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Human mesenchymal stromal cells modulate T-cell immune response via transcriptomic regulation

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Abstract

Background aims. Mesenchymal stromal cells (MSCs) have been identified as pan-immunosuppressant in various *in vitro* and *in vivo* inflammatory models. Although the immunosuppressive activity of MSCs has been explored in various contexts, the precise molecular signaling pathways that govern inhibitory functions remain poorly elucidated. *Methods.* By using a microarray-based global gene expression profiling system, this study aimed to decipher the underlying molecular pathways that may mediate the immunosuppressive activity of umbilical cord–derived MSCs (UC-MSCs) on activated T cells. *Results.* In the presence of UC-MSCs, the proliferation of activated T cells was suppressed in a dose-depended manner by cell-to-cell contact mode via an active cell-cycle arrest at the G_0/G_1 phase of the cell cycle. The microarray analysis revealed that particularly, *IFNG*, *CXCL9*, *IL2*, *IL2RA* and *CCND3* genes were down-regulated, whereas *IL11*, *VSIG4*, *GFA1*, *TIMP3* and *BBC3* genes were up-regulated by UC-MSCs. The dysregulated gene clusters associated with immune-response-related ontologies, namely, lymphocyte proliferation or activation, apoptosis and cell cycle, were further analyzed. *Conclusions.* Among the nine canonical pathways identified, three pathways (namely T-helper cell differentiation, cyclins and cell cycle regulation, and gap/tight junction signalling pathways) were highly enriched with these dysregulated genes. The pathways represent putative molecular pathways through which UC-MSCs elicit immunosuppressive activity toward activated T cells. This study provides a global snapshot of gene networks and pathways that contribute to the ability of UC-MSCs to suppress activated T cells.

Key Words: *cell-cycle arrest, gene expression, immunomodulation, mesenchymal stromal cells, T cells*

Introduction

In recent years, the use of human mesenchymal stromal cells (MSCs) in regenerative medicine, aesthetics care and immunotherapy has become more widespread. This is mainly due to the readily available sources of MSCs, their versatility in clinical settings and the fact that there are fewer ethical implications in the use of MSCs compared with embryonic stem cells [1,2]. Although MSCs were initially discovered in bone marrow, various tissue resident- or organ-specific MSCs have been isolated from humans and animals. Among these, umbilical cord MSCs (UC-MSCs) have received significant attention because the harvesting of UC-MSCs is neither invasive nor painful, and these cells demonstrate rapid *in vitro* expansion capabilities to meet therapeutic demands and superior retention of cellular plasticity even after several rounds of *in vitro* amplification compared with bone marrow–derived MSCs [3–5].

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The traditional view on the use of MSCs in regenerative medicine has changed drastically after the discovery of a specific immunosuppressive activity of bone marrow MSCs on T-cell immune responses [6]. Since then, the immunomodulatory functions of MSCs were extensively investigated, and it has been documented that MSCs have pan-immunosuppressive effects on various immune cells in equally divergent immune responses [7–10]. After the *in vitro* demonstration of MSCs' ability to inhibit the proliferation and effector functions of T cells, B cells, monocytes, neutrophils, natural killer cells and dendritic cells [9,11–14], animal models of various immune-based diseases have been developed to further validate the functional significance of MSC-mediated immunosuppression. Preclinical studies have demonstrated the immunosuppressive activity of MSCs' prolonged skin-graft survival [15], improved autoimmune type 1 diabetes [16] and prevention of tissue damage in collagen-induced arthritis [17]. In addition, in a number of human trials, MSC co-transplantation during allogeneic tissue transplantation has been shown to increase tolerance to graft-versus-host disease [18–20]. Although the immunomodulatory ability of MSCs at *in vitro* and *in vivo* is now being widely accepted, the underlying molecular and signaling mechanisms that govern this ability remain poorly delineated.

The cross-talk that exists between MSCs and their target immune cells via physical contact and/or soluble factors is necessary to facilitate an effective immunosuppression [7,21]. Because of the complexity of the signaling pathways and molecules involved in T-cell activation, MSCs potentially deliver their immunomodulatory signals via multiple molecules. These molecules include ligands of receptors and adhesion molecules that can halt T-cell activation at various points in diverse and interconnected pathways. It has been shown that MSCs inhibit mitogen-activated T-cell proliferation via the engagement of adhesion molecules (e.g., Program Death Receptor 1 [PD-1], Program Death Ligand 1 [PD-L1], Program Death Ligand 2 [PD-L2], Intracellular Adhesion Molecule-1 [ICAM-1], Vascular Cell Adhesion Molecule-1 [VCAM-1]) [8] and soluble factors (e.g., prostaglandin E2, nitric oxide, indoleamine 2,3-dioxygenase, human leukocyte antigen-G5, transforming growth factor-β, interleukin [IL]-10, hepatocyte growth factor) [22–24]. The cascade of signals through these molecular interactions ultimately results in cell-cycle arrest of activated T cells. Work by various groups, including ours, supports the notion that MSCs induce cell-cycle arrest at the G_0/G_1 phase by down-regulating cyclin D2 expression [25,26] and decreasing the transcription and translation levels of IL-2 and soluble IL-2 receptors in T cells [27]. However, a substantial number of reports also have documented different mechanisms that potentially administer MSC-mediated immunosuppression. The differences in reported mechanisms of MSC-exerted immunosuppression from various research laboratories might be attributed to the heterogeneity of MSC subpopulations. Despite this, there are divergent mechanisms, the universally observed pan-immunomodulatory activity of MSCs on immune cells suggest the presence of a common mechanism(s) that confer the immunosuppressive ability.

Therefore, this study aimed to create a global catalogue of T-cell gene expression changes that were driven by UC-MSCs to subsequently identify possible key genes and pathways that are associated with the immunosuppressive effects.

Methods

Generation and culture expansion of UC-MSCs

UC samples (n = 10) from full-term pregnancies were obtained during normal deliveries by an obstetrics and gynecology specialist at Brittannia Women & Children Specialist Centre, Kajang, Malaysia. Patients' written and informed consent were obtained in accordance with the regulation set out by the ethical committee of the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. One centimeter of UC tissue was excised from each sample. Blood vessels were removed and the tissues finely minced before incubating with an enzymatic mixture containing 0.4% collagenase type II (Worthington Biochemical) and 0.01% DNase I (Worthington Biochemical). The digested tissues were later mechanically dissociated using a handheld homogeniser (Wiggen Hauser). The cell suspensions were filtered through 100- and 70-μm strainers, washed and cultured in MSC complete media containing Dulbecco's Modified Eagle's Medium with nutrient mixtures F-12 (HAM) (1:1) with GLUTAMAX-I (Gibco), 10% fetal bovine serum (Stem Cell Technology), 1% penicillin and streptomycin (Gibco), 0.5% Fungizone (Gibco), 0.1% gentamicin (Gibco) and 40 ng/mL basic fibroblastic growth factor (Promega). The primary cultures were cultured in a 37°C humidified 5% CO_2 incubator for at least 1 week before the medium was changed. The plastic adherent cells were trypsinized with 0.05% trypsin-ethylenediaminetetraacetic acid (Invitrogen) and harvested. The cells were then further characterized for various cell surface markers and mesodermal differential potential as detailed previously [28].

T-cell proliferation assay

Peripheral blood mononuclear cells (PBMCs) were isolated from freshly withdrawn heparinized blood using 1.077 g/mL Ficoll-Paque PLUS (Amersham Biosciences) via density gradient centrifugation. The activation