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RESEARCH ARTICLE

Mesenchymal stem/stroma cell (MSC)-derived nanoscale exosomes inhibit T cell proliferation by negative regulation of PI3K/Akt and MAPK/ERK pathway

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ABSTRACT

Objective(s): Exosomes, as membrane-enclosed nanovesicles (30–150 nm), transports active biomolecules between various cells. As natural nanoparticles (NP), they serve a key role in the diagnosis, treatment, as well as prevention of diseases. Recently it has been verified that MSC-derived exosomes are capable of adjusting immune cells' biological processes. We investigated the effects of the MSCs-derived exosome on T cell proliferation.

Methods: xosomes isolated from the supernatant of bone marrow-MSC. The ultrastructure and shape of exosomes were evaluated via transmission electron microscopy (TEM), and CD9, CD63, and CD81 were detected by Western blotting. Then, we examined the effects of MSC-derived exosome on the proliferation of the T cells by MTT assay. Moreover, the expression levels of the PI3K, Akt, MAPK, and ERK were estimated at mRNA levels by Real-Time PCR.

Results: We showed that MSCs-derived exosome inhibited T cell proliferation based on the MTT assay results. Real-time PCR analysis also exhibited that exosome co-culture resulted in down-regulation of PI3K, Akt, MAPK, and ERK expression levels.

Conclusions: MSCs-derived exosome inhibits T cell proliferation by negative regulation of the survival- and proliferation-involved PI3K/Akt and MAPK/ERK pathway in vitro.

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INTRODUCTION

Mesenchymal stem/stromal cells (MSCs) as multipotent adult cells were firstly discovered by Friedenstein while investigating the BM [1]. Various studies have indicated that MSCs are capable of stimulating an immunosuppressive effect largely by suppressing T cell proliferation, averting B cell activation, and targeting dendritic cells (DCs) [2]. The inspiring competencies of MSCs to modify immune response confer their significance to decreasing immune responses by a diversity of mechanisms, more importantly, negative regulation of T cell induction and proliferation [3, 4]. MSCs also release extracellular vesicles (EVs), including nano-scale exosomes, and high quantities of cytokines and growth factors [5-7]. Exosomes, as nano-sized vesicles (30-100 nm in diameter), contains DNA, mRNA, miRNA, proteins, and other important molecules and ultimately modify recipient cells' biological process [8]. MSCs-derived nano-sized exosomes present biological actions like the parental cells while prohibiting various risks correlated with cell transplantation [9, 10].

Like parental MSCs, MSC-derived exosomes target T cells, B cells, NK cells, and macrophages activity mainly by miRNA delivery [11-13]. The MSCs activation results in is the secretion of anti-inflammatory biomolecules, such as indolamine 2,3-dioxygenase (IDO), prostaglandin E2 (PGE2), IL-10, NO and TGF-β [14-16]. These cytokines target immune cells and facilitate the inhibition of immune response. A clinical trial exhibited that MSC-derived exosomes attenuate the potential of peripheral blood mononuclear cells (PBMCs) to secret pro-inflammatory bimolecules in vivo [8, 17]. Based on the literature, MSC-derived nano-scale exosomes might promote IL-10 and TGF-β1 release from PBMCs, thus inducing Tregs activity [8]. This immunomodulatory competence of MSCs-derived exosomes opens new avenues for the management of inflammatory disease. In this light, MSC-derived exosomes have the unique capability to moderate immunological responses upon organ transplantation and graft-versus-host disease (GvHD) [18].

In the current study, we examine the potent effects of the MSCs-derived exosome on immune T cells in vitro and shed light on the possible corresponding mechanisms.

MATERIAL AND METHODS

Cell culture

Bone marrow-MSCs were acquired from Royesh Stem Cell Biotechnology Institute Cell Bank (Tehran, Iran) and cultivated in DMEM (Gibco Laboratories) with 10% FBS and 1% penicillin/streptomycin. Cells were kept at a 37°C humidified atmosphere with a 5% CO2 incubator.

For T cell isolation, human blood was procured from a healthy donor. Then, ficoll density gradient centrifugation was applied to separate the PBMCs by Lymphodex. T cells were isolated by MACS concerning the manufacturer's guidelines. Cell cultivated in RPMI 1640 media including 10% FBS, 1% penicillin/streptomycin, as well as 1 μg/mL phytohemagglutinin (PHA).

Exosome isolation

Non-scale exosomes were separated from the serum-free conditioned media utilizing the MagCapture™ Exosome Isolation Kit PS (FUJIFILM Wako). The medium was centrifuged at 10 000 × g for 30 min to eradicate other EV. Cleared supernatants were filtered by 0.22 mm filter membranes and finally concentrated.

Transmission electron microscopy (TEM)

Exosome morphology was assessed utilizing a transmission electron microscope (TEM).

Western blotting

To characterize exosome markers CD9, CD63, and CD81, exosomes and MSCs were lysed in RIPA buffer, including the protease inhibitor (Thermo Scientific). Then, the lysate proteins were run on 10% SDS-polyacrylamide gels and, after that, moved to PVDF membranes. Then, primary antibodies were applied to recognize proteins: anti-CD63, anti-CD9, and anti-CD81. Following incubation with secondary antibodies (HRP-conjugated secondary antibody), the detection phase was completed utilizing the Pierce ECL Western Blotting Substrate.

MTT assay

For exosome co-culture, 100 ng/ml exosomes isolated from MSCs were added to the T-cell containing medium. Within 24, 48, 72, and 96 hours, 20 μl of 5 mg MTT /ml medium was added, and cells were kept at 37 °C for 4h. Also, 100 μL of

Table 1. Candidate genes' primer pairs for Real-Time PCR

Gene	Primer (5′-3′)	
ERK	F	ACACCAACCTCTCGTACATCGG
	R	TGGCAGTAGGTCTGGTGCTCAA
MAPK	F	ACACCAACCTCTCGTACATCGG
	R	TGGCAGTAGGTCTGGTGCTCAA
PI3K	F	GCAGGTTCCTTCAGTCCTACTCCAGGC
	R	GCCCAGTCAGCTGATACCATTTAACCG
AKT	F	TCTATGGCGCTGAGATTGTG
	R	CTTAATGTGCCCGTCCTTGT
B-actin	F	AGAGCTACGAGCTGCCTGAC
	R	AGCACTGTGTTGGCGTACAG

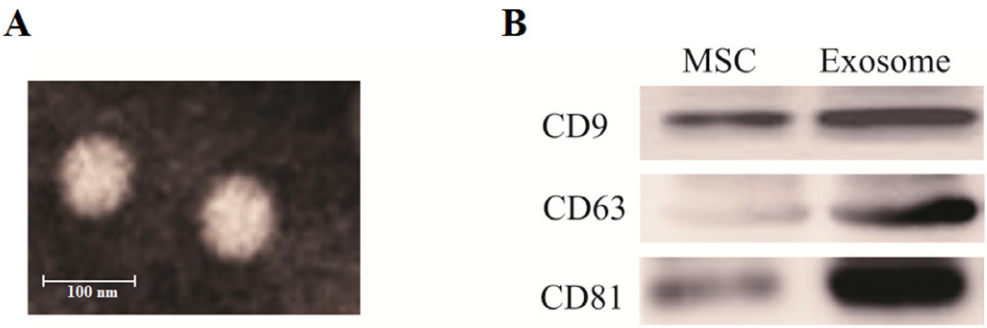

Fig. 1. Exosome characterizing by TEM image (A) and western blotting (B).

10% SDS in 0.01M HCl solution was used, aiming at dissolving crystals. Finally, the OD of wells was estimated at 570 nm using an ELISA reader.

RNA extraction and cDNA synthesis

Total RNA from T cells was extracted utilizing the RNX Plus solution kit (Sinaclon, Tehran, Iran). Upon cell lysis, the products were maintained at -70°C and thawed once RNA isolation was prerequisite. A high-capacity kit (Bioneer, CA) was applied to generate cDNA from the isolated RNA.

Real-Time PCR

Real-time PCR was conducted utilizing the SYBR Green reagent (Thermo Fisher Scientific). Relative gene expression was estimated by the Pfaffl method (Pfaffl, 2012). The designed primer pairs are cited in Table 1.

Statistical analysis

Consequences are indicative of three independent tests, and values are shown in mean ± SEM. B-actin was selected as an internal control. Student T-test was exploited to determine the statistical differences. All analyses were accomplished by SPSS software. P values < 0.05 were measured as statistically significant.

RESULTS AND DISCUSSION

Exosome characterizing

Then, TEM revealed isolated exosome had a spherical shape (Fig. 1A). The western blotting analysis also demonstrated the existence of CD63, CD9, and CD81 in isolated exosomes (Fig. 1B)

MSCs-derived non-scale exosome inhibits T cell proliferation

In this step, human T cells were co-cultured with exosomes to evaluate the potent effects of the exosome on their proliferation. Based on MTT assay results, exosome inhibits T cell proliferation in co-culture conditions (Fig. 2). Results showed that in col-culture conditions, nano-scale exosome resulted in a drop in proliferation of T cells within 48, 72, and 96 but not 24 hours of exposure (Fig. 2). This reduction was time-dependent.

Similarly, Li et al. found that Wharton's Jelly-derived MSCs (WJMSCs) derived exosomes could inhibit T cell proliferation in vivo [19]. Other studies have indicated that MSCs-derived microparticles (MPs) and also exosomes (NPs) induced an anti-

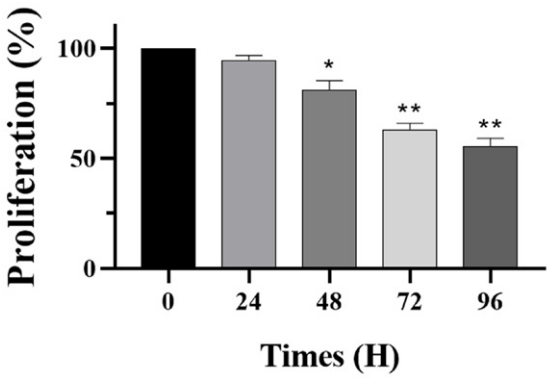

Fig. 2. MTT assay results of the effects of exosome co-culture on T cell proliferation.

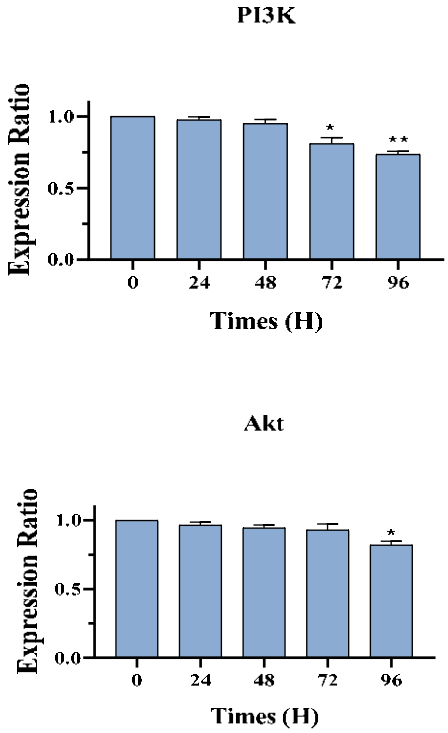

Fig. 3. Real-Time PCR results about the exosome effects on PI3K and Akt gene expression in T cells.

inflammatory role on T lymphocytes. However, NPs were more effective than MPs in deterring inflammation in vivo [20]. These competencies of MSCs-derived nano-scale exosome makes them a rational plan to treat COVID-19 [21, 22].

MSCs-derived non-scale exosome down-regulates PI3K/Akt pathway in activated T cell

Real-time PCR analysis was conducted to assess the effect of the nano-scale exosome on T cell proliferation-involved genes. Accordingly, T cells co-cultured with exosomes, and the expression of the PI3K and Akt genes at mRNA levels were estimated within 24-96 hours of exposure (Fig. 3). Based on the results, exosomes down-regulated the expression of PI3K genes within 72 and 96 but not 24 and 48 hours of co-culture (Fig. 3). Besides, exosome reduced Akt gene expression within only 96 hours of co-culture.

Previous studies signify the key role of PI3K/Akt pathway in activating and proliferation of T cells. The PI3K-AKT pathway exerts several biological

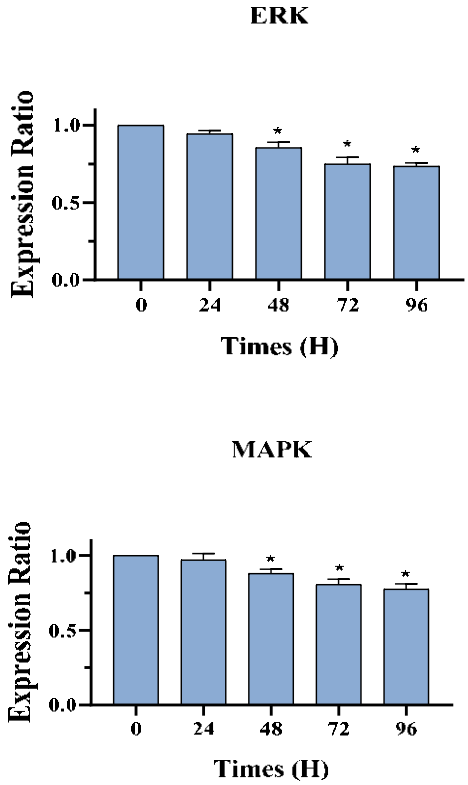

Fig. 4. Real-Time PCR results about the exosome effects on MAPK and ERK genes expression in T cells.

responses and is complicated in the immune responses and lymphocyte development [23, 24]. Lacking PI3K subunits or Akt results in the blockage of T cell development [25]. The production of PIP3 by PI3K triggers the recruitment and induction of other key proteins in T cells [26]. Thus, negative regulation of this pathway, as shown upon exosome co-culture, deters their inflammatory responses.

MSCs-derived non-scale exosome down-regulates ERK/MAPK pathway in activated T cell

Real-time PCR analysis was conducted to assess the effect of the nano-scale exosome on T cell proliferation-involved genes. Accordingly, T cells co-cultured with exosomes, and the expression of the ERK and MAPK genes at mRNA levels were estimated within 24-96 hours of exposure (Fig. 4). Results revealed that exosomes inhibited ERK and MAPK genes within 48, 72, and 96 but not 24 hours of co-culture (Fig. 4).

MAP kinase axes are major pathways triggered by TCR stimulation and thus serve a central role in T-cell responses [27]. Further, these pathways contribute to T cell survival, proliferation, and differentiation. Our findings are consistence with other reports indicating that MSCs-derived exosomes could inhibit various cell proliferation by targeting the MAPK pathway [28].

CONCLUSION

It has previously been found that MSCs-derived exosomes could inhibit T cell proliferation in vitro and in vivo, making them a rational strategy to induce immunomodulation. Herein, we showed that this effect is mediated by targeting two key signaling axis, PI3K/Akt and MAPK/ERK. It seems that exosome therapy could result in a promising outcome in the near future.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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