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Original Article

The Influence of Extracellular Vesicles from Human Peripheral Blood Mononuclear Cells and Umbilical Cord Mesenchymal Stem Cells on Acute Lymphoid Leukemia Cells

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Abstract

Objective: Many studies have suggested Mesenchymal stem cells, as a promising way to develop new treatment strategies for different types of disease. However, due to their possible tumorigenic effects, their clinical use has been limited. Since a great deal of MSCs' therapeutic benefits depend on MSC-derived extracellular vesicles, these particles have been receiving much attention in the past couple of years. With this in mind, we aimed to study the Effects of both peripheral blood mononuclear cells and human umbilical cord MSC-derived extracellular vesicles on cell growth, proliferation, and apoptosis of the Nalm6 cell line.

Materials and Methods: Isolated HUCMSCs were cultured, and PBMCs were acquired using the Ficoll-Hypaque technique. Their EVs were then extracted. The Nalm6 cells were divided into five groups: a control group and four treatment groups, which were treated with MSC- and MNC-derived EVs at different concentrations. Cell viability and metabolic assays were evaluated using trypan blue staining and the MTT assay, respectively. Thereafter, a flow cytometric assay was performed to detect cell cycle progression and apoptosis.

Results: Our research revealed that the level of metabolic activity between Nalm6-treated EVs and the control group was not significantly different after 3 days. Also, no significant changes were reported in the growth and apoptosis effects of EVs on Nalm6 cells compared to the control group. Furthermore, different concentrations of EVs didn't cause any inhibition on tumor growth.

Conclusion: Obtained EVs neither showed any anti-tumor effect nor caused any progression or aggressiveness in the Nalm6 cell line.

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1. INTRODUCTION

Pre-B-ALL is a highly invasive hematological malignancy, caused by an oncogenic transformation in an early B cell progenitor (1-3). Pre-B ALL is the most common form of malignancy in children (2, 4, 5). Significant breakthroughs have occurred over the past decades in Pre-B ALL treatment, such as immunotherapy. However, there are still some patients who suffer from relapse, which makes these treatments insufficient (1, 2).

Mesenchymal stem cells (MSCs) have been found to be a potential cell-based therapy for human diseases due to their multipotent differentiation ability, self-renewal, and immunomodulatory effects (6-8). Furthermore, many *in vivo* and *in vitro* studies have suggested the anti-cancer effect of MSCs on several particular cell lines. However, several studies have also reported that MSCs may possess tumorigenic effects (9, 10). Bone Marrow, adipose tissue, Wharton's jelly, placentae, and umbilical cord (UC) blood are significant sources of MSC isolation (11, 12). Among all these sources, MSCs obtained from human umbilical cords are an ideal source of MSCs due to some beneficial features such as low cost, ease of access, and low immunogenicity (13). More recent evidence proved that MSC-derived Extracellular vesicles (EVs) are better choices than MSCs since EVs have the same therapeutic effects but are cell-free (6).

Peripheral blood mononuclear cells (MNCs) are a heterogeneous population of immune cells that include lymphocytes, monocytes, and dendritic cells. MNCs play a crucial role in the immune response and the regulation of inflammation (14). MNC-derived EVs are small membrane vesicles that are released by MNCs. MNC-derived EVs have been shown to have immunomodulatory, anti-inflammatory, anti-fibrotic, and anti-angiogenic effects on various target cells and tissues. MNC-derived EVs have also been proposed as potential therapeutic agents for various diseases, such as cardiovascular diseases, autoimmune diseases, neurodegenerative diseases, and cancer (15).

Extracellular vesicles (EVs) have received much attention due to their ability to change protein translation and specific gene expression in their target cells (16). EVs are lipid bilayer surrounding particles secreted by a variety of cells including epithelial cells, lymphocytes, dendritic cells, mesenchymal stem cells, and tumor cells. They are also in biological fluids such as blood and saliva (17). EVs are classified into exosomes, microvesicles (MVs), and apoptotic bodies, according to their size, composition, and origin cells (6, 18). Apoptotic bodies are the biggest (>1000nm). These particles are released from the cells during apoptosis. MVs are nanoparticles with a size of 100-1000 nm that are derived from the plasma membrane (6). Exosomes are the smallest, with a size of 30-100 nm, that originate from endosomes (7). EVs can target both close-range and distant cells (19). Since EVs carry the genetic material of the origin cell, such as DNA, RNA, and microRNA, they are capable of modifying epigenetic properties of recipient cells, as well as initiating signalling cascades and affecting phenotypes. (20). More recent studies have proposed that EVs can affect both tumor suppressor genes and oncogenes, which leads to reprogramming the recipient cells (21).

A few studies have been published on how EVs derived from different origins affect various cell lines. The effect of MSC-EVs was evaluated on two leukemia cell lines, NB4 and K562, by a group of researchers from Thailand. MSC-EVs have been demonstrated to induce apoptosis in leukemic cells, suggesting that they may be applicable in supportive therapies for leukemia (22). In a recent study, researchers tried to evaluate the impact and mechanism of exosomes derived from human bone marrow mesenchymal stem cells (hBM-MSCs-Exo) on the proliferation and apoptosis of the acute myeloid leukemia (AML) cell line THP-1. THP-1 cells exposed to BM-MSCs-Exo demonstrated a decrease in cell viability, an increase in apoptosis, an increase in miR-222-3p expression, and a decrease in IRF1/INPP4B expression (23). In (24), the authors investigated the impact of microvesicles released from human embryonic stem cell-derived mesenchymal stem cells on the growth and proliferation of leukemia cells. The study's findings depicted that microvesicles secreted from human embryonic stem cell-derived mesenchymal stem cells hindered tumor proliferation and activated autophagy and that an excessive amount of autophagy possibly triggered apoptosis.

Multiple research investigations reveal that the tumorigenic or anticancer effects of EVs may be dependent on the MSC sources from which they are acquired, as well as the kind of malignancy(25, 26). With these explanations, this study aims to investigate the effects of extracellular vesicles from human peripheral blood mononuclear cells and umbilical cord mesenchymal stem cells on acute lymphoid leukemia cell line NALM6. These extracellular vesicles were hypothesized to have anti-cancer effects on NALM6 cells by modulating their growth, apoptosis, metabolic activity, and cell cycle progression. This study is significant because it could provide a novel and alternative therapeutic strategy for treating acute lymphoid leukemia using cell-free products derived from human cells.

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2. MATERIALS AND METHODS

This study employed an experimental research design. Nalm6, a Pre-B-ALL cell line, was obtained from the Pasteur Institute of Iran. The Nalm6 cells were split into five groups: a control group and four experimental groups, which were exposed to MSC- and MNC-derived EVs separately at the amounts of 50, 100, 200, and 500 µg/ml respectively. Control cells were cultured in the same medium as the test groups, with the only difference being that they were not exposed to varying concentrations of MSC and MNC-derived EVs.

2.1. Isolation of human peripheral blood mononuclear cells

Ten healthy volunteers (7 males, 3 females; ages 29-36) gave written informed consent to have their blood samples collected and de-identified. The mononuclear cells were then separated from the blood using a density centrifugation technique (Sigma-Aldrich, USA), washed twice with phosphate-buffered saline, and resuspended in RPMI 1640 (ATOcell, Austria) with 0.5% BSA (Invitrogen, USA) and 1% penicillin-streptomycin (Invitrogen, USA). This mixture was incubated for 48 hours at 37 ˚C in a 5% CO2 atmosphere.

2.2. Mesenchymal stem cell cultivation and characterization

Human umbilical cord MSCs were collected from a cord blood bank and maintained in a particular solution with 10% fetal bovine serum (FBS) (Invitrogen, USA). The cells were then kept in an incubator (Binder, Germany) at 37 ˚C with 5% CO2 and had their medium replaced every 3-4 days. Cells from passages 3-5 were chosen for the study.

MSCs were isolated and analyzed for morphological characteristics using an inverted microscope. Cells were detached from the flask with trypsin (Invitrogen, USA) and centrifuged at 400 g× for 5 minutes. Then washed twice with phosphate buffer and separated into test tubes. Monoclonal antibodies, including CD73-PE, CD29-PE, CD44-FITC, CD90-FITC, CD45-FITC, CD34-PE, and CD105-PE, were added to the test tubes. IgG-FITC/PE antibody was added to an isotype control tube. Subsequently, the cells were fixed with paraformaldehyde and analyzed with a flow cytometry device (Partec PAS III) and Flomax software.

2.3. Isolation of MNC-derived EVs

Supernatant was collected from incubating MNCs and centrifuged (Eppendorf, Germany) twice at 2500g for 30 minutes at 4 ˚C to remove cell debris. The obtained supernatant was then centrifuged at 20.000 g for two hours at 4 ˚C. Resulting pellet was resuspended in FBS-free RPMI 1640. This fraction was used as a source of MNC-derived EVs in this study (27).

2.4. MSC-derived EVs isolation

Cultured Human umbilical cord mesenchymal stem cells (HUCMSCs) were incubated in DMEM/Low glucose and 0.5% BSA for two to four days. Cells from passages 3-5 were used, then the supernatant was removed and RPMI 1640 supplemented with 0.5% BSA and 1% penicillin-streptomycin was added, and incubated for 24h at 37 °C. The collected supernatants were centrifuged (Sigma) twice at 2500 rpm for 30 minutes, then again at 20.000 g for one hour at 4 °C, which resulted in a pellet that was resuspended in FBS-free RPMI 1640 medium to be used as a source of MSC-derived EVs (27).

2.5. Characterisation of EVs

To assess the protein content of EVs, a Bradford experiment was performed using a NanoDrop spectrophotometer (WPA, UK). Firstly, different bovine serum albumin dilutions (125, 250, 500, 1000, and 2000 µg/ml) were mixed with 200 µl of Bradford solution and OD was measured at 595 nm. The results were used to plot a standard curve. Subsequently, 200 µl of Bradford solution, was mixed separately with 10µl of isolated EV samples from MSCs and MNCs. The OD was measured at 595 nm, and finally, using the standard curve, the protein content of EVs was assessed.

The size distribution of MSC and MNC-derived EVs was analyzed using dynamic light scattering (DLS) by a Malvern Master Sizer 5111 (Worcestershire, UK) laser diffraction system.

Furthermore, flow cytometry (Partec PAS III, Germany) was used to examine surface marker expression in MSC-derived extracellular vesicles (EVs). For this, EVs were mixed with fluorescently labeled antibodies specific to CD90, CD44, CD45, and CD34 (Dako, Denmark). After incubation in the refrigerator, the samples were analyzed with Partec CYFlow Space software.

2.6. Cell culture

Nalm6 as a Pre-B-ALL cell line was obtained from the Pasture Institute of Iran. Cells were cultured in a T25 flask containing RPMI 1640 medium (ATOcell, Austria),

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supplemented with 10% FBS and 1% penicillin-streptomycin solution. The cell cultures were incubated at 37 °C in a humidified 5% CO2 atmosphere.

2.7. Viability assay

Trypan blue staining (Sigma, USA) was used to test the possible cytotoxicity of the collected EVs on Nalm6 cells. The experiment was conducted three times using EVs taken from MSCs and PBMCs. Tests were conducted daily for 10 days, with the cells reaching an 80% confluency at the time. The media on the plates was replaced every three days, and extracellular vesicles obtained from mononuclear cells (MNC) and mesenchymal stem cells (MSC) were added. The cells were plated at a density of 200×10^3/well. Cells were cultured with different concentrations of MNC and MSC-derived EVs (50, 100, 200, and 500 µg/mL) for ten days in 6-well plates. The cells were treated separately with the indicated concentrations of EV-MNC and EV-MSC, not combined. After that, cells were centrifuged at 500g× for 5 min, and the supernatant was discarded. Resulting pellet was resuspended in 1ml of serum-free RPMI1640. Subsequently, 10 µl of 0.4% trypan blue was mixed with the same volume of cells. A Neubauer chamber and an inverted microscope (Nikon, Japan) were used to examine viable cells.

2.8. Metabolic activity

Subsequently, MTT assay was used to evaluate the metabolic activity of Nalm6 cells. The cells were plated in a 96-well plate at a density of 5×10^3/well in RPMI1640 media with 10% FBS and 1% penicillin-streptomycin solution. Next, cells were treated separately for 72h with different concentrations of MNC and MSC-derived EVs (50, 100, 200, and 500 µg/mL). The cells were treated separately with the indicated concentrations of EV-MNC and EV-MSC, not combined. The experiment was conducted three times. Every dose was tested in three wells each time. Then 100µl of MTT solution (Invitrogen, USA) was added to each well and incubated for 3-4h at 37 °C in 5% CO2. Following this step, the supernatant was removed, and 100µl of DMSO (Merck, Germany) was added to each well. Cells were then kept for 30 min in the dark at RT. An ELISA reader set to 570 nm was used to detect absorbance in each well. Nalm6 cells cultured in EV-free RPMI1640 were used as a negative control, and DMSO was used as a blank solution. To calculate cell metabolic activity, the following equation was used: (Treated cell OD/control OD) ×100.

2.9. Annexin/PI staining

To assess the apoptosis effect of MNC and MSC-derived EVs on the Nalm6 cell line, the Nalm6 cell line at a density of 100×10^3 cells/well was co-cultured with the MNC and MSC-derived EVs (500 µg/mL) in a six-well plate, for three days. The cells were treated separately with the indicated concentrations of EV-MNC and EV-MSC, not combined. The experiment was conducted three times using EVs taken from MSCs and PBMCs. Following this step, according to the manufacturer's protocol, cells were transferred to a falcon and centrifuged at 2000 rpm for 5 minutes. The supernatant was discarded, and the cell pellet was washed twice with ice-cold PBS. The cells were then suspended in 100µl of binding buffer. Next, 5µl of Annexin V-FITC and propidium iodide (PI) (BD, Biosciences, USA) was added to each well and incubated in the dark at RT for 15 minutes. Finally, the cells were analyzed via flow cytometry using Partec CYFlow Space software.

2.10. Cell cycle analysis

To assess cell cycle progression, the Nalm6 cell line at 100 cells/well was cultured in six-well plates in the presence of both MNC and MSC-derived EVs (500 µg/mL) for three days. The cells were treated separately with the indicated concentrations of EV-MNC and EV-MSC, not combined. After treatment, Phosphate buffered saline was used to wash the cells twice. The pellet was then resuspended in 50 µl of RNase A stock solution and 500 µl of propidium iodide staining solution and incubated for 15 min at 37°C. Finally, flow cytometry was used to determine the proportion of cells in each stage of the cell cycle, including G1, S, and G2/M, using Partec CYFlow Space software.

2.11. Data Analysis

The mean values of the experimental data were used to compare the differences among the experimental groups using SPSS version 18.0 (SPSS, Inc., Chicago, IL, USA). All tests were performed in duplicate or triplicate. A two-way ANOVA test was applied to analyze the MTT and Trypan blue data, while a One-way ANOVA test was employed to assess the flow cytometry data. The values were considered statistically significant at *p ≤0.05, **p ≤0.01 and ***p ≤0.001.

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3. RESULTS

3.1. Characterization of HUCMSCs

Characterization of MSC-derived umbilical cords was conducted using their surface marker expression and morphology. Flow cytometry analysis indicated that HUCMSC exhibits CD90, CD105, CD44, CD73, and CD29 but is negative for CD34 and CD45 (**Fig. 1**). MSC analysis using an inverted microscope showed a spindle-shaped morphology and monolayer growth.

3.2. Characterization of MNC and MSC-derived EVs

Flow cytometry was employed to examine the surface marker expression of MSC EVs.The flow cytometric assay showed the presence of CD90 and CD44 but not CD34 and CD45 (**Fig. 2**). A DLS technique was used to quantify EV size in order to characterize isolated EVs. The majority of MNC- and MSC-derived EVs were 324 nm and 340 nm in size, respectively (**Fig. 3**).

3.3. Cell viability

To determine the effect of MNC and MSC-derived EV on the viability of the Nalm6 cell line, the Nalm6 cell line was co-cultured with the obtained EVs for ten days, and the trypan blue exclusion dye was yielded every day during these ten days. Nalm6 cells with no EV treatment were used as controls. The analysis didn't show any significant differences between co-cultured cells in comparison to the control group (P-value>0.05) (**Fig. 4**). The control group exhibited 97% viability. In contrast, cells treated with MNC and MSC-derived EVs displayed viability of 95±1%.

3.4. Metabolic activity

Nalm6 cells were treated with both MNC and MSC-derived EVs at different concentrations for 72h. The MTT assay did not reveal any significant differences between the metabolic activity of treated cells and the control group (P-value>0.05) (**Fig. 5**). The control group exhibited a metabolic activity of 100%. In contrast, cells treated with MNC and MSC-derived EVs revealed a metabolic activity of 96% ± 2%.

3.5. Annexin/PI staining

The apoptotic effect of obtained EVs was detected by co-culturing both MNC- and MSC-derived EVs (500 µg/mL) with the Nalm6 cell line for five days. Afterward, annexin V/PI staining was performed. No significant correlation was found between EV-exposed Nalm6 cells and the control group (P-value>0.05) (**Fig. 6**).

3.6. Cell viability

The cell cycle distribution of the Nalm6 cell line was determined after being exposed to both MNC- and MSCs-derived EVs (500µg/ml) for three days. The PI staining technique and flow cytometry were used to assess the influence of MNC and MSC-derived EVs on cell cycle progression. We did not observe significant changes in cell cycle phases compared with the control (P-value>0.05) (**Fig. 7**).

4. DISCUSSION

The current experiment is one of the first to investigate the effects of EVs obtained from peripheral blood mononuclear cells and HUCMSC on acute lymphoid leukemia cell line NALM6. This approach could potentially offer a new therapeutic strategy for treating leukemia using cell-free products derived from human cells (28). This research demonstrated no significant changes in Nalm6 cell growth or apoptosis after exposure to the EVs obtained from peripheral blood mononuclear cells and HUCMSC. The anti-cancer effect of MSCs has been revealed by several studies (11, 29). However, there is still some controversy surrounding these results (30). In a few studies, it has been proven that MSCs can cause tumor progression. MSCs were not only able to form tumor microenvironments but also promote tumor growth and metastasis (31, 32). In a recent study, co-culture of both the BV173 cell line and the Jurkat cell line with MSCs resulted in growth inhibition of leukemic cell lines (33).

Several studies have demonstrated that alternative sources of MSC-derived EVs can replicate these cells' immunomodulatory and anti-inflammatory activities(6, 21). A promising alternative to whole-cell therapy for many hematological malignancies could be MSC-derived EVs due to their lower immunogenicity and higher safety (34, 35). EVs are critical mediators in cell-to-cell communications (13, 29, 31, 36). They can transfer proteins, functional mi-RNAs, and mRNAs to the target cell (7, 37, 38). This leads to a genetic exchange of information between cells and causes functional and phenotypical alterations in the recipient cells (16, 20). Shuai Wu and co-workers revealed that UCMSC-derived EVs can cause growth inhibition in the T24 bladder leukemic cell line *in vitro* and *in vivo* (39). In this paper, we have evaluated alterations in apoptosis and growth of the Nalm6 cell line following treatment with both HUCMSC and MNC-derived EVs. Contrary to expectations, after treatment of the Nalm6 cell line with the isolated EVs for ten days, no significant differences were observed in apoptosis or proliferation of the cell line. Afterward, the

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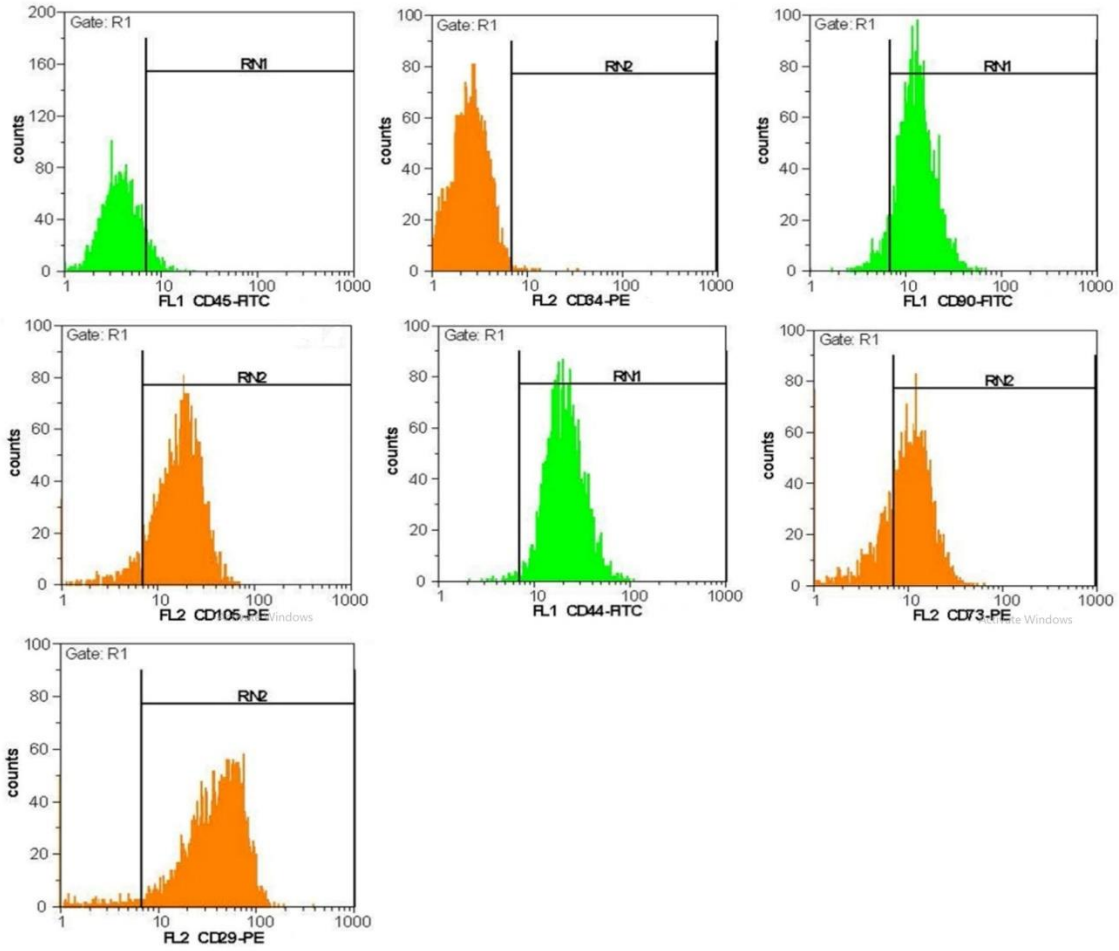

Figure 1. The expression of surface molecules on HUCMSCs was wxamined by flow cytometry and revealed that they were positive for CD90, CD105, CD29, CD73, which are typical markers of MSCs, and negative for CD34, CD45, which are hematopoietic markers.

metabolic activity of the treated cells was also measured by MTT assay. After three days of exposure to the isolated EVs, the results did not reveal any significant changes in Nalm6 cells' metabolic activity (P value > 0.05). In contrast to previous research that found mesenchymal stem cell-derived EVs trigger apoptosis in the hepG2 cell line (40). In the current study, the Nalm6 cell line was treated with both MNC and MSC-derived EVs (500 µg/ml) for five days, and then the apoptosis rate was measured using flow cytometry and annexin V/PI staining. The analysis showed no significant variations between the treated Nalm6 cells and the untreated group. Furthermore, Du T and colleagues discovered that hWJMSCs-derived EVs induced the RCC cell line to proliferate and become more aggressive (31, 41). These inconsistent findings that have been reported regarding the role of MSC-EVs in tumor progression can be due to variations in MSC source, tumor type, animal model, and other factors (42). Therefore, further data collection would be needed to determine precisely how EVs affect leukemia cell line growth and apoptosis.

Wong et al. discovered that following exposure to MSCs, both the BV173 and Jurkat cell lines were stopped in the G0/G1 phase (33). Our results do not seem to confirm their observations. In our study, Nalm6 cells were treated with both MSC and MNC-derived EVs at a concentration of 500 µg/ml for three days to evaluate cell cycle progression. No significant differences were found in cell cycle analysis between treated cells and the control group (P <0.05).

The significance of the current study is that it challenges the previous assumptions that MSC-derived EVs have anti-cancer effects on various types of tumors. The findings revealed that neither PBMC-derived EVs nor HUCMSC-

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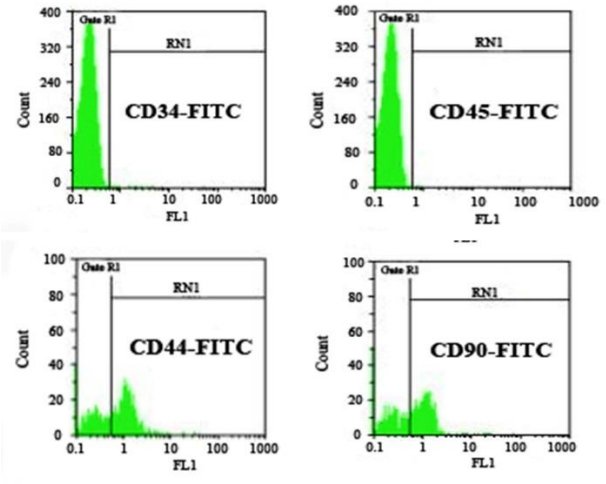

Figure 2. Flow Cytometric validation of cell surface markers on isolated EVs derived from mesenchymal stem cells. EVs derived from MSCs were positive for surface expressed molecules, CD44 and CD90, and negative for surface expressed molecules, CD45 and CD34, expressed on hematopoietic stem cells.

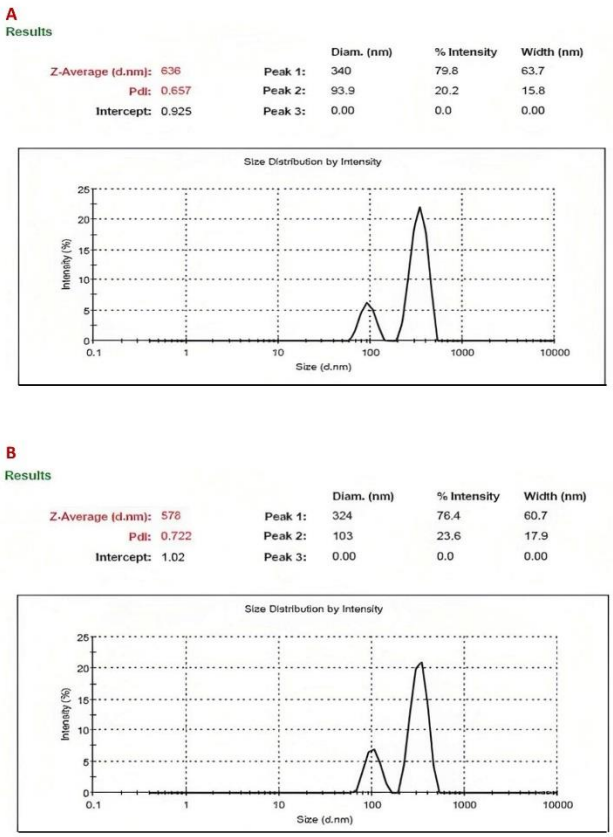

Figure 3. Representing the Size distribution of MNC (a) and MSC (b) -derived EVs. The size of EVs was determined by DLS analysis. The mean distribution was reported to be in the range of 324nm and 340 nm, respectively.

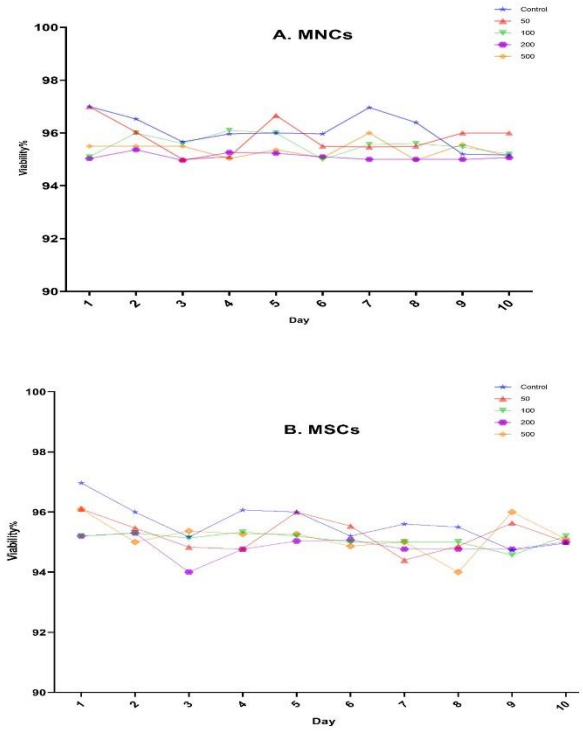

Figure 4. Effect of MNC (a) and MSC (b) -derived EV on Nalm6 cell line viability. Nalm6 cell lines were treated with obtained EVs (50, 100 ,200, and 500 µg/ml) for ten days. A trypan blue exclusion assay was used to determine Cell viability. The experiment was conducted three times using EVs taken from MSCs and PBMCs (n=3). The results of the viability test were presented as a percentage relative to the control group of each experiment prior to statistical analysis (mean). No significant variations were observed between co-cultured cells and the control group. (P-value > 0.05). Two-way ANOVA test was applied to analyze trypan blue assay.

derived EVs have a significant influence on NALM6 cell proliferation, apoptosis, metabolic activity, or cell cycle progression. This suggests that the effects of EVs on leukemia cells may depend on various factors, such as the source of EVs, the type of leukemia, the dose and duration of treatment, and the interaction with other microenvironmental components (43).

More recent evidence shows that malignant cells are capable of releasing EVs. Tumor microvesicles (TMVs) carry nucleic acids, proteins, and lipids. They have the ability to transfer these chemicals to surrounding cells, trigger signaling pathways, and impact the tumor microenvironment (29, 44). They have a critical role in creating a malignant phenotype in normal cells. TMVs can significantly increase cell proliferation by delivering

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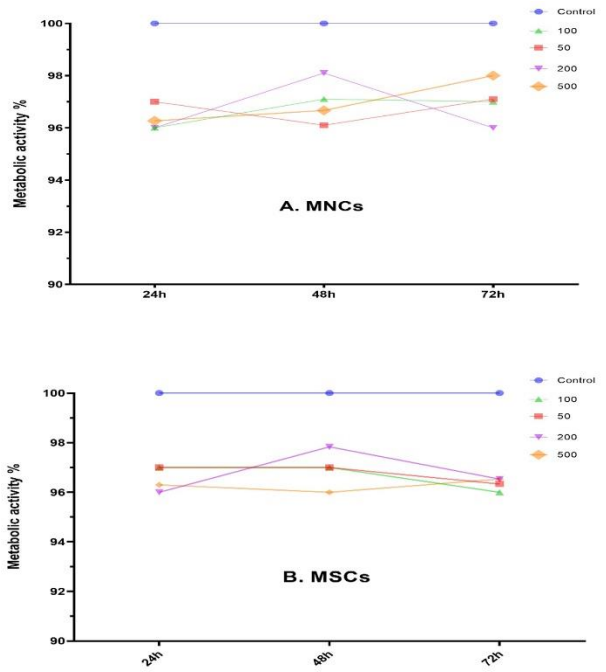

Figure 5. MNC (a) and (b) MSC -derived EVs effect on the metabolic activity of the Nalm6 cell line was determined by MTT assay. Different concentrations of the obtained EVs were considered. The experiment was conducted three times using EVs taken from MSCs and PBMCs (n=3). The results of the MTT test were presented as a percentage relative to the control group of each experiment prior to statistical analysis (mean). The result didn't show any significant alteration in metabolic activity (P-value > 0.05). Two-way ANOVA test was applied to analyze MTT.

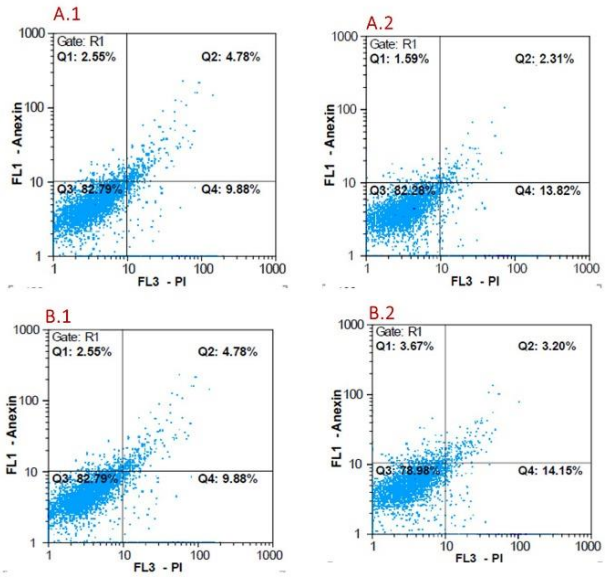

Figure 6. Apoptotic percentages of Nalm6 cells. (A.1) Untreated cells as a control. (A.2) Co-cultured with MSC derived EVs (B.1) Untreated cells as a control. (B.2) Co-cultured with MNC derived EVs for 5 days. After co-culturing with the obtained EVs, cell death was not significantly increased in comparison to the control group (P-value > 0.05). One-way ANOVA test was applied to analyze flowcytometry data.

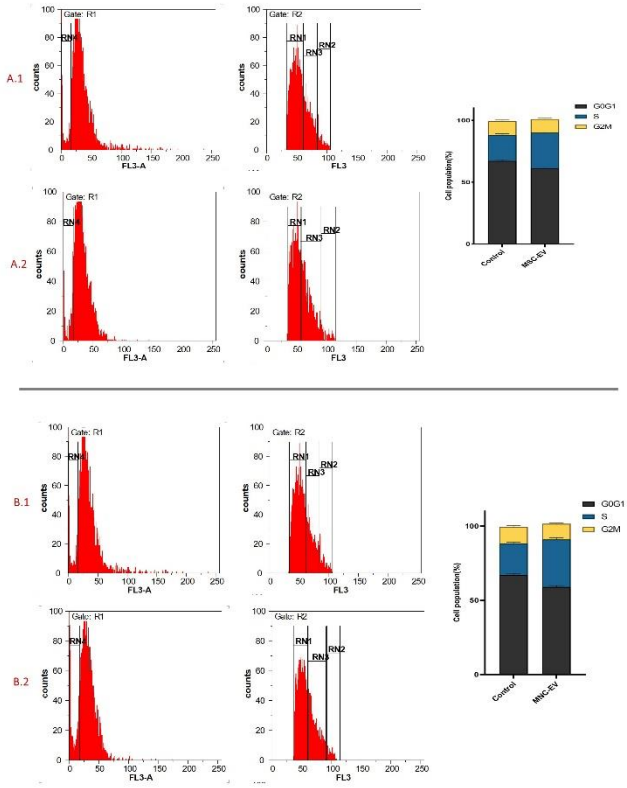

Figure 7. Cell cycle distribution. (A) Cell cycle distribution of Nalm6 cells 1) untreated cell as a control. 2) Co-cultured with MSC- derived EVs (B) cell cycle distribution of Nalm6 cells 1) untreated cell as a control. 2) Co-cultured with MNC derived EVs for three days. The experiment was conducted three times using EVs taken from MSCs and PBMCs (n=3). Co-cultured cells didn't show any significant alterations in cell cycle progression in comparison to the control group. (P-value > 0.05) One-way ANOVA test was applied to analyze cell cycle data.

oncogenes to tumor and normal cells (44). By secreting TMV, malignant cells are also taking drugs and apoptotic factors out, leading to a higher survival rate in these cells (45). Interestingly, TMVs could significantly prompt tumor metastasis even at the concentration of 0.1 µg/ml (46). Hence, future studies on TMVs are required to enlighten their mechanisms and effects on malignant cells and therapeutic interventions.

Our results are in contradiction with those reported in the literature. Moreover, some controversial results were reported from the previous studies. These studies demonstrated the enhancement of proliferation in malignant cells following treatment with MSC or MSC-derived EVs. Many mechanisms have been suggested to justify these findings, but none have been able to do so.

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One of the main reasons is suggested to be the heterogeneity and various tissue recourses of MSCs (42). According to many researches, MSCs may have a dual function in cancer progression. Despite being effective in cancer therapy, a few studies have proven that these can cause tumor advancement (30).

This experiment has important implications for future research and clinical applications of EVs in leukemia therapy. It highlights the need for more comprehensive and systematic studies to elucidate the molecular mechanisms and pathways involved in the EV-mediated modulation of leukemia cells. It also calls for more careful evaluation and optimization of the optimal conditions and parameters for EV isolation, characterization, delivery, and monitoring. Furthermore, it emphasizes the importance of considering the heterogeneity and variability of leukemia cells and their response to EVs in different settings and scenarios.

4. CONCLUSION

The current paper demonstrated no significant alterat ions in Nalm6 cell line proliferation and advancement after treatment with MSC and MNC-derived EVs. We proposed that further research be done on patient samples to get a better conclusion.

Acknowledgment

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Conflict of interest

None

Ethical approval statement

This study was approved by the Research Ethics Committee of the Iran University of medical sciences. All participants gave their written informed consent prior to the collection of blood samples. The study was conducted in accordance with the Deceleration of Helsinki and the International Ethical Guidelines for Biomedical Research Involving Human Subjects. No personal or identifiable information was collected or disclosed in this study.

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