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Tumor Microenvironment Changing through Application of MicroRNA-34a Related Mesenchymal Stem Cells Conditioned Medium: Modulation of Breast Cancer Cells toward Non-aggressive Behavior

Katayoun Bahman Soufiani[1], Ali Akbar Pourfathollah[1], Mahin Nikougoftar Zarif[2], and Ehsan Arefian[3]

[1] Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[2] Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran
[3] Department of Microbiology, School of Biology, College of Science, University of Tehran, Tehran, Iran

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ABSTRACT

Conditioned medium (CM) derived from mesenchymal stem cells (MSCs) contains bioactive molecules including microRNAs (miRs) that could be a potential tool for controlling cancer cells' behavior. Due to the properties of CM, this study assesses the effects of miR-34a related MSC-CM on tumor behavior through the evaluation of migration, invasion, apoptosis, and PDL1 expression in breast cancer cell lines.

The miR-34a overexpression vector or scramble control was produced using lentiviral vectors, DNA cloning, and the transfection of the HEK-293T cell line. It was then transduced into human adipose-derived mesenchymal stem cells (hAD-MSCs). MSC-CMs were collected and added onto MDA-MB-231 cell lines. The functional evaluations were performed by transwell, wound healing, and Annexin V/PI methods on the treated MDA-MB-231 cell lines. The PDL1 expression was also assessed by Real-time PCR and western blot.

The findings of this study showed that ectopic miR-34a expression was significantly upregulated in manipulated hASC with miR-34a (p<0.0001). Treatment of MDA-MB-231 cell line with miR-34a-hAD-MSC-CM, scramble-hAD-MSC-CM, or hAD-MSC-CM displayed not only a reduction in the number of migrated or invaded cells (p=0.01) but also an increase in the apoptotic cells in the test group (p=0.02) when compared to the control groups. It also showed down-regulation in the gene (p=0.05) and protein expression levels of PDL1 in the test group.

The results of the present study showed that simultaneous application of miR-34a and MSC-CM can be considered as a new method for changing the cancerous microenvironment; and therefore, as a potential strategy in breast cancer therapy.

Keywords: Adipose-derived mesenchymal stem cells; Breast neoplasms; Conditioned medium; MIRN34 microRNA

INTRODUCTION

Breast cancer is the most common cause of death among women worldwide.[1] In recent years researchers have emphasized that the microenvironment plays an important role in the growth and metastasis of tumor

Corresponding Author: Ali Akbar Pourfathollah, PhD; Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, P.O.Box: 14115-331, Tehran, Iran. Tel: (+98 21) 8288 3874, E-mail: pourfa@modares.ac.ir

cells.[2] It has been proved that tumorigenesis is strongly affected by the tumor microenvironment.[3] Tumor microenvironment includes a variety of cells such as endothelial cells, fibroblast, cancer cells, immune cells, and in particular mesenchymal stem cells, [4] that induce inflammatory states in tumor tissues by releasing cytokines, chemokines, and other inflammatory substrates.

Mesenchymal stromal cells (MSCs) are unique cells. In response to cell destruction signaled by their homing receptors such as chemokine receptor type 4 (CXCR4), selectins, and integrins, MSCs enter into tumors and cause inflammation in tumor sites.[5,6] There are contradictory views on the functions of MSCs. While some studies showed that MSCs can facilitate tumor growth by releasing inflammatory substances such as interleukin-6 (IL-6), nitric oxide (NO), prostaglandin D2 (PGD2), and Indoleamine 2, 3-dioxygenase (IDO), other reports showed that MSCs inhibit tumor growth.[7] Therefore, an understanding of the conditions in which MSCs enhance or inhibit tumor growth and metastasis is very crucial. In 1968 Fridenstein et al discovered MSCs and identified them as fibroblast-like cells.[8] MSCs are non-hematopoietic stem cells that can differentiate into three lineages of adipocyte, osteocyte, and chondrocyte.[9,10]

In recent years extensive researches have been carried out to study the fate of stem cells and the behavior of tumors through epigenetic factors. MicroRNAs are small non-coding RNA molecules that consist of 17-27nucleotides and originate from a precursor of approximately 70 nucleotides.[11,12] These molecules are found in various species such as plants, animals, and some viruses. They also play a role in RNA silencing, post-transcription, and gene expression. These molecules are also involved in many other biological processes such as stemness properties, proliferation, development, and apoptosis.[13] There are also a few reports on the disruption of miRNAs expression in some diseases such as cancers. Genetic changes, epigenetic silencing, and disorders in the biosynthesis of miRNAs are three important mechanisms of abnormal expression of these molecules.[14]

There is growing evidence that miRNAs are involved in tumor growth and survival. The comparison of cancerous tissues with normal tissues often indicates the variable expression level of mature miRNAs.[15] The miRNAs in the tumor microenvironment can transfer between adjacent cancer cells and other cells especially MSCs; therefore, it seems that MSCs can modulate the behavior of tumor cells. However, controversial results were reported about the effects of CM achieved from MSCs on the morphology and proliferation of tumors.[16]

Due to the presence of miRNAs in conditioned medium and their vital role in the regulation of gene expression, this study was carried out to assess the molecular role of MSCs-CM containing miR-34a in breast cancer cell line behavior. Further, this study aimed to apply the lentiviral delivering system to increase the expression of miR-34a in human adipose-derived mesenchymal stromal cells (hAD-MSCs).[17] and enhance anti-tumorigenic properties in MSC-CM. After the treatment of the cancer cell line with modified mesenchymal stem cells-CM including miR-34a, the degree of migration, invasion, and apoptosis was assessed in the breast cancer cell line. The miR-34a encoding gene is located on chromosome 1 and widely localized in all normal tissues except lung tissues.[18] miR-34a also acts as a tumor suppressor in a variety of tumors.[19] It has been reported that miR-34a regulates the genes which are associated with cell cycle control, cell senescence, proliferation, and apoptosis.[20]

In addition, this study examined the effect of miR-34a related CM on PDL1 expression as one of the immune checkpoint ligands. Recent clinical trials showed that the inhibition of the PDL1-PD1 pathway enhances the immunity against some tumors such as melanoma, and renal cell carcinoma. In some cancer patients receiving such kinds of drug inhibitors[21] the lack of immunity response[21] displays a requirement for the use of more effective factors such as miR-34a in cancer therapy.

MATERIALS AND METHODS

Cell Lines and Cell Culture

Human embryonic kidney 293(HEK-293T) and MDA-MB-231 cell lines were purchased from Pasteur Institutes of Iran, and hAD-MSCs were obtained from the Iranian Blood Transfusion Organization. The MDA-MB-231 or hAD-MSCs were cultured in Dulbecco's Modified Eagle's Medium (DMEM)-F12 and HEK-293T were cultured in a DMEM high glucose medium (Gibco Co., US). All media were supplemented with 10% Fetal bovine serum (Gibco Co., US) and penicillin-streptomycin (100X). All cells were grown at 37°C with 5% CO2 in a humidified

incubator. The study proposal was approved by the Ethics Committee of the Faculty of Medical Sciences (ID Code: IR.TMU.REC.1395.500), Tarbiat Modares University, Tehran, Iran.

hAD-MSCs Immunophenotyping and Differentiation Assays

To confirm that hAD-MSCs were multipotent mesenchymal stromal cells, we used anti- CD73, CD166, CD29, CD34, and CD45 antibodies conjugated to phycoerythrin (BD Co., US) and analyzed MSCs by flow cytometry (PartekCo., German).

Adipocyte-derived MSCs were grown to 80-90% confluency in 6 well plates, the medium of each well was replaced with adipocyte or osteocyte differentiation medium (Bio Idea Co., Iran) to evaluate the differentiation capacity of MSCs to adipocyte and osteocyte lineages. After 21 days, osteocyte and adipocyte differentiation efficiency were analyzed using alizarin red and oil red staining for calcium accumulation and lipid droplets, respectively.

Transduction of Adipocyte-derived MSCs with Lentiviral Vectors

hsa-miR-34a precursor sequence was cloned into the PCDH-CMV-MCS-EF1-GFP-T2A-Purolentiviral vector (Bioscience Co., US). The transfer vector was transmitted to the HEK-293T cells with packaging vector (psPAX2; Bioscience Co., US) and envelope vector (pMD2. Bioscience Co., US) by calcium phosphate transfection method. In the next stage, virus particles were collected and centrifuged. After the transduction of HEK-293T, the viral titer was determined by flow cytometry on the fourth day (Data not shown). hASC cells in MOI 20 were transduced by viral particles. The manipulated hASCs were assessed for green fluorescent protein expression after 48 hours under a fluorescent inverted microscope (Nikon Co., Japan) and compared with optical microscopic images (Figure 1A and B). On the fourth day and in 80-90% confluency, puromycin was added to eliminate transducted cells. The conditioned medium of stem cells was replaced with DMEM-F12 with 1% FBS and was incubated at a temperature of 37°C with 5% CO2 in a humid atmosphere.

Collection of MSC Conditioned Medium

After 72 hours of the culture of unmanipulated or manipulated hAD-MSCs with scramble or miR-34a in DMEM-F12 supplemented with 1% FBS in standard conditions (5% CO2; 37° C), conditioned medium was collected from three samples: i.e., hAD-MSC (control), scramble-hAD-MSC (control), and miR-34a –hAD-MSC (test). The CM was centrifuged at 3,000 rpm for 20 min at 4°C to remove cell debris. After filtering with 0.22 µm syringe flitter, CM was frozen at -70^{0}C.

Wound Healing Assay

200,000 cells from the MDA-MB-231cell line were cultured in 6-well plates and maintained in DMEM supplemented with FBS 10% and P/S over night. A single scratch was created with a 200 µL sterile tip in the center of the cell monolayers. To eliminate detached cells, the wells were rinsed with phosphate buffer saline (PBS, 1X) and cultured in miR-34a-hAD-MSC-CM, scramble-hAD-MSC-CM, or hAD-MSC-CM for 48 hours. Then treated MDA-MB-231 were assessed on the third day to determine the proliferation rate. The area was occupied by moving cells when observed under an optical inverted microscope. Each sample was repeated 3 times.

Transwell Assay

To assess invasion, a transwell insert with an 8 µm pore size was used. Transwells were purchased from Corning Co. (Cat No: 3428).5× 10^4 cancer cells were seeded in 4-well plates and kept in DMEM-F12 medium without FBS and incubated in standard condition for 24h. After the detachment of cells, 2×10^4 cells were seeded in transwells that coated with sterile gelatin 0.1% and were cultured with 200CM from miR-34a-hAD-MSC-CM,scramble-hAD-MSC-CM and hAD-MSC-CM. Then transwells were put into 24-well plates which contain DMEM medium supplemented with 10% FBS. The next day, the cells were washed with PBS and fixed in 4% paraformaldehyde and then stained by violet crystal dye. Before visualization and photography under an optical inverted microscope, the non-migrated cells were eliminated using a swab. Five microscopic fields were counted for each sample.

Annexin V/PI Assay

Annexin V/PI and protein binding buffer (4X) were purchased from Bioscience Co. In brief, 2× 10^5 MDA-MB-231 cells were cultured in three samples' CM; i.e., miR-34a–hAD-MSC-CM, scramble-hAD-MSC-CM, and hAD-MSC-CM for 72h. The cells were detached, washed with cold PBS, and 100µL of cells resuspended

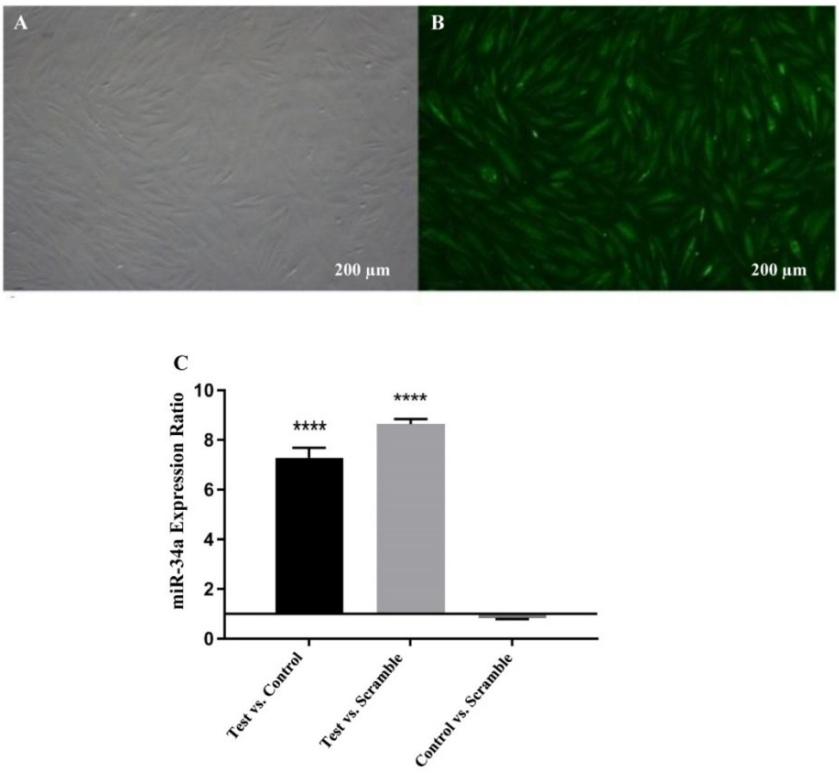

Figure 1. Transduction of human adipose-derived mesenchymal stromal cells (hAD-MSCs) with viral vectors in the second day. The images were taken by an optical (A) and fluorescence (B) inverted microscope (scale bar=200 µm). (C) Bar graph showing the level of miR-34a in manipulated hAD-MSC (test) was up-regulated 8.7 and 7 fold compared with scramble-hAD-MSC and hAD-MSC in Q Real-time PCR, respectively. Data are represented as mean±SD in three biological repeats and the asterisk is marked according to the *p* values;***p*<0.0001. The baseline is based on reference gene reaction efficiency (RE=1).**

in 300 µL of diluted protein binding buffer. In addition, 5 µL AnnexinV-fluorescein isothiocyanate (FITC) was added to each tube and incubated for 20-30 minutes at room temperature (RT) in darkness. Finally, 5 µL of PI was added to each tube and detected by flow cytometry. Each sample was evaluated three times.

Western Blot Analysis

Total protein was extracted from MDA-MB-231 after being treated with CM of all three samples on the third day. All subsequent experiments were conducted on ice. At first, the cells were lysed by radioimmunoprecipitation assay (RIPA) buffer containing protease inhibitor mixture. After centrifugation for 15 minutes at low temperature and at 20000 x g, the supernatant was collected. Proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes using a semi-dry system (Bio-Rad Co., US). The membranes were blocked with 5% low fat dried milk for 2h, then incubated overnight at 4 °C with corresponding primary antibodies directed against PDL1 (Rabbit, 1:1000, ab213524, Abcam) and β-actin (Rabbit,1:1000, ab190476, Abcam). Subsequently, the membranes were incubated with horseradish peroxidase (HRP)-conjugated sheep anti-rabbit secondary antibody (Sina Biotech Co., Iran) for 75 minutes at RT. The protein bands were appeared using an enhanced chemiluminescent reagent (Thermo Scientific Co., US). β-actin was set as the internal control.

RNA Isolation, Reverse Transcription, and Quantitative Real-time PCR

On the sixth day, hAD-MSCs were lysed, and total RNA was extracted; using Rnx-Plus buffer (SINAClone Co., Iran) according to the kit protocol. The purity and concentration of RNA were detected using a nanophotometer (Implen instrument, German). The cDNA was synthesized from 1 µg of total RNA using random hexamer, specific primers (miR-34a, snord47), and reverse transcriptase M-MLVenzyme (TermofisherCo., US) based on the kit protocol.

In addition, on the third day, the MDA-MB-231 cancer cell-line was treated with miR-34a-hAD-MSC-CM, scramble-hAD-MSC-CM, and hAD-MSC-CM according to the aforementioned method, RNA extraction and cDNA synthesis was performed to assess PDL1.

For cDNA synthesis, the reaction condition was as following: 25°C (10 min), 37°C (15 min), 42°C (45 min), 75°C (10 min) and the total volume of the reaction system was 20 µL. The reaction systems for Q-real-time PCR consisted of 12.5 µL cyber-green master mix (2X), Rax (YTA Co.), 0.5 µL of forwarding primer, 0.5 µL of reverse primer, 1 µL of cDNA, and 10.5 µl of RNase Free dH2O. The reaction conditions were as follows: pre-denaturation at 95°C for 2 min, denaturation at 95°C for 2 Secs, and extension/annealing at 60°C for 30 Secs in PCR thermocycler (step one TM Real-time PCR system, ABI instrument, US). Three repeated parallel wells were prepared for each sample. The primers for miR-34a, snord-47, PDL1, and β2 microglobulin (β2MG) were synthesized by Pishgaman Company. Snord-47 was the internal reference for miR-34a, and β2MG was the internal reference for the PDL1 gene. The expression level of the product was calculated by REST 2009 software. This method was applied to measure miR-34a and PDL1 mRNA expression in cells. The primers of PDL1 and β2MG for RT-PCR are listed as following:

PDL1 forward: 5'-ATC AAG TCC TGA GTG GTA AGA C-3';

PDL1 reverse: 5'- GAG GTA GTT CTG GGA TGA CC-3';

β2MG forward: 5'-ATG CCT GCC GTG TGA AC-3';

and β2MG reverse: 5'-ATC TTC AAA CCT CCA TGA TG -3'.

Statistical Analysis

Values were shown as Mean±SD. Graph Pad Prism was used to analyze data by one or two-way analysis of variance followed by Tukey's multiple comparison tests. A $p<0.05$ was considered significant.

RESULTS

hAD-MSC Definition Criteria

The assessment of differentiation cluster was done by using phycoerythrine-conjugated antibodies and flow cytometry technique following ISCT minimal definition criteria. HASCs did not express hematopoietic lineage markers such as CD34, and CD45. On the other hand, they were positive for CD73, CD166, and CD29 (Figure 2A and 2B). In addition, their differentiation ability into osteogenic, and adipogenic lineages was demonstrated after dying with alizarin red and oil red, respectively (Figure 2C and 2D).

Increased Expression Level of MiR-34a in hAD-MSC

This study showed that mature miR-34a was elevated in transduced hAD-MSC compared to the control groups; i.e., scramble transduced and untransduced hAD-MSC. The data analyses based on cycle threshold (CT) and REST2009 software showed the up-regulation of the expression level of miR-34a in the manipulated hAD-MSC with the recombinant virus. The up-regulation levels were 7 and 8.7 fold higher than the control groups ($p<0.0001$, Figure 1C).

MiR-34a Related CM Inhibited Migration and Invasion in Cancer Cell Line

Migration and invasion of MDA-MB-231 breast cancer cell lines were decreased after treatment with miR-34a-hAD-MSC-CM, compared with MDA-MB-231 cell lines that were treated with CM of the control groups; i.e., scramble-hAD-MSC-CM and hAD-MSC-CM. In vitro scratch assay revealed that miR-34a in CM can reduce the migration rate of cancer cell lines which were grown in miR-34a related CM (Figure 3). Moreover, the invasion assay showed that the potency of invasiveness of MDA-MB-231 was markedly attenuated during growth in miR-34a related CM (Figure 4A). In comparison with the control groups, the number of migrated cells after treatment with miR-34-

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hAD-MSC-CM showed significant decreases in the test group (*p=0.01, **p=0.004, Figure 4B). Taken together, these results suggested that miR-34a may play a critical role in the behavior of breast cancer cells.

MiR-34a-related CM Induced Apoptosis in Cancer Cell Lines

To discriminate apoptotic cells from normal or necrotic cells, we used Annexin V, propidium iodide (PI) staining, and flow cytometry. First, MDA-MB-231 was treated with miR-34a-hAD-MSC-CM, scramble-hAD-MSC-CM, and hAD-MSC-CM for 72 h. Then, they were stained with Annexin-V-FITC/PI dyes. Data analyses with flowJo software showed the percentage of apoptotic cells in the MDA-MB-231 cell line treated with miR-34a-hAD-MSC-CM, significantly increased compared with the control groups in Annexin assay (p=0.02, Figure 5). A low percentage of necrotic cells observed in each of the three groups but did not have significant value in the PI test analysis.

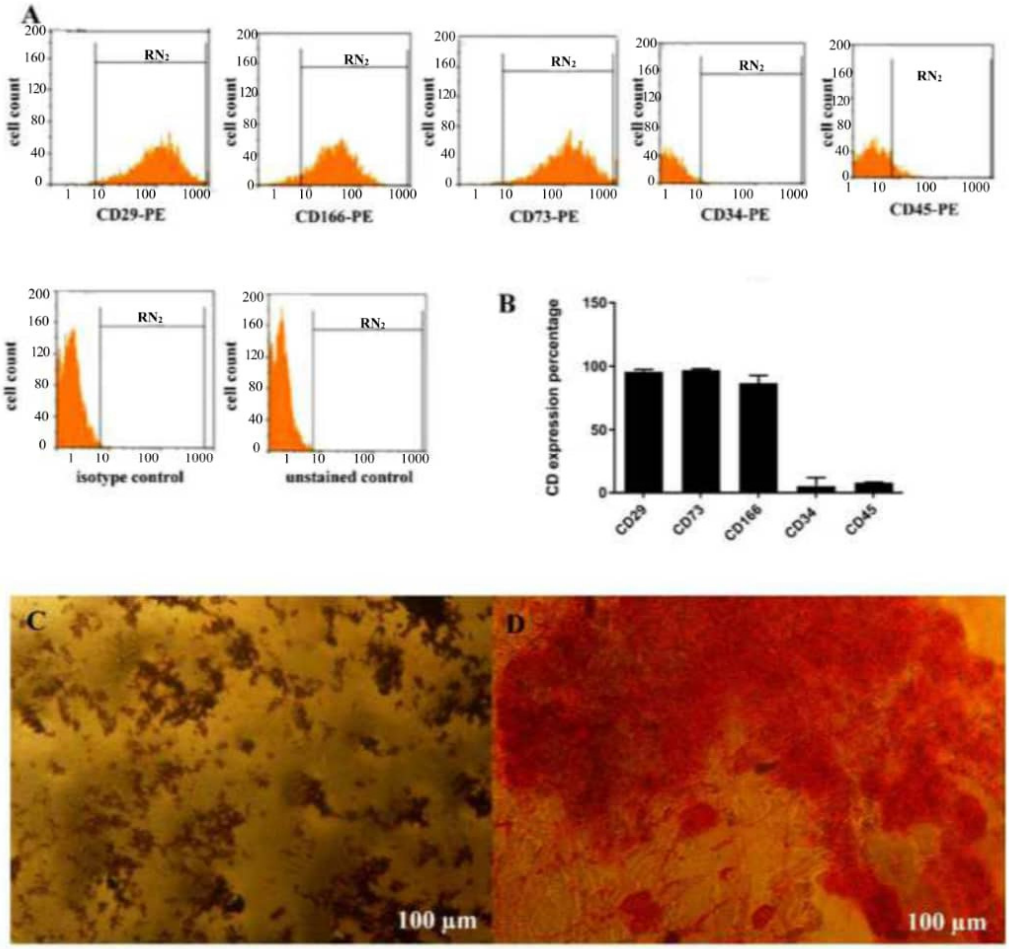

Figure 2. Characterization of human adipose-derived mesenchymal stem cells. (A) The expression level of CD34, CD45, CD29, CD73, and CD166 was analyzed by FACS; using a specific antibody conjugated with phycoerythrin against each indicated CD marker. (B) The histogram results show a low expression level of CD34, CD45, and high expression level ofCD29, CD166 and CD73. Images show in vitro differentiation of human adipose-derived mesenchymal stromal cells (hAD-MSC) to (C) adipocytes and (D) osteoblast with differentiation medium. Results of histogram and differentiation capacity verified MSCs identity. The images were taken by a camera fixed to a Nikon optical inverted microscope (scale bar=100 µm).

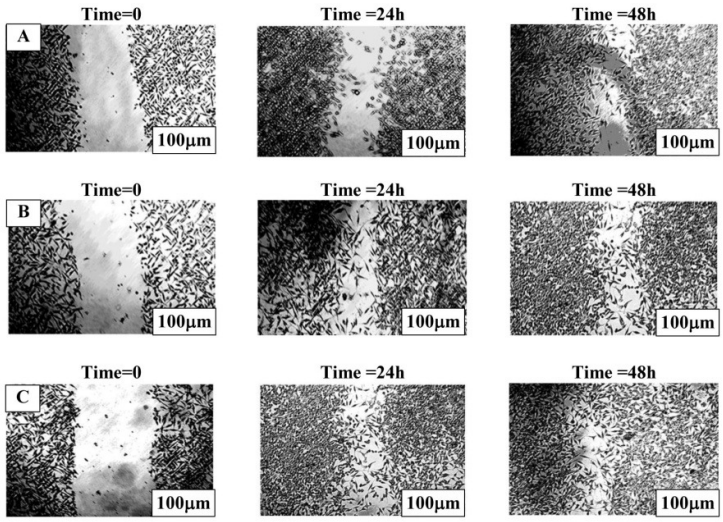

Figure 3. Wound healing assay of inhibited migration by miR-34a related conditioned medium (CM). Uniform scratches were made in MDA-MB-231, which were treated with (A) miR-34a-hAD-MSC-CM, (B) scramble-hAD-MSC-CM or (C) hAD-MSC-CM at 0 h, 24 h, and 48 h, and then photographs were captured by a camera fixed to a Nikon inverted microscope (scale bar=100 µm). The images indicate a delay in filling the scratch area after treatment with miR-34a related CM on the cancer cell line comparing with both control groups.

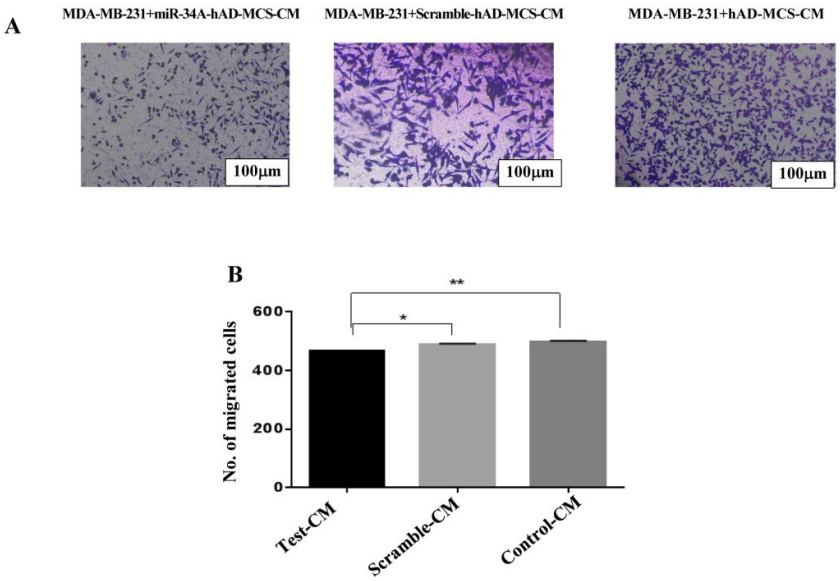

Figure 4. MiR34a-CM reduces invasion of MDA-MB-231 breast cancer cell lines. Transwell migration assays were used to measure cell invasion in MDA-MB-231 after 48 h of treatment with miR-34a-hAD-MSC-CM, scramble-hAD-MSC-CM, and hAD-MSC-CM. (A) Photomicrographs (scale bar=100 µm) showing crystal violet stained cells present on the lower membrane of the transwell. (B) Bar graph showing the mean number of MDA-MB-231 cells present on the lower membrane after 48 h. The asterisk is marked according to the *p* values; **p*=0.01, ***p*=0.004.

MiR-34a-related CM, Reduced mRNA and Protein Expression of PDL1

The results of the assessment using the CT and REST 2009 software showed a significant reduction of PDL1 mRNA expression in cancer cell lines treated with miR-34a-hAD-MSC-CM, compared with the control groups (p=0.05, Figure 6C). On the other hand, the results of western blot revealed a hardly detectable band in MDA-MB-231 treated with miR34a-hAD-MSC-CM compared with two control groups;i.e., scramble-hAD-MSC-CM and hAD-MSC-CM (Figure 6A and 6B). Thus, the results suggested that miR-34a can decrease PDL1 expression level through transcription and translation status.

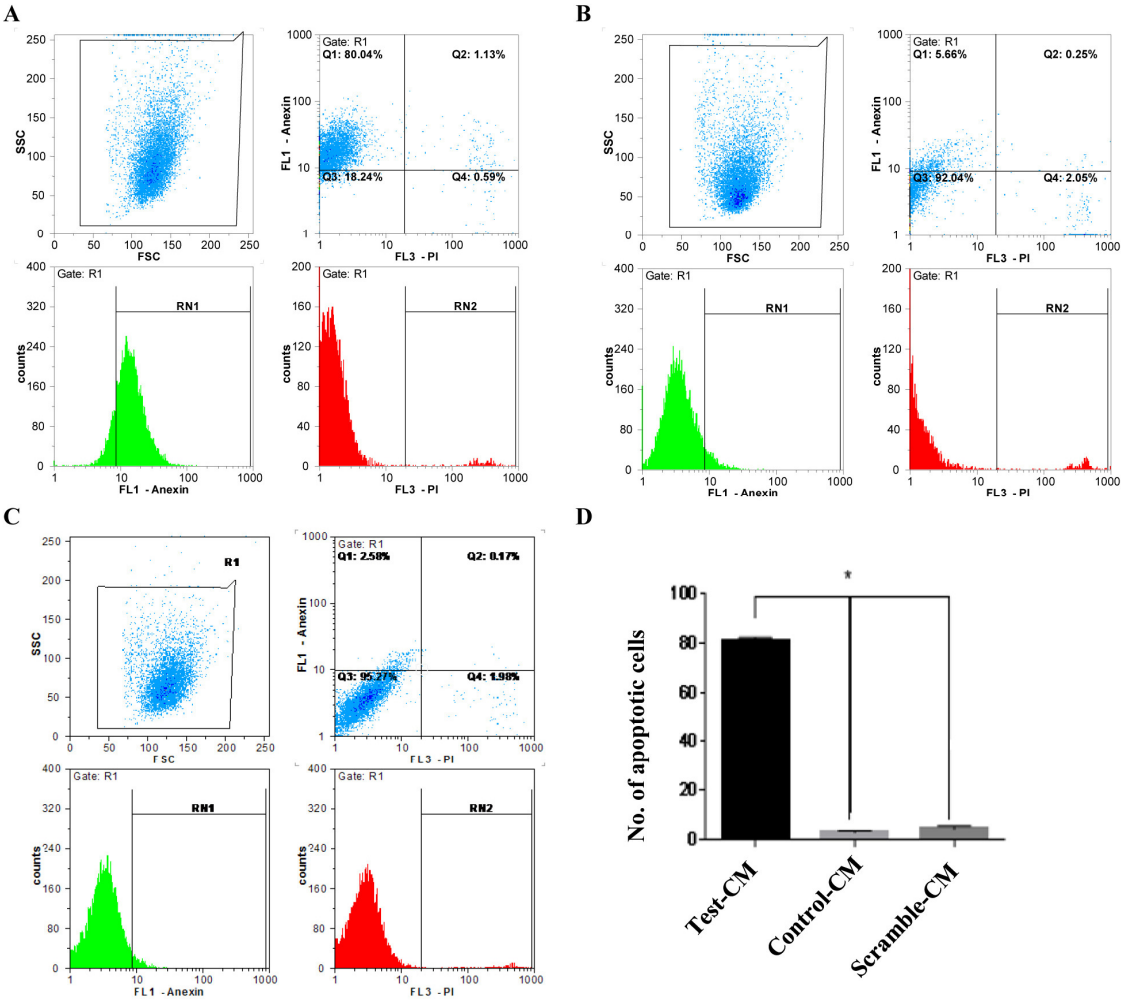

Figure 5. FACS analysis of the apoptosis effects of hAD-MSC-CM. MDA-MB-231 cells treated with (A) miR-34a-hAD-MSC-CM, (B) Scramble-hAD-MSC-CM, or (C) hAD-MSC-CM. The cell death was monitored by annexin V-FITC and propidium iodide staining by flow cytometry. Q1 and Q4 showing the apoptotic and necrotic cells percentage, respectively. (D) The histogram shows the percentage of apoptotic cells in MDA-MB-231 after treatment with CMs (*p=0.02).

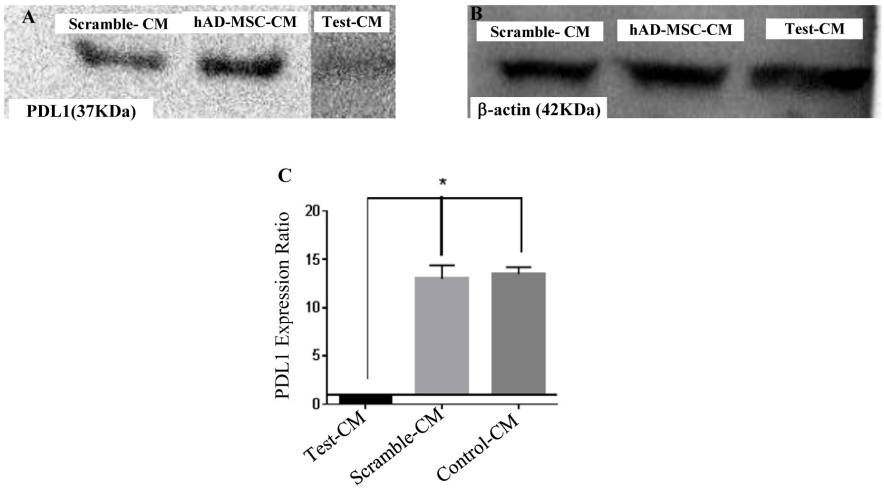

Figure 6. PD-L1 mRNA and protein expression analyses by Q real-time PCR and western blot. Western blot analysis of cell lysates from MDA-MB-231 cell lines loaded to SDS-PAGE transferred to PVDF membrane and labeled with the PD-L1 antibody. (A) PDL1 protein showed hardly detectable band (~37kDa) in extracted protein from a treated MDA-MB-231 cell line with miR-34a-hAD-MSC-CM comparing with control groups (i.e., scramble-hAD-MSC-CM and hAD-MSC-CM. (B)β-actin (42kDa) was set as an internal control. (C) The histogram represents the low expression level of mRNA after treatment with miR-34a-CM in cancer cell lines; using real-time PCR. The asterisk is marked according to the *p*-values; **p*=0.05. The baseline is based reference gene on reaction efficiency (RE=1).

DISCUSSION

MSCs have a vital role in the development and repair of tissues, cancerous metastases through the secretion of extracellular vesicles (EVs), and the release of biologic substances such as growth factors and cytokines.[21] Recent studies have indicated that EVs can regulate gene expressions by delivering miRNAs into adjacent cells.[22,23] MiRNAs are stable in an extracellular environment and can be circulated in a soluble form via binding to lipoproteins, proteins, or through encapsulation in vesicles derived from cellular membrane. Several studies have also been conducted on the effectiveness of MSC-CM on diseases. It is important to note that some of these studies have reported the positive effects of MSC-CM in controlling diseases.[24]

Because of biological substances such as miRNAs, MSC-CM can be considered as effective therapeutic protocols in the treatment of diseases especially cancer. Previous studies have demonstrated that miRNAs serve crucial functions in tumor biology, and miR-34a has been suggested to be associated with breast cancer migration and invasion. It has also been reported that low expression of miR-34a is involved in many cancers especially in breast cancer metastasis and invasion.[25-27] miR-34a is a noncoding RNA that regulates cell cycle, cell senescence, and apoptosis-dependent genes.[20] Therefore, this study aimed to investigate the effects of miR-34a related hAD-MSC-CM on the behavior, functional properties, and expression of PDL1 on breast cancer cell lines. In other words, this study aimed to use miR-34a to shift the functions of MSC-CM toward tumor cell inhibition.[16]

First, the findings of this study revealed that the lentiviral vectors are suitable means for the insertion of external miRNA genes such as miR-34a into the MSCs genes. This study also showed a decrease in the invasiveness potency of cancer cells after treatment with miR-34a-MSC-CM and demonstrated the presence of miR-34a in MSC-CM. This finding is in contrast with F.T. Martin et al. research which showed an increase in invasion potency after treatment of breast cancer cell lines with MSC-CM.[28] Another finding of this study was that there is a reduction in the number of migrated cancer cells in wound healing assay. Thus, it can be argued that delay in wound healing repair after treatment of MDA-MB-231 cell lines with miR-34a-

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hAD-MSCs-CM in comparison with the control groups (i.e., scramble-hAD-MSC-CM and hAD-MSC-CM) is an indication of the important role of miR-34a in cancer proliferation and invasion. This finding is to some extent similar to the findings of research conducted by Rui et al who showed that miR-34a suppresses breast cancer proliferation and invasion after cell line transfection; using miR-34a.[26]

Induction of apoptosis; using miRNAs can be considered as a mechanism for eliminating cancer cells.[29] Apoptosis is physiological and programmed cell death in multicellular organisms. Apoptosis leads to characteristic changes in cell morphology and death as a response to stress shocks such as infections, drugs, and epigenetic changes. The findings of this study confirmed that the use of miR-34a related conditioned medium can induce apoptosis in the cancer cell lines. The increase in anti-apoptosis activity following treatment of CM obtained from MSC on cancer cell line was also reported by other researchers,[16,28] whose results were similar to the results of our control groups. The present study suggests that the inhibitory effects of miR-34a in breast cancer cells may be TP53 dependent. TP53 is a tumor-suppressor transcription factor that, in response to many activators, leads to cell cycle arrest and apoptosis induction.[30] Therefore, it seems that a parallel increase in miR-34a and TP53 can be a novel therapeutic protocol in the treatment of resistant cancers. Previous reports showed that the activation of TP53 led to the expression of miR-34a.[31]

PDL1 regulation is one of the most attractive and controversial subjects in cancer therapy. PDL1 (CD274) is presented on the surface of tumor cells even though it is also observed on the surface of a subset of myeloid lineage and antigen-presenting cells (APCs). Programmed cell death protein 1 (PD1) is an inhibitory co-receptor for PDL1 which is present on the surface of hematopoietic cells such as activated B and T cells, NK cells, and myeloid cell lines. The PDL1-PD1 interaction induces apoptosis in effector T cells, inhibits the activity of cytotoxic T cells, and reduces the production of cytokines. This process causes tumor cells to escape from immunoediting cells and continue growth and metastasis.[32] Preclinical and clinical studies have suggested that PDL1 can be considered as a potential therapeutic or diagnostic marker.[33] Numerous studies have shown that MDA-MB-231 has a high degree of cell surface PDL1 (CD274) in comparison with other cell lines.[32,34,35] Therefore, we decided to use MDA-MB-231 cell lines in this study. The results of our analyses indicated that the presence of miR-34a in CM can reduce PDL1 expression in both transcription and translation levels in the MDA-MB-231 cancer cell line. Xi-Wang et al demonstrated that transfection of two cancer cell lines; i.e., human leukemia-60 (HL-60) and Kasumi-1 with miR-34a, is associated with the reduction of PDL1 expression.[36] The results of the present study demonstrated that the overexpression of miR-34a in MSC-CM can reduce PDL1 expression, migration, and invasion.

The current study aimed to shed light on cancer therapy through the application of miR-34 and CM. The decrease in invasion, migration, and reduced expression of PDL1 along with the increase in apoptosis of MDA-MB-231 cancer cell lines indicated that miR-34a was able to successfully target genes related to migrations, invasion, and apoptosis. In addition, the reduction of PDL1 expression in cancer cell lines suggested that miR-34a can help to increase immune cells' potency in immunoediting processing. Therefore, it is suggested that tumor microenvironment changing with simultaneous application of miR-34a and MSC-CM can induce anti-tumorigenic effects in conditioned medium. Finally, MSC-CM may be used as a therapeutic strategy in breast cancer therapy in the future.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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