学 位 論 文 の 要 旨

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学　位　論　文　名　Human Mesenchymal Stem Cell-Transplantation Changes Proinflammatory Gene Expression Through NF-κB-Dependent Pathway in a Rat Focal Cerebral Ischemic Model

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論 文 内 容 の 要 旨

INTRODUCTION

In cerebral ischemia, macrophage/microglia accumulate in the lesion area, and play important roles by producing cytokines, growth factors, reactive oxygen species and phagocytosis of dead tissue. The overactivated macrophage/microglia increases production of proinflammatory cytokines, which deteriorate neuroinflammation and increase of apoptotic neuronal loss.

In our previous reports, we have demonstrated that transplantation of human mesenchymal stem cells (B10 MSC) decreased lesion size and neurological deficits in transient cerebral ischemic rats. These changes were accompanied by decreased macrophage/microglia accumulation and expression of proinflammatory factors, suggesting that transplanted B10 cells play a key role in regulation of the activation status of macrophage/microglia. However, the underlying mechanisms of such regulation are still elusive.

Transcription factor, such as nuclear factor κB (NF-κB) has been reported to express in cerebral ischemia where it can modulate the expression of several proinflammatory genes including inducible nitric oxide synthase (iNOS), interleukin-1β (IL-1β), tumor necrosis factor α (TNFα), cyclooxygenase-2 (Cox-2) and monocyte chemoattractant protein-1 (MCP-1). Our previous studies demonstrated that B10-transplantation inhibits iNOS expression in macrophage/microglia. As iNOS was the downstream factors of NF-κB signaling pathway, we

hypothesized that B10-transplantation might regulate these proinflammatory genes through the modulation of NF-κB signaling in macrophage/microglia in cerebral ischemic rats.

MATERIALS AND METHODS

Transient focal cerebral ischemia was induced by middle cerebral artery occlusion (MCAO) in adult male Sprague-Dawley rats (8 weeks old). After 24 hours, vehicle (PBS) or human MSCs (B10) were transplanted intravenously. The neurological deficits were monitored by modified neurological severity score system (mNSS), and the infarct volume was monitored by MRI. The rats were sacrificed 3 and 5 days after MCAO. Transplanted B10 cells (human origin) were identified by immunofluorescence staining of human nuclei. The accumulation of macrophage/microglia was analyzed by immunofluorescence staining of ionized calcium binding adaptor molecule 1 (Iba-1) and CD68 (ED1). The transcription factors including C/EBPβ and NF-κB were quantified by Western blotting, and their localizations were analyzed by double immunofluorescence staining using ED1 as a macrophages/microglia marker. The ED1-positive macrophage/microglia in the core and the Iba-1-positive macrophage/microglia in penumbra were microdissected by laser capture microdissection, and their mRNA expressions were analyzed by real time-PCR. The localizations of TLR2 and CD40 were analyzed by double immunofluorescence staining of CD40 and ED1, or TLR2 and ED1. To analyze the signaling pathway, double immunofluorescence staining of NF-κB and CD40, or NF-κB and TLR2 were employed.

RESULTS AND DISCUSSION

The evaluation of infarct volume and neurological deficits revealed that they were decreased in the B10 group at Day 5 ($p < 0.05$). The immunofluorescence results revealed that transplanted B10 cells migrated to the ischemic core and the penumbra, which were in the vicinity of macrophage/microglia. The number of B10 cells peaked at Day 3 and gradually decreased. The activated morphology and increased number of macrophage/microglia were displayed in the ischemic region. In the penumbra, only Iba-1-positive macrophage/microglia was found, which had ramified morphology, and the cell number was decreased in the B10 group ($P < 0.05$). In the core, a plentiful of both ED1-positive and Iba-1-positive macrophage/microglia were found, which had round shaped morphology. Moreover, the 77.7% of Iba-1-positive cells were positive for ED1 in the PBS group, whereas that was decreased to 42.7% in the B10 group. The ED1-positive cells include blood-borne macrophages and phagocytic microglia. Hence,

these results indicated that the B10 cells inhibited the recruitment and the transformation of the phenotype of macrophage/microglia.

The Western blotting results revealed that the transcription factor NF-κB protein level was selectively decreased in the core region in the B10 group at Day 3 and Day 5 ($p < 0.05$). In the core region, the NF-κB was localized mainly in macrophage/microglia, and consistent with the Western blotting results, the number of NF-κB-expressing macrophages/microglia was decreased. Then we analyzed the mRNA levels of NF-κB dependent gene in macrophage/microglia in the core and the penumbra of MCAO rat brains, and found the IL-1β, TNFα, iNOS and MCP-1 were decreased in ED1-positive and Iba-1-positive cells in the B10 group. On the other hand, the mRNA of the cytokines that inhibits NF-κB signaling, such as IL-4 and IL-10 were increased in ED1-positive cells in B10 group, therefore, the B10-transplantation might induce an autocrine inhibitory effect on NF-κB signaling. Taken together, our results suggested that B10-transplantation causes changes in the activation of the macrophages/microglia, rendering it to acquire an anti inflammatory phenotype.

As cell surface receptors including TLR2 and CD40 play a crucial role in the activation of NF-κB signaling pathway in the early periods of MCAO, the expression of TLR2 and CD40 were analyzed. Our immunofluorescence results revealed that TLR2 and CD40 were abundantly expressed in ED1-positive macrophage/microglia in the core area. The percentage of TLR2-positive and CD40-positive cells in macrophage/microglia population was significantly decreased in the B10 group at Day 5 ($P < 0.05$). Moreover, the percentage of NF-κB-positive cells in TLR2-positive or CD40-positive cells population was significantly decreased in the B10 group at Day 5 ($P < 0.05$). As a result of decreased expression of the receptors that activate NF-κB, the possibility of such pathway activation is reduced.

Therefore, B10 could affect NF-κB signaling at least three levels, a) at receptor level by inhibiting the expression of TLR2 and CD40, b) at activation level by decreasing proinflammatory cytokines expression and increasing anti-inflammatory cytokines expression, and finally, c) by inhibiting the expression of NF-κB itself through inhibiting its activator such as IL-1β and TNFα.

CONCLUSION

In conclusion, our study clearly demonstrates the inhibitory effects of B10 MSC on NF-κB signaling in the cerebral ischemic condition, and decreased proinflammatory gene expression. This inhibitory effect might be a key feature that ensues the beneficial effects of B10 MSC transplantation.