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The genes involved in asthma with the treatment of human embryonic stem cell-derived mesenchymal stem cells

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ABSTRACT

Background: Asthma is affecting more than 300 million people worldwide, which represents the most common chronic disease among children. We previously found that mesenchymal stem cells (MSCs) derived from induced pluripotent stem cells (iPSCs) modulated the immune response on Th2-mediated asthma *in vivo* and *in vitro*. This study further evaluated the immunomodulatory effects of MSCs from human embryonic stem cells (hESCs) on asthma.

Methods: Multipotent hESC-MSCs were obtained using a feeder-free method. The hESC-MSCs were analysed for the expression of stem cell surface markers by flow cytometry, their differentiation potentials were analysed using *in vitro* trilineage differentiation methods hESC-MSCs were transplanted into the murine model with ovalbumin (OVA)-induced airway allergic inflammation. The expression levels of allergic related genes were measured by the mRNA PCR arrays.

Results: The hESC-MSCs expressed classical MSC markers and held the capability of differentiation into multiple mesoderm-type cell lineages. hESC-MSCs were able to suppress allergic inflammation by modulating Th2 cells and eosinophils in the mice, and reversed the reduction of regulatory T cells. By using PCR array, 5 mRNAs-chemokine (C-C motif) ligand 11 (*Ccl*11), Ccl24, interleukin13 (*Il*13), *Il*33 and eosinophil-associated, ribonuclease A family, member 11 (*Ear*11) were identified the most relevant in murine airway allergic inflammation and hESC-MSCs treatment.

Conclusions: The therapeutic effects of hESC-MSCs were identified in the murine model of airway allergic inflammation with key mRNAs involved. This study will provide a better understanding regarding the mechanisms underlying hESC-MSCs therapeutic application in airway allergic inflammation.

1. Introduction

Asthma is a chronic, reversible airway disease that affecting more than 300 million people worldwide and causing substantial medical and financial burdens (Martinez and Vercelli, 2013; Asher and Pearce, 2014). Most allergic asthma have common characters such as high serum immunoglobulin E (IgE) antibodies, increased cellular infiltration and excessive activation of type 2 T helper (Th2) cells (Wills-Karp, 1999). The amount of mRNA and protein expression in the related cells have also changed with the progress of asthma (Pascoe et al., 2015; Plager et al., 2010). For example, Th2-associated cytokines including IL-4, IL-5 and IL-13 play a key role in asthma occurrence (Steinke and Borish, 2001; Deo et al., 2010) and the increase of programmed cell death 1 (PDCD1) can reduce the activation, proliferation

Abbreviations: APC, allophycocyanin; aMEM, medium eagle alpha modification; ANOVA, one-way analysis of variance; BALF, bronchoalveolar lavage fluid; bFGF, basic fibroblast growth factor; BM-MSC, bone marrow-derived mesenchymal stem cell; Ccl, chemokine (C-C motif) ligand; CD, cluster of differentiation; CGH, comparative genomic hybridization; Ear11, eosinophil-associated, ribonuclease A family, member 11; EGF, epidermal growth factor; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence activated cell sorting; FBS, fetal bovine serum; H&E, haematoxylin and eosin; hESCs, human embryonic stem cells; hESC-MSCs, human embryonic stem cells derived mesenchymal stem cells; Il, Interleukin; Ig, immunoglobulin; iPSC-MSCs, induced pluripotent stem cells derived mesenchymal stem cells; iPSCs, induced pluripotent stem cells; MSCs, mesenchymal stem cells; NEAA, non-essential amino acid; OVA, ovalbumin; PAS, periodic acid–Schiff; PBS, phosphate buffer; PCR, polymerase chain reaction; PDCD1, programmed cell death 1; PE, phycoerythrin; SNP, single nucleotide polymorphism; Th2, type 2 helper T cell; Treg, T regulatory; WGS, whole genome sequencing

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and the survival of T cells (Davies et al., 2016). Therefore, targeting the genes involved in asthmatic responses and immunomodulation would be helpful for further asthma treatment.

Mesenchymal stem cells (MSCs) show the rosy prospect in clinical therapeutic application owing to their differentiation potential (Fehrer and Lepperdinger, 2005) and immunomodulatory properties. MSCs have been found to be able to modulate the immune response in allergic diseases via both soluble factors and cell–cell contact (Shi et al., 2010; Keating, 2012). MSCs can increase the phagocytosis of neutrophils by inducing Th2 cells secreting IL-17 (Duffy et al., 2011). Not only inhibit the activation and the proliferation of T cells, MSCs also inhibit the differentiation of Th1 and Th17 cells with the promotion of Treg cells (Luz-Crawford et al., 2013a). MSCs can promote the Ig secretion of B cells *in vitro* and *in vivo*. Furthermore, animal studies and clinical trials have demonstrated that MSCs hold great potential in clinical applications for the treatment of immune-related diseases (Goodwin et al., 2011; Xiao et al., 2012).

However, there are several inherent defects such as senescence (Giuliani et al., 2011), quality variation (Galipeau, 2013) and genomic mutation (Wang et al., 2013) exist in MSCs derived from bone marrow or other adult tissues, hindering their regeneration efficient for standard clinical applications (Law and Chaudhuri, 2013). In contrast, according to previous studies, MSCs derived from human embryonic stem cells (hESC-MSCs) or induced pluripotent stem cells (iPSC-MSCs) not only showed similar biological characteristics to bone marrow mesenchymal stem cells (BM-MSCs) including surface markers, multilineage differentiation and immunomodulation, but also had higher proliferation, regenerative capacities and less heterogeneity (Frobel et al., 2014; Lian et al., 2007).

Pluripotent stem cells potentially offered an unlimited source for functional and qualified MSCs generation. However, iPSCs after reprogramming show genetic instability and tumorigenicity *in vivo* (Barrilleaux and Knoepfler, 2011; Tan et al., 2014). To meet the safety standards, it would be necessary for iPSCs to finish some complex and expensive evaluations such as whole genome sequencing (WGS) and comparative genomic hybridization (CGH) single nucleotide polymorphism (SNP) analysis before clinical application. Over the disadvantages of iPSCs, ESCs will be more suitable for clinical large-scale applications.

Additionally, we have demonstrated that the treatment of iPSC-MSCs significantly suppressed the pathology and permitted a re-balance in the immune response in a mouse model of airway allergic inflammation (Sun et al., 2012). Therefore, this study will continue investigating the role of ESC-MSCs treatment in murine airway allergic inflammation model. In this study, we developed an efficient method for generating hESC-MSCs, the effects of hESC-MSC administration were then investigated in a mouse airway allergic inflammation model. By using PCR array, the genes involved in the process of hESC-MSCs immunomodulation were identified.

2. Materials and methods

2.1. Cell culture

H9 human embryonic stem cells (hESCs) obtained from WiCell (Madison, Wisconsin) were cultured as previously described (Lian et al., 2007; Sagar et al., 2014). When the cells reached 60% confluence in the Matrigel-coated 6-well-plate, the hESCs culture medium was replaced by fresh MSCs differentiation medium comprised of Medium Eagle Alpha Modification (aMEM) medium (Gibco, Gaithersburg, MD), 10% fetal bovine serum (FBS) (Gibco), 50 μmol/L-ascorbate-2-phosphate (Gibco), 100 IU/mL penicillin (Gibco), 100 μg/mL streptomycin (Gibco). After 12 days culture in the MSCs differentiation medium that was changed every day, the cells were harvested with Accutase™ (Innovative Cell Technologies, San Diego, CA) and seeded onto 0.1% gelatin-coated 6-well-plate with MSCs differentiation medium. The cells were defined as hESC-MSCs passage 0 (P0). When the cells reached 80–90% confluence, they would be trypsinized and seeded onto non-coated T25 flasks containing MSCs growth medium comprised of Dulbecco's modified eagle medium (DMEM)/High Glucose (Gibco), 10 % FBS (Gibco), 100 μM Nonessential amino acids (NEAA) (Gibco), 10 ng/mL Human basic fibroblast growth factor (bFGF) (Gibco), 1 ng/mL Human epidermal growth factor (EGF) (Gibco), 55 μM 2-mercaptoethanol (Gibco), 100 IU/mL penicillin (Gibco), 100 μg/mL streptomycin (Gibco) (defined as hESC-MSCs P2). These MSC-like cells were continued culturing with MSCs growth medium in feeder-free condition and passaged at 1:4 splits every 3 days when the clones reach an optimal density. The differentiation of hESCs has been performed three times to evaluate the robustness of the protocol.

2.2. Flow cytometry analysis of cell surface marker

Flow cytometry analysis of hESC-MSCs surface antigen expressions was carried out with P4 and P8 hESC-MSCs. The cells were harvested and resuspended to 5×10^5 cells in 100 μl FACS buffer. The cells were incubated with phycoerythrin (PE)-conjugated mouse anti-human Cluster of differentiation (CD)73, CD90, CD166, PerCP-Cy5.5-conjugated mouse anti-human CD44, CD146 and allophycocyanin (APC)-conjugated mouse anti-human CD45, CD105 monoclonal antibodies on ice for 30 min respectively (BD Biosciences, San Jose, NJ). All antibodies were diluted 1:20 for incubation according to the manufacturer's instructions. Then, 2.5×10^5 cells in 400 μl FACS buffer were analysed using the BD FACSCanto flow cytometer (BD Biosciences, NJ). All data were analysed using FlowJo v10.0.7 software (Tree Star, Ashland, OR).

2.3. Multilineage differentiation of hESC-MSCs

The differentiation potential of hESC-MSCs was assessed using the OriCell™ Adipogenesis, Osteogenesis and Chondrogenesis Kit (Cyagen Biosciences, Shanghai, China). All procedures were carried out according to manufacturer's instruction. Oil Red O staining analysis, Alizarin Red staining analysis and Alcian Blue staining analysis were used to validate hESC-MSCs adipogenic differentiation, osteogenic differentiation and chondrogenic differentiation, respectively.

2.4. Animals

Female BALB/c mice (4–6 weeks) were purchased from the Guangdong Medical Laboratory Animal Centre (Guangzhou, China). All procedures were performed in accordance with the Guidelines for Animal Experiments and using Committee (Approval No. SCXK 2013-0002) approved by The University Animal Ethics Committee of Sun Yat-sen University Institutional Animal Care.

2.5. Mouse airway allergic inflammation model and the transplantation of hESC-MSCs

The mouse airway allergic inflammation model was established by sensitization and challenge with ovalbumin (OVA, grade V, Sigma, St. Louis, MO) according to previous descriptions with minor modification (Sun et al., 2012). Briefly, 40 μg OVA/mouse for the sensitization and 5% OVA in phosphate-buffered saline (PBS) for the challenge were employed to induce allergic airway inflammation in the mouse. The hESC-MSCs (P7 or P8) were suspended in sterile PBS at a density of 5×10^6 cells per ml and 0.2 ml of the cell suspension was intravenously injected via the tail vein on day 20 before the challenge (Supplementary Fig. 1). An equal volume of PBS was injected as the control. Mice were divided into control, model and treatment three groups: Control group (PBS/PBS/PBS) that mice were sensitized and challenged with PBS, then injected with PBS on day 20 (n = 19); Model group (OVA/OVA/PBS) that mice were sensitized and challenged with OVA, then treated with PBS on day 20 (n = 17); hESC-MSCs treatment group (OVA/OVA/