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RESEARCH ARTICLE

Mesenchymal stem/stromal (MSCs)-derived exosome inhibits retinoblastoma Y-79 cell line proliferation and induces their apoptosis

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ABSTRACT

Objective(s): Based on the literature, mesenchymal stem/stromal cell-derived exosome has a dual role in tumor therapy or progress. We evaluated the possible anti-tumor effect of the MSCs-exosome on retinoblastoma Y-79 cell cells in vitro.

Methods: To verify the efficient isolation of exosomes, the MSCs-exosome structure and the expression of CD9 and CD81 were examined using transmission electron microscopy (TEM) and western blotting, respectively. Y-79 cell proliferation and apoptosis were evaluated using MTT assay and flow cytometry. The Real-time PCR estimated the expression levels of Bcl-2 and microRNA-143.

Results: MSCs-exosome inhibited Y-79 cell proliferation as shown by MTT assay results. Also, MSCs-exosome induced apoptosis in Y-79 cells within 72 hours of treatment. Real-time PCR results also showed that exosome therapy decreased Bcl-2 expression while up-regulating microRNA-143 expression.

Conclusions: MSCs-derived exosomes could deter retinoblastoma Y-79 cells tumorigenicity, thereby providing a novel modality to manage retinoblastoma patients.

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INTRODUCTION

Retinoblastoma (RB) is a curable neoplasm mainly in developed countries, with about 99% patient survival [1, 2]. Conversely, in developing countries, usually many patients are detected with distributed and advanced disorders with high fatality. The treatment paradigm of RB, the most shared intraocular condition in adolescents, has recently altered severely [3-5]. Using novel methods to deliver therapeutic agents locally, such as safer approaches for intravitreal chemotherapy administration concomitant with ophthalmic artery chemosurgery, has yielded promising outcomes [6, 7]. Small drugs, oncolytic viruses, and immunotherapy have caused a paradigm shift in intraocular RB therapy [8, 9]. Prominently, the first stages toward novel therapeutic methods for metastatic disorders could be provided by the extensive examinations of the arrangement of tumor distribution.

Mesenchymal stem/stromal cell (MSC) delivers groundbreaking therapeutic approaches for the treatment of immune-related diseases, transplant

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surgery, and cancers [10, 11]. MSCs show CD90, CD105, CD73, and MHC-I, without presenting CD11b, CD45, CD34 and CD14 [12]. Such stem/stromal cells can be efficiently procured from bone marrow, adipose, liver, muscle, endometrium, umbilical cord, etc. Then, they can be applied to generate cartilage, bone, etc. [13, 14]. MSCs secret various biomolecules with pro-and anti-cancer influences, eventually influencing the survival, angiogenesis, metastasis, and proliferation of human cells. For instance, Lu et al. showed that MSCs induce apoptosis and partially deter glioma cell proliferation in vitro by inhibiting AKT activity [15].

Recent reports have signified that the therapeutic impacts of MSCs are usually mediated by secreting paracrine factors like cytokine and miRNAs [16, 17]. Exosomes (Exo), well-known natural nanoparticles (NPs), are implicated in the MSCs-stimulated paracrine effect [18-20]. They have been broadly examined due to their tumor-homing capacity and yield benefits [21]. Nonetheless, they play a controversial role in tumor cell progression and metastasis [22]. The anti-tumor influences are likely mediated by targeting tumor viability, growth, angiogenesis, and invasion [22].

Herein, we evaluated the potent anti-tumor effects of the MSCs-derived exosome on the survival and proliferation of the retinoblastoma cell lines in vitro.

MATERIAL AND METHODS

Cell culture

MSCs were purchased from Royan Institute (Tehran, Iran). Following culture in DMEM (Capricorn) containing 10% fetal bovine serum (FBS) (Sigma-Aldrich, Germany) and 1% pen/strep, cells were maintained at a 37°C humidified atmosphere with CO2 5%.

Also, the retinoblastoma Y79 cell line (Sigma-Aldrich, Germany) was cultivated in RPMI 1640 medium, including 10% FBS and 1% pen/strep. Then cells were maintained at a 37°C humidified atmosphere with CO2 5%.

Exosome procurement

Exosomes were procured from the culture media by exoEasy Maxi Kit (QIAGEN). The culture media was centrifuged at 10000 × g for 50 min to eliminate other types of extracellular vesicles and finally were filtered by 0.2 mm filter membranes.

Transmission electron microscopy (TEM)

The exosome morphology was assessed using a transmission electron microscope (TEM).

MTT assay

The potent effect of the exosome at concentrations of 50 ng/ml and 100 ng/ml on the Y-79 cell line was examined by the MTT assay based on the MTT kit instructions (Abcam, UK). The 5×10^4 cells were firstly seeded in 96-well plates. After 24 hours, cells were exposed to 50 ng/ml and 100 ng/ml exosomes for 24-96 hours. After that, 10 µL of 5 mg MTT/ml medium was added to the wells. Following 4 hours, the OD of wells was measured at 570 nm wavelengths.

Western blotting

To evaluate the expression of the well-defined markers of the exosome, CD9 and CD81, the MSC and derivative exosome were firstly lysed by RIPA buffer. Upon lysate proteins running on 10% sodium dodecyl sulfate-polyacrylamide gels, these proteins were conveyed to polyvinylidene difluoride (PVDF) membranes. Specific primary and secondary antibodies (Abcam, UK) were exploited, and the detection phase was performed.

Fluorescence-activated cell sorting (FACS)

Concisely, MSCs-exosome in the concentration of 50ng/ml and 100 ng/ml were added to the plates containing the Y-79 cell line. Based on kit instructions, an Annexin V-FITC Apoptosis Detection Kit (Sigma-Aldrich, Germany) was employed to define the apoptosis of Y-79 cells within 72 hours of treatment.

RNA extraction and cDNA synthesis

The RNA isolation from the retinoblastoma Y-79 cell line was performed utilizing the RNX Plus solution kit (Sinaclon, Iran). Concisely, cells were lysed, and a high-capacity kit (Bioneer, USA) was employed to manufacture complementary DNA (cDNA) from the harvested RNA.

Real-Time PCR

Using PrimeTime™ Master Mix (Idtdna, USA), the expression levels of the Bcl-2 and microRNA-143 were measured in the Y-79 cell line following treatment with MSCs-exosome in concentrations of 50ng/ml and 100 ng/ml. This process was conducted utilizing the pre-set cycling parameters. The GAPDH genes were used to normalize the mRNAs expression levels. The primer pairs for Bcl-

2 were "GGATTGTGGCCTTCTTTGAG" as the forward and "CAGCCAGGAGAAATCAAACAG" as the reverse. For microRNA-143, primer pairs were "GCAGTGCTGCATCTCTG" as forward and "GAACATGTCTGCGTATCTC" as the reverse.

Statistical analysis

Data are shown in mean ± SEM and analyzed using the Student T-test. All analyses were managed using Graph Pad Prism software.

RESULTS AND DISCUSSION

The characterizing of exosome

The TEM images (Fig. 1A) and study of the CD9 and CD81 expression levels by western blotting (Fig. 1B) verified the efficient isolation of the exosome.

MSCs-exosome inhibits Y-79 cells proliferation

Based on the MTT assay consequences, MSCs-exosome in concentrations of 50ng/ml and 100 ng/ml inhibited the proliferation of the Y-79 cell line within 24, 48, 72, and 96 hours (Fig. 2). The inhibitory effects of the exosome on Y-79 cell proliferation were more evident at 72 and 96 hours of treatment than at 24 and 48 hours (Fig. 2). Also, MSCs-exosome in the concentration of 100ng/ml was more effective in decreasing cell proliferation than exosome of 50ng/ml.

Likewise, other studies have indicated that MSCs-exosome may hinder tumor cell proliferation. Meanwhile, Bruno et al. found that MSCs-exosome negatively regulates cell cycle, induces cell apoptosis, and reduces their proliferation in hepatocellular carcinoma and Kaposi's sarcoma [23, 24].

MSCs-exosome induces Y-79 cells apoptosis

The apoptosis rates of Y-79 cells were estimated within 72 hours of treatment with MSCs-exosome in concentrations of 50ng/ml and 100 ng/ml using Annexin-V/PI staining and FACS analysis. Both 50ng/ml and 100 ng/ml exosome induced apoptosis significantly in Y-79 cells within 72 hours of exposure ($P<0.05$) (Fig. 3A, B). The apoptosis percentages in Y-79 cells (control) and Y-79 cells treated with exosome 50ng/ml and Y-79 cells treated with exosome 100ng/ml were 3.98±1.09, 16.30±2.05, and 28.27±3.08, respectively (Figs. 3A, B).

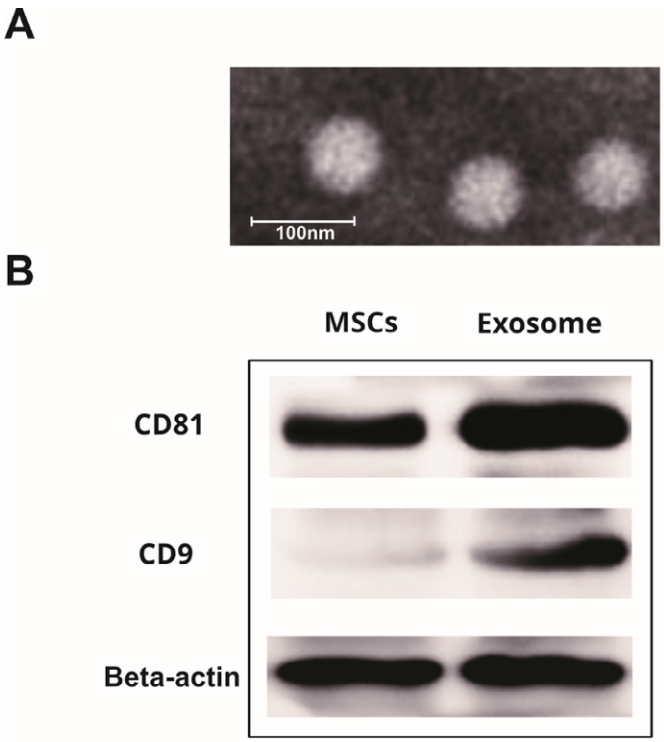

Fig. 1. The characterizing of MSCs-exosome by TEM image (A) and western blotting (B).

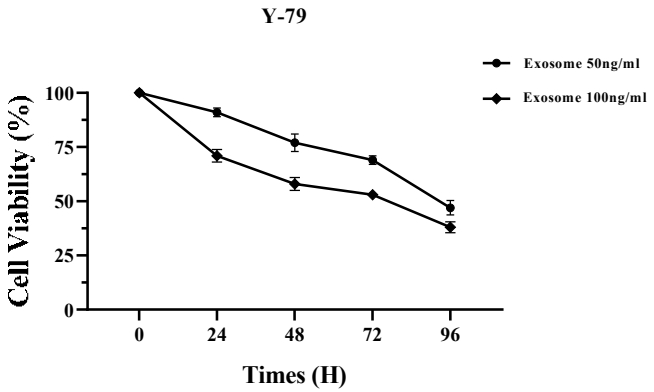

Fig. 2. Study of the effects of the exosome on Y-79 cell proliferation using MTT assay.

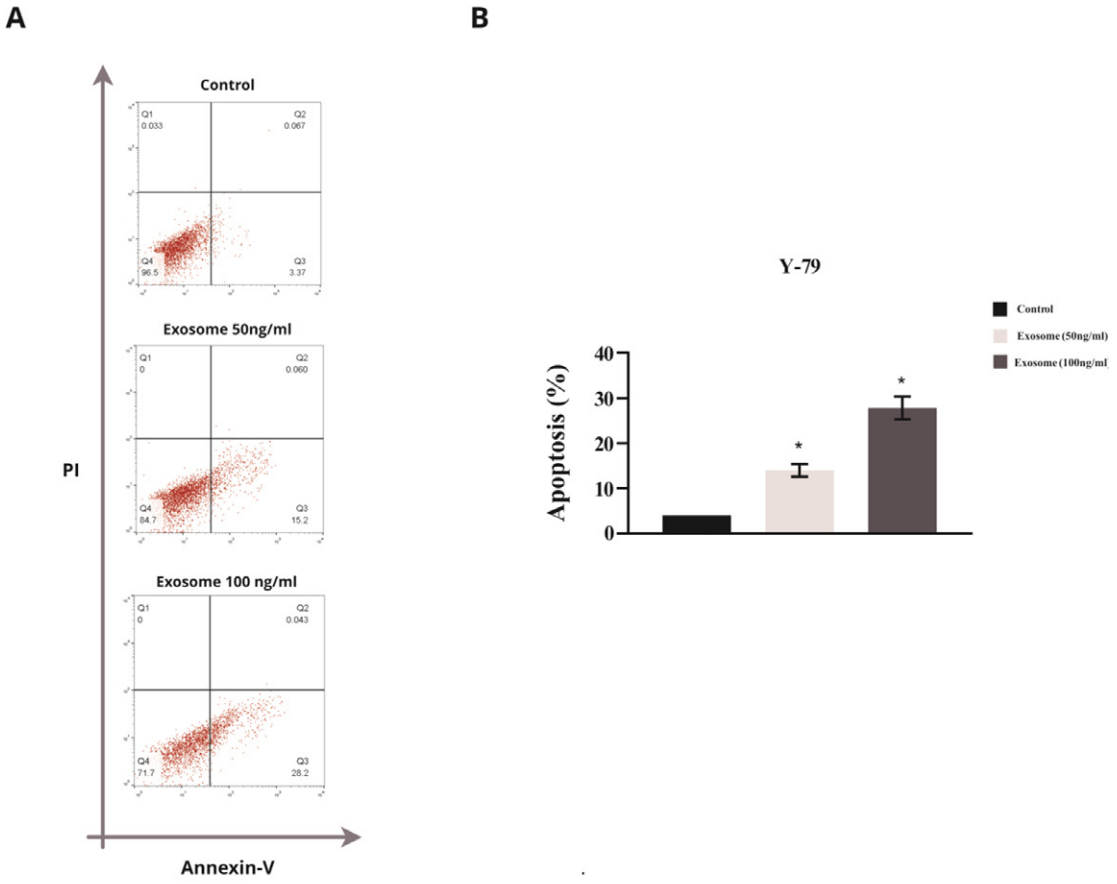

Fig. 3. Study of the Y-79 cell survival following exposure with exosome using flow cytometry.

Other studies have indicated that MSCs-exosome could induce apoptosis in bladder cancers by targeting Akt pathways [25]. Besides, exosomes diminished ovarian cancer cell proliferation and provoked apoptosis by targeting oncogene-associated miRNAs [26].

MSCs-exosome down-regulates Bcl-2 expression in Y-79 cell

The real-time PCR test was accomplished to evaluate the Bcl-2 expression in the retinoblastoma Y-79 cell line following exposure with exosome at 24 and 72 hours of exposure. Based on the results,

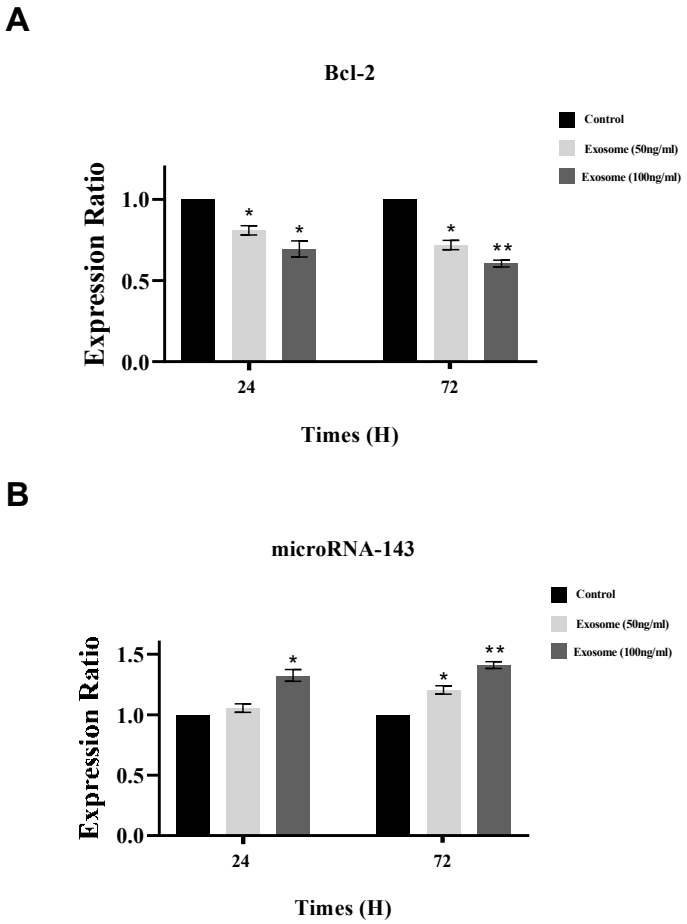

Fig. 4. The Real-Time PCR results based on the Bcl-2 and microRNA-143 expression in the Y-79 cell line treated with exosome.

exosome treatment in both 50ng/ml and 100 ng/ml concentrations resulted in a reduction in Bcl-2 expression in the Y-79 cell line ($P<0.05$) (Fig. 4A).

MSCs-exosome up-regulates microRNA-143 expression in Y-79 cell

Real-time PCR test was applied to assess the microRNA-143 expression in the retinoblastoma Y-79 cell line upon treatment with exosome at 24 as well as 72 hours. Accordingly, 50ng/ml exosome treatment improved microRNA 143 expression within 72 but not 48 hours of treatment ($P<0.05$) (Fig. 4B). Also, 100 ng/ml exosome promoted microRNA-143 expression in the Y-79 cell line at both 24 and 72 hours of treatment ($P<0.05$) (Fig. 4B).

Studies have reported that microRNA-143 is usually down-regulated in retinoblastoma [27]. In contrast, its activation inhibits these cells' proliferation [28, 29]. Thus it could be applied as a novel strategy for retinoblastoma diagnosis and treatment.

CONCLUSION

Based on this study's results, MSCs-exosome could partially inhibit retinoblastoma cell tumorigenicity by negatively regulating the anti-apoptotic expression and up-regulating microRNA-143 expression.

CONFLICT OF INTEREST

There is no conflict of interest.

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