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An invited review following *the Soujinkai Award*: Human Induced Pluripotent Stem Cell Modeling of the Blood-Brain Barrier Identifies Intrinsic Barrier Dysfunction in Multiple Sclerosis

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Abstract Blood-brain barrier (BBB) dysfunction found in the multiple sclerosis (MS) cases is generally considered as a consequence of neuroinflammation. In this study we challenge this view by developing and analyzing novel BBB model from MS patients using induced pluripotent stem cells (iPSCs). We differentiated iPSCs into brain microvascular endothelial cell (BMEC)-like cells to establish an *in vitro* BBB model. We found that BMEC-like cells from MS patients exhibited compromised barrier integrity, characterized by weakened junctions, heightened permeability, and an elevated inflammatory profile when compared to cells from healthy individuals. Notably, the activation of the Wnt/β-catenin signaling pathway led to improvements in barrier function and a reduction in inflammatory responses, indicating potential therapeutic targets for reinforcing BBB stability in MS.

Key words: blood-brain barrier, multiple sclerosis, hiPSCs, Wnt/β-catenin signaling

Introduction

Multiple sclerosis (MS) is considered as an autoimmune disease that affects the central nervous system (CNS), leading to chronic inflammation, demyelination, and neurodegeneration.[1] While the exact cause of MS remains elusive, current treatments typically focus on modulating the immune response. Emerging evidence suggests that blood-brain barrier (BBB) breakdown is a significant factor in the progression of the disease.[2] The BBB serves as a selective barrier that protects the CNS by preventing harmful substances in the bloodstream from entering the brain while allowing essential molecules to pass through. In MS, early-stage lesions often involve focal disruptions of the BBB, as detected by gadolinium-enhanced MRI. Furthermore, extravasation of serum proteins such as IgG or fibrin has been detected in autopsy samples of MS patients, even in lesions where MRI shows no abnormal signal (normal appearing white matter: NAWM).[3] These findings underscore that BBB breakdown is a consistent feature throughout the course of MS. Interestingly, serial MRI scans of MS patients and animal models suggest that BBB disruption may precede immune cell infiltration and subsequent demyelination,[4,5] positioning the BBB as a potential therapeutic target for preventing relapse and even disease progression. However,

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the lack of effective disease models for analyzing BBB function has made it difficult to study the active involvement of the BBB in MS pathogenesis. Therefore, we developed a BBB model derived from MS patients to better understand the mechanisms underlying BBB breakdown in MS and to explore whether the BBB could serve as a therapeutic target for MS.

iPSC Modeling Recapitulates BBB Breakdown in MS

We first investigated whether the BBB breakdown observed in MS autopsy samples could be replicated *in vitro* using two established methods: the defined medium method (DMM)[6] and the extended endothelial cell culture method (EECM).[7] We focused on two major hallmarks of BBB dysfunction found in MS autopsy samples: (1) tight junction disruption and (2) upregulation of adhesion molecules.[8,9] Indeed, we showed that tight junction proteins, such as claudin-5 and occludin, were disrupted in MS-derived cells, whereas they remained intact in healthy control cells. Additionally, cell surface adhesion molecules, including ICAM-1 and VCAM-1, were upregulated in MS-derived cells compared to healthy controls (Fig. 1). These results highlight the effectiveness of using iPSC-based disease modeling to study morphological changes at the BBB.

MS-Derived BMEC-Like Cells Exhibit BBB Leakiness and Enhanced Immune Cell Recruitment

We then investigated two critical functions of the BBB related to observed morphological changes: (1) serum protein leakage and (2) immune cell recruitment, to determine whether the BBB actively contributes to MS pathogenesis. By measuring transendothelial electrical resistance (TEER) and assessing permeability to sodium fluorescein as an indicator of diffusion barrier integrity, we observed that MS-derived BMEC-like cells exhibited lower TEER values and higher permeability to small molecules compared to healthy controls (Fig. 2). Furthermore, the upregulation of ICAM-1 and VCAM-1 in MS-derived BMEC-like cells was found to be functional, as a greater number of allogeneic T cells and autologous PBMCs not only adhered to but also transmigrated through the MS-derived BMEC-like cells (Fig. 3). Importantly, we

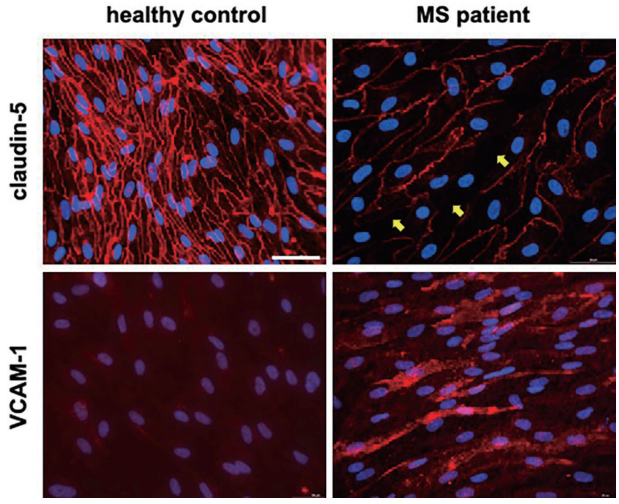

Fig. 1 MS-patient derived EECM-BMEC-like cell recapitulate BBB abnormalities observed in MS autopsy sample.

BMEC-like cells were differentiated using the extended endothelial cell culture method (EECM). Tight junction protein claudin-5 and adhesion molecule VCAM-1 were assessed by immunocytochemistry. In MS-derived cells, claudin-5 shows discontinuity (indicated by yellow arrows) and VCAM-1 expression is upregulated following proinflammatory cytokine stimulation. Scale bar shows 50 μm.

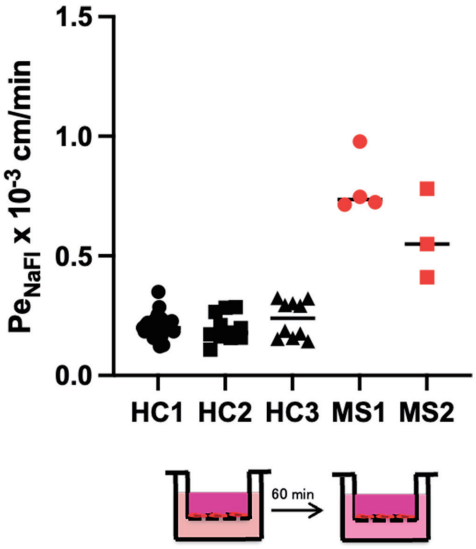

Fig. 2 MS-patient derived EECM-BMEC-like cell exhibit BBB leakiness.

EECM-BMEC-like cells were cultured on a Transwell system. The permeability of these cells was assessed by measuring the clearance of sodium fluorescein. MS patient-derived EECM-BMEC-like cells demonstrated higher permeability compared to healthy controls (HC).

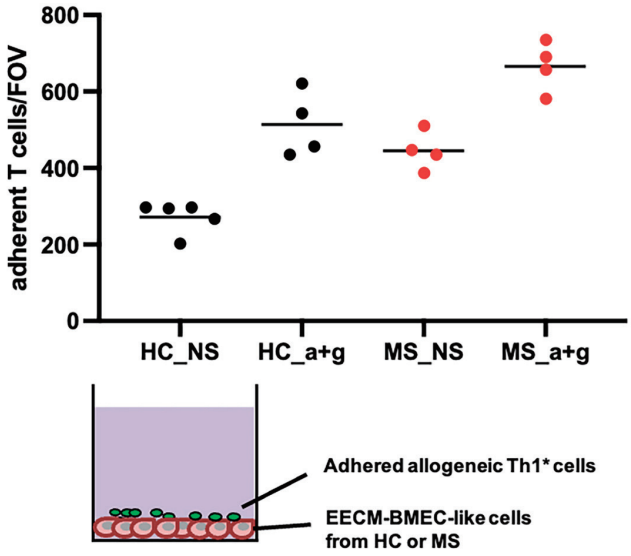

Fig. 3 Increased immune cell adhesion to MS patient-derived EECM-BMEC-like cells compared to healthy controls.

EECM-BMEC-like cells were cultured on chamber slides, and fluorescently labeled T cells were allowed to adhere to the BMEC-like cells. Allogeneic T cells showed increased adhesion to MS patient-derived EECM-BMEC-like cells compared to healthy controls.

observed these abnormalities in the absence of neuroinflammatory conditions, as the assay was simplified to avoid interactions with CNS resident cells. These results underscore that BMEC-like cells actively contribute to MS pathogenesis by promoting protein leakage and facilitating immune cell migration.

Wnt/β-Catenin Signaling Enhancement Rescues BBB Abnormalities in MS

We next investigated whether our novel model could serve as a tool to develop therapeutic strategies targeting BMECs. Given the critical role of Wnt/β-catenin signaling in BBB development and maintenance,[10,11] we focused on enhancing the barrier properties of BMEC-like cells by activating this pathway. CHIR99021, a known activator of Wnt/β-catenin signaling, was used on MS-derived endothelial progenitor cells (EPCs) in a plastic state. Treatment with 4 μM CHIR99021 restored claudin-5 expression in MS-derived EECM-BMEC-like cells (Fig. 4A). This treatment also reduced permeability to small molecule tracers to levels comparable with healthy controls (MS-derived EECM-BMEC-like cells from EPCs treated with CHIR99021: $0.147 \pm 0.081 \times 10^{-3}$ cm/min versus HC-derived EECM-BMEC-like cells: $0.208 \pm 0.058 \times 10^{-3}$ cm/min). Additionally, activation of Wnt/β-catenin signaling led to a reduction in cell surface VCAM-1 expression (Fig. 4B). These results demonstrate that our novel MS patient-derived BMEC-like cells are valuable for identifying compounds that can artificially enhance BBB properties.

Conclusion

This study highlights the critical role of BBB integrity in MS pathogenesis and underscores the potential of targeting BBB dysfunction in therapeutic approaches. The use of iPSC-derived BMEC-like cell models offers a powerful tool for investigating the molecular mechanisms underlying BBB dysfunction in MS and identifying novel therapeutic targets. Future research should aim to further elucidate the involvement of BBB dysfunction

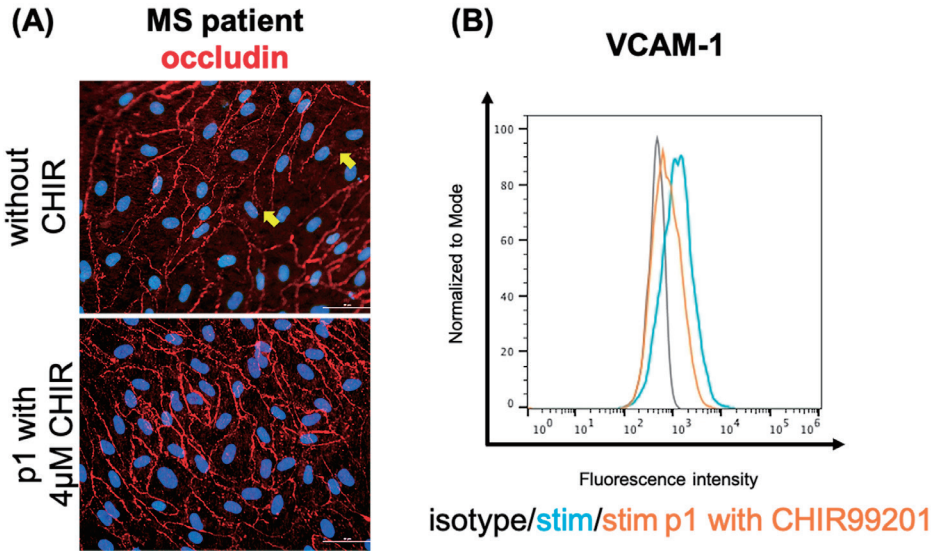

Fig. 4 Wnt/β-catenin signaling activation restores BBB integrity in MS patient-derived EECM-BMEC-like cells.

Endothelial progenitor cells undergoing differentiation into EECM-BMEC-like cells were cultured in the presence or absence of the GSK3 inhibitor CHIR99021, a Wnt/β-catenin signaling activator. (A) MS patient-derived EECM-BMEC-like cells exhibited disrupted occludin staining, which was restored upon Wnt/β-catenin signaling activation. (B) Cell surface VCAM-1 expression was assessed by flow cytometry. Pretreatment with CHIR99021 reduced VCAM-1 expression on the cell surface under proinflammatory cytokine stimulation.

in MS and explore the therapeutic potential of Wnt/β-catenin signaling activation in clinical settings.

Conflict of Interest

The author declares no conflict of interest.

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