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Research Report

Improvement of deficits by transplantation of lentiviral vector-modified human amniotic mesenchymal cells after cerebral ischemia in rats

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ABSTRACT

Amniotic membrane is known to have the ability to transdifferentiate into multiple organs and is expected to stimulate a reduced immunologic reaction. Human amniotic membrane-derived mesenchymal stem cells (hAMCs) do not express the major histocompatibility complex (MHC) class I molecule and may be expected to show immunologic tolerance. A good deal of research has explored the clinical therapeutic potential of hAMCs. In the present study, we isolated hAMCs and transfected them with the brain derived neurotrophic factor (BDNF) gene using lentiviral vectors. These cells were then transplanted into the brains of rats subjected to a transient middle cerebral artery occlusion (MCAO). The hAMCs survived for three weeks in the brains of the ischemic rats, and some of the transplanted hAMCs expressed the neuronal marker MAP2 and the neuronal progenitor marker Nestin. Furthermore, caspase-3 activity and iNOS expression were decreased in the vicinity of the graft and injection site. Importantly, intracerebral grafting of EGFP-modified hAMCs and BDNF-transduced hAMCs significantly ameliorated behavioral dysfunction in ischemic rats. BDNF-hAMCs ameliorated the behavioral dysfunction of rats more rapidly and effectively relative to EGFP-hAMC-treated rats. Finally, the grafts also reduced the infarct volume. hAMCs survived in the brain tissue and improved functional recovery.

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Abbreviations: hAMCs, Human amniotic mesenchymal cells; BDNF, brain derived neurotrophic factor; iPS, induced pluripotent stem; MCAO, transient middle cerebral artery occlusion; HAM, human amniotic membrane; ECA, external carotid artery; ICA, internal carotid artery; TTC, 2,3,5-triphenyltetrazolium chloride

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Because of the lack of ethical concerns and the high supply of these cells, hAMCs represent a promising clinical treatment for gene delivery similar to stem cell strategies.

1. Introduction

Stroke remains a leading cause of death and disability; approximately 20% of people who suffer from a stroke die within the first week (Towfighi et al., 2010). However, although 70% of stroke patient survive considerably longer after a stroke, and despite intensive research, few treatment options are available.

Experimental animal models and human trials have demonstrated great promise for stem cell transplantation to restore brain function post-injury (Barinaga, 2000; Gogel et al., 2011; Lee et al., 2010). Embryonic stem cells and induced pluripotent stem (iPS) cells can be differentiated into cells of various organs (Wernig et al., 2007; Yamanaka, 2007; Yamanaka and Blau, 2010), including cardiomyocytes, there are many underlining problems to overcome before clinical applications can be used. Among them, tumorigenicity is a serious concern. Autografts of iPS cells may not cause immunologic rejection; ironically, this will also cause possible neoplasm formation to escape from immune surveillance (Ludwig et al., 2006; Yamanaka, 2009), ethical and political concerns have limited the use of fetal stem cells and ES cells.

Adult stem cells that are capable of contributing trophic support or integrating into functional synaptic networks with host tissues are being developed for therapeutic use. Several studies have reported the successful use of various adult stem cell lines to treat animal models of ischemia, including rodent mesenchymal stem cells (Chen et al., 2001; Gogel et al., 2011; Zhang et al., 2011), conditionally immortal clonal stem cell line (Pollock et al., 2006), human bone marrow stem cells (Zhao et al., 2002), human umbilical cord blood cells (Chen et al., 2001a,b; Ou et al., 2010), and human teratocarcinoma-derived neurons (Borlongan et al., 1998). However, risk of forming neoplasm and host immune response to some cell lines is serious concerns for the clinical use of these cells. Therefore, in the present study, we evaluated the use of human amniotic mesenchymal cells (hAMCs) as a therapeutic cell resource and as gene carrier in the ischemic brain.

The human amniotic membrane (HAM), formed from the epiblast on about the eighth day post-fertilization, plays a significant role in the suppression of the semiallo immune response against the fetus, and HAM also excretes various nutritive factors (Diaz-Prado et al., 2011; Insausti et al., 2010). HAM has been used clinically for transplantation to promote the regeneration of mesenchymal cells in burns and skin ulcers, or as a dressing for wounds or skin grafts (Kim et al., 2009; Subrahmanyam, 1995). Brain derived neurotrophic factor (BDNF) can promote the survival and differentiation of neuronal tissue through its influence on receptor kinases (Boesmans et al., 2008; Horne et al., 2010; Lim et al., 2008; Schabitz et al., 2000). BDNF may increase neuronal survival following ischemia, as intraventricular administration of BDNF prior to focal cerebral ischemia or intraparenchymal BDNF infusion post-ischemia both significantly reduced cerebral infarct volume (Blaha et al., 2000; Nomura et al., 2005; Takeshima et al., 2011). However, due to the acute nature of these administration routes, this therapeutic effect cannot not be continuously maintained. To allow a long term exposure of neuronal cells to BDNF post-ischemia, one potential approach is to implant stem cells that stably express BDNF into specific brain regions.

In the present study, we have modified hAMCs with a lentiviral-based packaging system, which has previously been used to infect non-dividing cells and primary tissue, as well as to generate transgenic animals from several species (Lois et al., 2002; Pfeifer et al., 2002; Wiznerowicz and Trono, 2005). Genes carried by lentiviral vectors are stably and efficiently expressed in the host without expressing immunogenic viral components (Imren et al., 2004).

In the present study, we transplanted EGFP (enhanced green fluorescence protein)-labeled and BDNF overexpressing hAMCs into the ischemic rat brain after middle cerebral artery occlusion (MCAO) and observed the graft integrated and migrated in the rat brain, and gaft also significant decreases behavioral dysfunction and infarct volume. So, lentiviral vector-modified hAMCs can be used as both a stem cell therapy and a gene delivery system. Moreover, their use in clinic may avoid potential immunological reactions and reduces the leaky expression of the lentiviral proteins *in vivo*. hAMCs are easy to obtain and do not present the same political and ethical issues of embryonic stem cells. Therefore, this model represents a safe and effective option for stem cell-mediated gene therapy.

2. Results

2.1. *Three germ layers differentiation of human hAMCs*

hAMCs exhibited differentiated cell type characteristics (Fig. 1). We used various antibodies to rigorously assess the multipotent character of the hAMCs: the stem cell marker (Nanog, Oct-4, SOX2), mesoderm markers (vimentin, Bhy), and endoderm markers (AFP), ecdoderm markers (beta3-tubulin, nestin, GABA). Results showed that hAMCs can be labeled with three germ layer markers (Vimentin, Bhy, AFP, beta3-tubulin, nestin and glutamate). hAMCs can therefore yield differentiated cells corresponding to each of the three embryonic germ layers and serve as precursors to a broad spectrum of differentiated cell types.

2.2. *Survival of transplanted hAMCs in the ischemic rat brain*

In the EGFP-hAMC transplanted group, EGFP-positive hAMCs were detected surrounding the injection tracts in the consecutive 3 weeks following the cell implantation (Figs. 2A–C; A: 7 days, B: 14 days, C: 21 days, separately), and the whole graft in low magnification image with EGFP was shown (Fig. 2D). The RT-PCR results confirmed that EGFP was expressed in the hAMC-EGFP graft but not hAMC graft (Fig. 2E). For the functional gene, such as BDNF, can be overexpressed in graft successfully after 21 days (Figs. 2F, G), and G was the low magnification image of F and BDNF can't be labeled in hAMC-EGFP graft (Fig. 2H).