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Research Report

Human umbilical mesenchymal stem cells enhance the expression of neurotrophic factors and protect ataxic mice

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ABSTRACT

Cerebellar ataxias, which comprise a wide spectrum of progressive disorders, are incurable at present. It has been reported that human umbilical mesenchymal stem cell (HU-MSC) transplantation has a protective effect on neurodegenerative diseases. In this study, we investigated the effect of HU-MSCs on ataxic mice induced by cytosine beta-D-arabinofuranoside (Ara-C). The ataxic mouse received an intravenous injection of 2×10^6 HU-MSCs once a week for three consecutive weeks. Neurological function was scored weekly by rotarod test and open field test. The mouse cerebellar volume and weight were also measured. The apoptotic cells, pathological alternations and distribution of HU-MSCs were determined by TUNEL assay and immunohistochemistry staining respectively. Double immunostaining was carried out to investigate the dynamics of HU-MSCs in the host animals. Neurotrophic factors in cerebellar tissue and serum were measured by Q-PCR and ELISA. Our results showed that HU-MSCs implantation significantly improved the motor skills of ataxic mice 8 weeks after application. HU-MSCs also alleviated cerebellar atrophy and decreased the number of apoptotic cells in the therapeutic group. Implanted HU-MSCs stayed in cerebellum for at least three months with no obvious differentiation. HU-MSC treated mice had enhanced expression of insulin-like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF) in cerebellum extraction and blood serum. Double immunostaining revealed that a few MAB1287 positive cells co-localized with IGF-1 or VEGF express cells. Our results suggest that HU-MSC treatment is capable of alleviating the motor impairments and cerebellar atrophy in the ataxic mouse model, probably via promoting particular neurotrophic factors.

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Abbreviations: Ara-C, cytosine beta-D-arabinofuranoside; BDNF, brain derived neurotrophic factor; FBS, fetal bovine serum; GDNF, glial cell line derived neurotrophic factor; HU-MSCs, human umbilical mesenchymal stem cells; IGF-1, insulin-like growth factor-1; IGL, internal granular cell layer; ML, molecular layer; PBS, phosphate-buffered saline; PCL, Purkinje cell layer; PFA, paraformaldehyde; VEGF, vascular endothelial growth factor

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1. Introduction

Cerebellar ataxia is a heterogeneous group of incurable disorders with ataxia as the leading symptom (Ogawa, 2004). As different phenotypes have specific pathogenesis (Teive, 2009), it is difficult to envisage a cure to them based on etiological factors until the pathogenic mechanism for each type is well detailed. This provides an impetus for us to search for symptomatic treatment. Since this series of disorders have several clinical and pathologic manifestations in common, including ataxic gait, cerebellar atrophy, Purkinje cell damage etc., thus the ataxic model induced by cytosine beta-D-arabinofuranoside (Ara-C) which resembles the clinical impairments of ataxic patients (Tatsuoka et al., 1985), was utilized in this study.

Nowadays, stem cell graft has emerged as a prospective therapeutic approach for neurodegenerative diseases. Human umbilical mesenchymal stem cells (HU-MSCs), derived from Wharton's jelly, have the potential to differentiate into neural cells, cartilage, muscle and adipocytes (Troyer and Weiss, 2008). In contrast to other stem cells, HU-MSCs own several merits, such as well-tolerated by immune system, greater expansion ability, rich and uncontroversial source (Troyer and Weiss, 2008; Weiss et al., 2006). These attractive advantages of HU-MSCs inspire us to assess the benefit of HU-MSCs in various diseases, in particular for those that are fatal and difficult to cure. Recently, HU-MSCs transplantation was reported to exhibit neuroprotection on neurological diseases in animal models (Koh et al., 2008), such as Parkinson diseases. Interestingly, a preliminary study on the application of HU-MSCs on a multiple sclerosis patient demonstrated HU-MSCs are feasible and effective (Liang et al., 2009). However, the research of stem cells on cerebellar ataxia is limited. Fetal cerebellar grafts were reported to be profitable in reversing the motor dysfunction of ataxic mice (Cendelin et al., 2009; Kaemmerer and Low, 1999; Sotelo and Alvarado-Mallart, 1987). It is speculated that cerebellar ataxia, which is also a progressive neurodegenerative disease, may gain benefits from HU-MSCs transplantation.

Besides cell replacement, the enhanced expression of bioactive substances, including insulin-like growth factor 1 (IGF-1), vascular endothelial growth factor (VEGF), brain derived neurotrophic factor (BDNF) and glial cell line derived neurotrophic factor (GDNF), are thought to nourish and repair the host damages as well (Wakabayashi et al., 2010). The evidence that the trophic factor gene modified MSCs could achieve better outcome than primary transplantation highlights the pivotal role of trophic factors (Nomura et al., 2005).

Therefore, we performed this study with the attempt of developing an effective therapy for ataxia on the basis of HU-MSCs, functions and applications.

2. Results

2.1. Model establishment

With the injection of Ara-C during the first three postnatal days, the mice manifested ataxic gait and cerebellar atrophy when compared to their littermate at ten weeks of age, which were in accordance with previous study (Tanabe et al., 2009).

2.2. Properties of HU-MSCs in vitro

HU-MSCs were isolated from neonatal umbilical cord. When cultured to P3, the cells were fibroblast-like (Fig. 1A). These cells exhibited CD105, CD73, CD90 positive, and CD34, CD45, CD14, CD79, HLA-DR negative phenotypes on flow cytometric analysis (Fig. 1B).

2.3. HU-MSCs alleviated the behavior impairment of ataxic mice induced by Ara-C

Rotarod test was performed to evaluate the balance and coordination of animals. The results indicated that ataxic mice (n=6) remained on the apparatus much shorter than normal ICR-mice (n=6) (68.11±9.59 s vs. 330.11±51.87 s, $p<0.01$). The slightly better behavior performance of ataxic mice appeared at week 7 following HU-MSCs treatment and achieved improvement significantly by the 9th week (HU-MSCs vs. control: 90.56±13.75 seconds vs. 58.00±9.43 s, $p<0.05$) until the 12th week (Fig. 2A). In line with the rotarod test, the open field test demonstrated that differences between the control group (n=6) and HU-MSCs group (n=6) began to emerge at week 6 post-transplant and showed statistical significance at the 8th week (74.33±10.05 vs. 128.67±16.98, $p<0.05$). This difference continued during the following four weeks (Fig. 2B).

2.4. HU-MSCs therapy alleviated cerebellar atrophy and inhibited cell apoptosis in ataxic mice

To determine whether HU-MSCs were able to improve cerebellar morphology of ataxic mice, the volume and weight of mouse whole brain and cerebellum were calculated. Ataxic mice showed apparent reductions of cerebellar weight and volume compared to normal mice (28.73±1.33 mg vs. 72.37±5.68 mg in weight, $p<0.05$; 26.56±9.42 mm^3 vs. 59.65±3.65 mm^3 in volume, $p<0.01$) (Table 1). However, in contrast to the control mice, HU-MSCs treated mice had improvements on both body weight (54.61±10.60 mg vs. 28.73±1.33 mg, $p<0.05$) and cerebellar volume (37.51±4.76 mm^3 vs. 26.56±9.42 mm^3, $p<0.05$) (Table 1).

The effect of HU-MSC on Purkinje cells in the cerebellum was investigated by calbindin staining. Purkinje cells are located between the molecular layer (ML) and the internal granular cell layer (IGL) regularly in normal mice (Fig. 2C), but the arrangement of Purkinje cells in vehicle treated mice was chaotic (Fig. 2D). Also, the cell density decreased in the IGL and the IGL looks much thinner. With the therapy of HU-MSCs, ataxic mice manifested proliferation of Purkinje cells and higher cell population in IGL (Fig. 2E). Accordingly, the IGL seems thicker in contrast to the control mice. In order to estimate the apoptotic changes, TUNEL-positive cells were counted in normal mice, control mice and HU-MSCs treated mice respectively (Fig. 2F). The enhanced cell apoptosis triggered by Ara-C was partially abrogated by HU-MSCs treatment (TUNEL-positive rate: 14.9%±1.5% in control mice, 6.8%±0.09% in 1 month post HU-MSCs treatment, $p<0.01$; 10.23%±1.89% in 2 months post HU-MSCs treatment, $p<0.05$; 9.63%±1.45% in 3 months post HU-MSCs treatment, $p<0.05$) (Fig. 2G).