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Establishment of Adult Stem Cell from Human Umbilical Cord Vein and its Therapeutic Effect on Ischemic Stroke Model

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Introduction

Cell replacement therapy has been recognized as a new therapeutic strategy in neurodegenerative disorders. Embryonic stem (ES) cells, neural progenitor cells, and adult stem cells might be used in cell therapy for the replacement of lost neuronal cells. However, ethical concern as well as the potential dangers of teratoma formation and infection render it difficult to use pluripotent ES cells and immortalized adult stem cells as a therapeutic option. We have established human umbilical cord vein-derived stem cells (HUSCs), which are new adult stem cells. These cells are similar to previously reported other adult stem cells derived from human umbilical cord, and these cells were shown to be able to differentiate into adipocytes and osteocytes. However, whether these cells could differentiate into neuronal cells in vitro and in vivo has not yet been investigated. In the present study, we examined whether HUSCs could be used for cell replacement therapy of ischemic stroke model. We first analyzed the characteristics of the HUSCs, and then investigated whether the HUSCs could differentiate into neuronal cells in vitro and in vivo. Finally, we studied whether the HUSCs could improve neurological symptoms, when implanted into the brain of the ischemic stroke rat model.

Materials and Methods

The isolation and culture of HUSCs from human umbilical cord vein were carried out by the method previously described. HUSCs were labeled with PKH26. Cells cultivated on chamber slides were fixed with 2% paraformaldehyde in PBS for 2 hours at 4°C. After washing with PBS, cells were incubated with 3% hydrogen peroxide and blocked.

Results

Characteristics of the HUSCs are followings: Morphologically, the HUSCs showed fibroblast-like appearance, closely resembling bone marrow-mesenchymal cell (BM-MSC). RT-PCR analysis revealed that HUSCs expressed genes of BMP-4, PAX-6, Oct-4, SCF, ADAM12, nestin, and HLA class I. The HUSCs showed distinct immunoreactivity against fibronectin, collagen type I, II, III, IV and XII, intracellular cell adhesion molecule-1, vascular cell adhesion molecule-1, homing cell adhesion molecule, vimentin, von Willebrand factor, and TRA-1-60. After culture for 20 days in a condition allowing neuronal differentiation, 77.4% of HUSCs exhibited immunoreactivity against the neuron-specific beta III tubulin antibody. When HUSCs were implanted into the brain of the ischemic stroke rat (HUSC group, n=10), the neurobehavioral deficit severity score after 3 weeks significantly decreased in the HUSC group (2.22 ± 0.62), compared to the control group (n=7) (-0.29 ± 0.42) ($p<0.007$). Histochemical studies showed localization of implanted HUSCs in the ischemic damaged hemisphere, including areas of ipsilateral corpus callosum, circumference of nucleus accumbens, and subventricular zone of third ventricle, and these cells were immunochemically positively stained with neuron-specific markers.

Conclusion

Based on these observations, it is suggested that the HUSCs derived from the human umbilical cord vein could be used for therapeutic modality of various incurable neurologic diseases, particularly, ischemic stroke.