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Resveratrol augments therapeutic efficiency of mouse bone marrow mesenchymal stem cell-based therapy in experimental autoimmune encephalomyelitis

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ABSTRACT

Experimental autoimmune encephalitis (EAE) is an inflammatory demyelinating disease, which served as a useful model providing considerable insights into the pathogenesis of multiple sclerosis (MS). Mouse bone marrow mesenchymal stem cells (mBM-MSC) were shown to have neuroprotection capabilities in EAE. Resveratrol is a small polyphenolic compound and possess therapeutic activity in various immune-mediated diseases. The sensitivity of mBM-MSCs to resveratrol was determined by an established cell-viability assay. Resveratrol-treated mBM-MSCs were also characterized with flow cytometry using MSC-specific surface markers and analyzed for their multiple differentiation capacities. EAE was induced in C57BL/6 mice by immunization with MOG35-55. Interferon gamma (IFN-γ)/tumor necrosis factor alpha (TNF-α) and interleukin-4 (IL-4)/interleukin-10 (IL-10), the hallmark cytokines that direct T helper type 1 (Th1) and Th2 development, were detected with enzyme-linked immunosorbent assay (ELISA). In vivo efficacy experiments showed that mBM-MSCs or resveratrol alone led to a significant reduction in clinical scores, and combined treatment resulted in even more prominent reduction. The combined treatment with mBM-MSCs and resveratrol enhanced the immunomodulatory effects, showing suppressed proinflammatory cytokines (IFN-γ, TNF-α) and increased anti-inflammatory cytokines (IL-4, IL-10). The combination of mBM-MSCs and resveratrol provides a novel potential experimental protocol for alleviating EAE symptoms.

1. Introduction

Multiple sclerosis (MS), also known as disseminated sclerosis or encephalomyelitis disseminata, is a heterogeneous inflammatory and neurodegenerative disease, in which perivascular inflammation, demyelination and axonal loss happen in the central nervous system (CNS) (Hemmer et al., 2002; Sospedra and Martin, 2005). It is the most common autoimmune disorder affecting the CNS. MS exhibits exacerbation (sudden increases in symptoms that remit weeks or months later) and progression (a slower steady increase in symptoms other than exacerbation). While the cause of MS is not clear, immune system destruction and myelin-producing cell failure were considered the underlying mechanisms (Nakahara et al., 2012). Genetics and environmental factors like infections were all proposed to be the potential causes of MS (Ascherio and Munger, 2007; Compston and Coles, 2002).

To date no efficient cure for MS has been developed. Treatments are limited to improving function after attack, as well as preventing new attacks (Compston and Coles, 2002). Medications showing modestly effective may also have intolerable adverse effects. While lacking enough clinical evidence, psychological stress has long been considered the key factor for the exacerbations of MS. Experimental studies in animal models of MS such as experimental autoimmune encephalomyelitis (EAE) has promoted the understanding of the immune-endocrine response to stress (Heesen et al., 2007; Martin, 1997; van den Broek et al., 2005). EAE as an animal model of brain inflammation is an inflammatory demyelinating disease of the CNS and a T-cell-mediated autoimmune disease, which is widely studied as an animal model of the human CNS diseases including MS, because it could mimic certain aspects of MS.

Stem cell transplantation is a potential promising therapy to treat MS by inducing neuron regeneration (Constantin et al., 2009; Gerdoni et al., 2007). Stem cells can differentiate into various cell

Abbreviations: MS, multiple sclerosis; CNS, central nervous system; EAE, experimental autoimmune encephalitis; mBM-MSC, mouse bone marrow mesenchymal stem cells; IFN-γ, interferon gamma; TNF-α, tumor necrosis factor alpha; IL-4, interleukin-4; IL-10, interleukin-10; ELISA, enzyme-linked immunosorbent assay.

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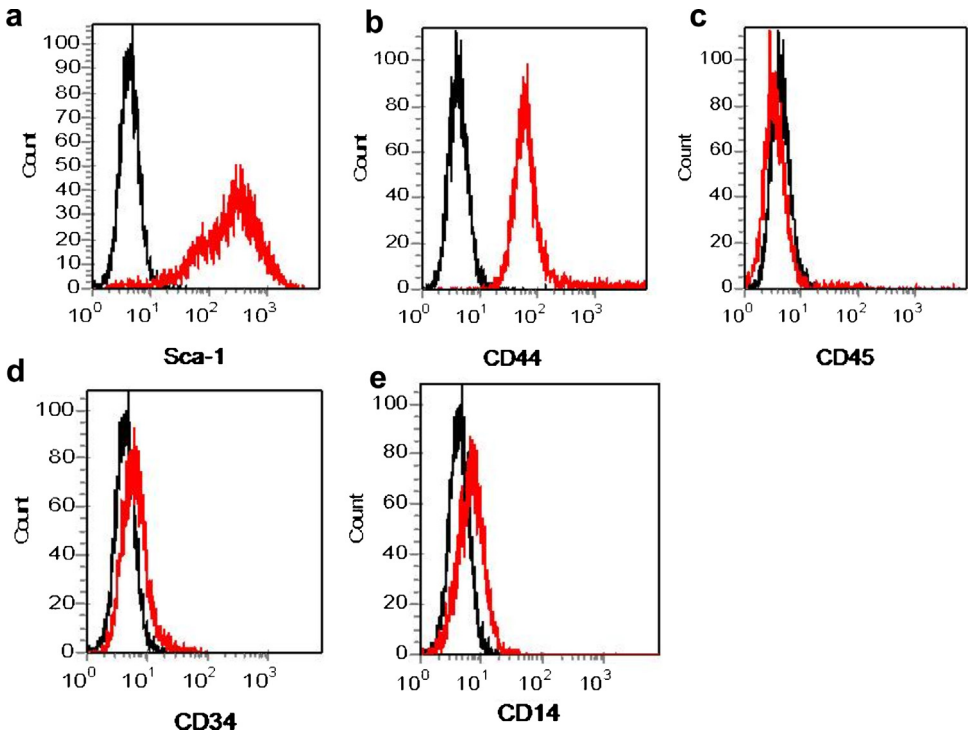

Fig. 1. Charaterization of mouse bone-marrow derived MSCs. (a–e) Flow cytometry revealed that the majority of the cells are Sca-1$^+$, CD44$^+$, CD45$^-$, CD34$^-$ and CD14$^-$, which are characteristic phenotypes of MSCs.

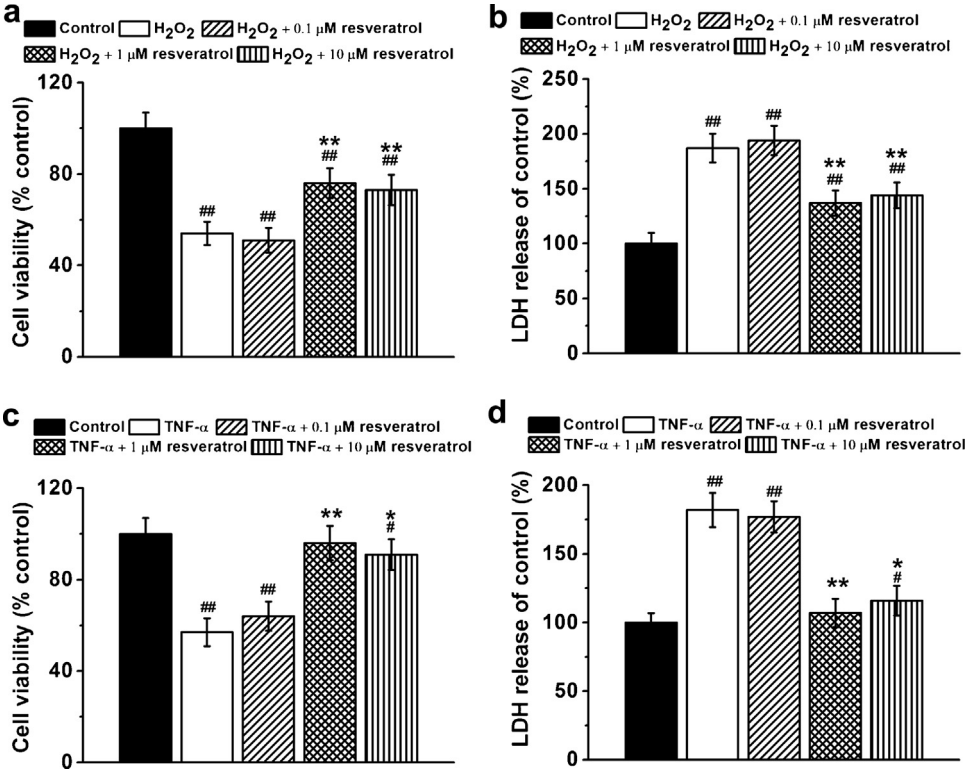

Fig. 2. Effects of resveratrol treatment (0.1, 1 and 10 μM) on cell viability and LDH release of MSCs under 20 ng/mL TNF-α exposure for the entire duration of culture (a, b) or 4-h 500 mM H_2O_2 exposure (c, d) at DIV 5, respectively. Cell viability was measured by MTT assay. All the values were normalized to those of control. Data were presented by mean ± SEM. *P < 0.05 and **P < 0.01 versus the corresponding TNF-α or H_2O_2 exposure group. #P < 0.05 and ##P < 0.01 versus control.

lineages and repair damaged tissue by reconstructing the tissue with new cells, and eventually recover lost functions. There are mainly two types of stem cells used in the treatment of EAE: mesenchymal stem cells (MSC) and neural stem cells (NSC). MSCs, derived from adult tissues, could suppress the encephalitogenic T cells that mediate neuronal inflammation and damage, therefore attenuate the encephalitogenic manifestation of MS (Bai et al., 2009; Einstein et al., 2006; Gerdoni et al., 2007; Kassis et al., 2008;