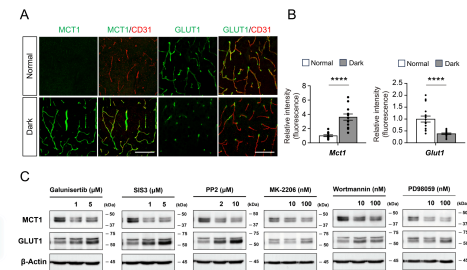
Dark rearing abolished the alternations of light-responsive genes in the visual cortex BECs at P30. (A) Schematic diagram illustrating workflow (B) Representative images showing the expression of *Mal*, *Hspb1*, and *Slc16a1* (green) in the BEC (red) of the visual cortex in mice reared in normal or dark cages. Scale bar, 100 μ m. (C-D) Quantification of fluorescence intensity in (B). Mean $\pm$ SEM; **** $P < 0.0001$ by two-way ANOVA with Tukey's post hoc correction for multiple comparisons (C), $n = 12$ or Student's *t* test (D), $n = 12$.

Result IV

Dark rearing impaired the development of the BBB



Opposing modulation of MCT1 and GLUT1 by TGF- β signaling during development



Current conclusion

✓ Through transcriptome analysis using the RiboTag method and bioinformatic analysis, 32 genes have been identified as light-responsive in developing brain endothelial cells (BECs) of the visual cortex.

✓ 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 may be involved in the pathway that regulates the opposing modulation of MCT1 and GLUT1 expression.

Hypothesis

