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Original Article

Cytokines secreted from bone marrow-derived mesenchymal stem cells promote apoptosis of CD34$^+$ leukemic stem cells as anti-cancer therapy

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ABSTRACT

Objective: The effect of mesenchymal stem cells (MSCs) on the immortal characteristics of malignant cells, particularly hematologic cancer cells, remains a topic of debate, with the underlying mechanisms still requiring further elucidation. We explored the *in vitro* effect of the bone marrow-derived MSCs (BM-MSCs) on CD34$^+$ leukemic stem cells (LSCs) enriched from the KG1-a cell line by assessing apoptosis, measuring cytokine levels, and examining TERT protein expression. Additionally, the potential signaling pathways implicated in this process, such as P53, PTEN, NF-κB, ERK1/2, Raf-1, and H-RAS, were also investigated.

Methods: CD34$^+$ LSCs were enriched from the KG1-a cell line with the magnetic activated cell sorting (MACS) method. Two cell populations (BM-MSCs and CD34$^+$ LSCs) were co-cultured on trans well plates for seven days. Next, CD34$^+$ LSCs were collected and subjected to Annexin V/PI assay, cytokine measurement, and western blotting.

Results: BM-MSCs caused a significant increase in early and late apoptosis in the CD34$^+$LSCs. The significant presence of interleukin (IL)-2 and IL-4 was evident in the co-cultured media. In addition, BM-MSCs significantly increased the protein expression of P53, PTEN, NF-κB, and significantly decreased p-ERK1/2, Raf-1, H-RAS, and TERT.

Conclusion: The mentioned effects of IL-2 and IL-4 cytokines released from BM-MSCs on CD34$^+$ LSCs as therapeutic agents were applied by the components of P53, PTEN, NF-κB, p-ERK1/2, Raf-1, and H-RAS signaling pathways.

1. Introduction

Although the role of Mesenchymal stem cells (MSCs) is not completely defined, they secrete various cytokines with multifunctional properties [1]. MSCs are particularly intriguing due to their unique attributes, like multipotency and self-renewal capabilities. They hold promise for clinical applications and can transform into diverse cell types, like neuron-like cells, osteocytes, and adipocytes [2]. Their plasticity and lack of immunogenicity have made them a consideration for cell-based therapies [3]. In terms of MSCs' impact on leukemic cells, various signaling pathways like extracellular signal-regulated kinase (ERK) 1/2, mitogen-activated protein kinases (MAPK), AKT, and glycogen synthase kinase (GSK)-3α/β have been studied. However, the effects of MSCs on other signaling pathways such as Raf, PTEN, RAS, and NF-kB have not been extensively explored.

The use of MSCs in cell transplantation has garnered increased attention in the context of hematological disorders and blood

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malignancies. Several investigations have used bone marrow (BM)-MSCs, and no instances of tumor formation post-BM-MSC transplantation have been documented, a trend observed across different human and animal sources [4,5]. Moreover, BM-MSCs could potentially promote tumor growth by either boosting the invasive capabilities of tumor cells or shielding them from immune cell detection [6]. In the other words, there are apprehensions regarding these cells, and the uncertainties surrounding the risks associated with cell therapy persist, especially concerning patients with pre-existing cancer. Reports have highlighted the pivotal role of interactions between cancer cells and MSCs in fueling both tumor progression and invasiveness [7]. Tumor cells might induce alterations in the surveillance and molecular composition of MSCs as stromal cells during tumor advancement, potentially influencing the properties of cancer cells [8]. Numerous studies have explored the impact of MSCs on cancer cell lines [9]. MSCs may stem from progenitor cells in the tumor-resident stroma and possess the ability to migrate toward injured tissues, guided by chemotactic gradients of cytokines secreted from those same injured tissues [10]. Additionally, researches have demonstrated that BM-MSCs can induce immunosuppression through the secretion of soluble cytokines [11]. However, there is limited documentation on the impact of the type and quantity of BM-MSC-derived cytokines and growth factors and the mechanisms involved. In the existence of cancer cells, MSCs neither promote tumor angiogenesis *in vivo* nor differentiate *in vitro*. Farahzadi et al. (2022) and Fathi (2019) et al. explored the impacts of released cytokines from BM-MSCs on acute myeloid leukemia (AML) cells (KG1-a cell line) and chronic myeloid leukemia (CML) cells (K562 cell line), respectively [9,12]. These findings introduce intriguing new perspectives on the interplay between cancer and stem cells.

We explored the effect of factors secreted from BM-MSCs on $CD34^+$ leukemic stem cells (LSCs) through the EMT-related signaling pathways. As KG1-a cell line efficiently expressed $CD34^+$ cells, this cell line has been selected for this study [13]. This objective was achieved by cultivating $CD34^+$ LSCs alone and co-culturing $CD34^+$ LSCs with BM-MSCs (10:1), and analyzing the apoptosis assay using flow cytometry and some proteins related to EMT-signaling pathways.

2. Materials and methods

2.1. Reagents

With the exception of the cell culture materials procured from SPL and Gibco, the remaining materials are detailed within the manuscript text.

The cells were divided into the control group (culture of $CD34^+$ LSCs alone), and the experimental group (co-cultured $CD34^+$ LSCs and BM-MSCs).

2.2. Isolation of BM-MNCs

Isolation of mononuclear cells (MNCs) was done based on Fathi et al. (2022) [14]. Briefly, ethical approval was obtained from the Tabriz University of Medical Sciences (IR.TBZMED.VCR.REC.1402.836), and 5 (5–8 weeks old) Rattus rats were humanely euthanized by ketamine and Xylazine. Subsequently, BM was washed using washing buffer (PBS treated with 5% FBS) and layered onto Ficoll-Paque (Innotrain, Germany). Following centrifugation (850×*g*/25 min/4 °C), the MNC layer was harvested and washed with washing buffer.

2.3. Culturing and characterization of BM-MSCs

To culture the BM-MSCs, the MNCs collected in the previous step underwent suspension in Dulbecco's Modified Eagle Medium (DMEM) with low glucose (Gibco-BRL) and 10% FBS. The plates were placed in an incubator (37 °C) for three days. After the third day, the cells were rinsed with warm PBS and passaged using 0.25% trypsin/EDTA [15]. BM-MSCs from passages 3–6 were utilized. Flow cytometry was employed to characterize the BM-MSCs [16]. In this process, 10×10^4 cells were trypsinized and exposed to FITC-conjugated CD73 and CD34 antibodies, as well as PE-conjugated CD105 and CD56 antibodies (BD Bioscience, USA) for 40 min on ice. Following the incubation period, a flow cytometry device (Becton, USA) measured the fluorescence intensity of the cells.

2.4. Enrichment of $CD34^+$ LSCs

$CD34^+$ LSCs were isolated using the Magnetic Activated Cell Sorting (MACS) technique following the guidelines provided by Miltenyi Biotech Co. (Germany) [17]. In brief, after culturing the KG1-a cells, an acute myelogenous leukemia cell line, the cells were washed and suspended in PBS buffer supplemented with 3–5% FBS. Subsequently, 1×10^7 cells were tagged with CD34 microbeads for 20 min at 4 °C on a rotator. The cells were then rinsed with washing buffer and separated using an LS column connected to a MidiMACS Seperator. Following the separation process, the $CD34^+$ cells were collected for further analysis. Flow cytometry with CD34 antibodies (PE, BD Bioscience) assessed the purity of the isolated $CD34^+$ cells.

2.5. Co-culture of $CD34^+$ LSCs with BM-MSCs

Cryopreserved BM-MSCs from passage 3 were thawed, and Trypan blue staining determined the viability of the cells. The cell concentration was adjusted to $150 \times 10^3/cm^2$ by DMEM low glucose culture medium treated with 1% (v/v) streptomycin/penicillin solution and 10% FBS. Following culturing, the BM-MSCs were detached with trypsin, harvested, and seeded into *trans*-well inserts in a 6-well plate. Following 24 h, 10×10^5 $CD34^+$ LSCs in 2 mL of RPMI 1640 complete culture medium were separately added to two groups of BM-MSCs: the control group (cultured with $CD34^+$ LSCs alone) and the experimental group (co-cultured with $CD34^+$ LSCs and BM-MSCs). On day 7, the $CD34^+$ cells cultured alone and those co-cultured with BM-MSCs (at a ratio of 10:1) were collected for annexin/PI analysis and western blotting. Subsequently, the supernatants from both cell groups were gathered for cytokine measurement.

2.6. Flow cytometric detection of apoptosis using Annexin V/PI

To explore the potential induction of apoptosis in $CD34^+$ cells by the co-culture with BM-MSCs, Annexin V/PI analysis was conducted on both groups. After the co-culture phase, $CD34^+$ cells were harvested and gently resuspended in 1X binding buffer (reference number: 00-0055-56, e-bioscience) and stored at 4 °C. Subsequently, the cells underwent staining with binding buffer, FITC-conjugated Annexin V (reference number: 11-8005-74, e-bioscience) was added, and the mixture was incubated for 15 min at 25 °C. Following a wash, the cells were resuspended in a binding buffer with 5 mL of PI and incubated for an additional 15 min in a dark environment at 25 °C. Fluorescence levels were assessed using FACSCalibur (BD Bioscience), and the FlowJo software X.0.7.49 analyzed the data.

2.7. Western blotting analysis for determination of protein expression

Upon completion of the co-cultivation period, cells from the two groups were collected, and protein extraction was carried out following the methodology outlined by Bagheri et al. (2021) [18]. In brief, the cells were rinsed using PBS and then lysed using RIPA buffer. Subsequently, the protein level was determined using the Pierce BCA Protein Assay kit (USA). The cell protein specimens were then isolated on a 12% SDS-PAGE gel, followed by transferring onto polyvinylidene difluoride (PVDF) membranes, which were exposed to primary antibodies at a 1:1000 dilution against β-actin (sc-47778), P53 (sc-126), ERK1/2 (sc-292838), PTEN (sc-7974), NFκB (sc-8008), p-ERK1/2 (sc-16981-R), Raf-1 (sc-7267), H-RAS (sc-35), TERT (E-AB-33070), followed by secondary antibody exposure at a 1:5000 dilution. Lastly, using the ECL Kit (Roche, UK), the protein bands were visualized. The ImageJ 1.6 software quantified protein band intensity, and the signal intensity was normalized to the relevant β-actin control.

2.8. Cytokine measuring by ELISA

The culture media from both the experimental and control groups, as previously mentioned, was collected. ELISA was conducted following the producer's instructions (R&D Systems, China). Briefly, a 96-well plate was coated with detection Reagent A and left to incubate for 16 h at 4 °C. Subsequently, cell culture media containing IL-2, IL-4, and IL-15 antibodies was added to the 96-well plate and analyzed using the ELISA sandwich technique [19].

2.9. Statistical analysis

The results were analyzed using the software program Graph Pad Prism version 6.01. We used two-way ANOVA followed by Sidak post hoc test to determine the significant difference among groups, respectively.

3. Results

3.1. Phenotypical characterization of BM-MSCs

The BM-MSCs were assessed for the expression of cell surface markers (Fig. 1). BM-MSCs exhibited positivity for mesenchymal markers CD105 (94.6%) and CD73 (95.2%), while they were negative for CD56 (0.16%) and CD34 (0.12%) hematopoietic markers.

3.2. Identification of $CD34^+$ cells

For identifying enriched cells by the MACS method, flow cytometry was done. As shown in Fig. 2, the enriched $CD34^+$ cells showed high CD34 expression levels (91.6%).

3.3. Apoptosis assay by Annexin V/PI assay

Late apoptotic cells were positive for both PI and Annexin V ($Annexin^+$, PI^+), while early apoptotic cells exhibit Annexin V positivity and PI negativity ($Annexin^+$, PI^-). To evaluate the impact of cytokines released by BM-MSCs on the apoptosis of $CD34^+$ LSCs, cells from both groups were collected, and Annexin V/PI analysis was conducted using flow cytometry according to Farahzadi et al. (2023) [20]. As illustrated in Fig. 3, the early and late apoptosis was seen in the experimental group at the end of the co-culture period.

In other words, early apoptotic stage ($Annexin^+$, PI^-) was 23.4% for experimental group compared with control group. While about 21.8% of the cells were in late apoptosis ($Annexin^+$, PI^+). The results suggest that in the co-culture condition, early apoptosis as well as late apoptosis in the experimental group would occur in a significant level (****$p < 0.0001$).

3.4. Cytokines secreted from BM-MSCs changed the protein expression of P53, ERK1/2, p-ERK1/2, PTEN, NF-κB, Raf-1, H-RAS, and TERT

The protein expressions of signaling pathway components were assessed to explore the impact of BM-MSCs on the $CD34^+$ LSCs. In this panel, P53, ERK1/2, p-ERK1/2, PTEN, NF-κB, Raf-1, H-RAS, and TERT as components in the signaling pathways were assessed. According to Fig. 4, the protein levels of P53, PTEN, and NF-κB were significantly increased 2.09-fold, 2.30-fold, and 1.66-fold in the experimental group compared to the controls, respectively (****$p < 0.0001$). Also, the expression of p-ERK1/2, RAF-1, H-RAS, and TERT protein levels was significantly decreased by 0.36-, 0.32-, 0.27-, and 0.25- fold in the experimental group compared to the controls, respectively (****$p < 0.0001$). No significant change was detected in the ERK1/2 protein expression level.

3.5. Measuring cytokine secretion

The findings of this study indicate that the co-culture environment leads to an elevation in the secretion of various cytokines that

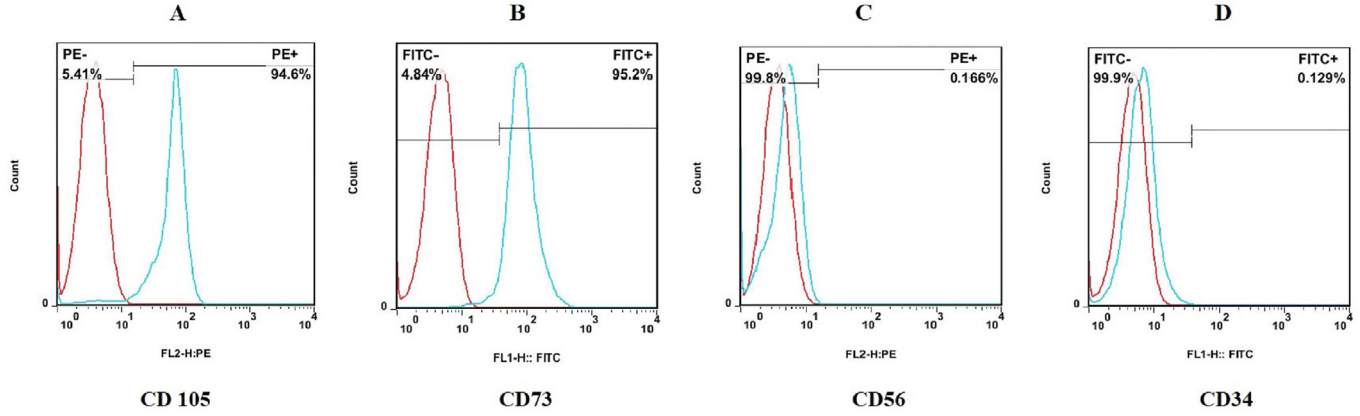

Fig. 1. Phenotypical characterization of BM-derived MSCs by flow cytometry. (A) The BM-derived MSCs were positive for (A) CD105 (94.6%) and (B) CD73 (95.2%) and negative for (C) CD56 (0.16%) and (D) CD34 (0.12%). FlowJo software (version 6.2) was used for analyzing of flow cytometry data.

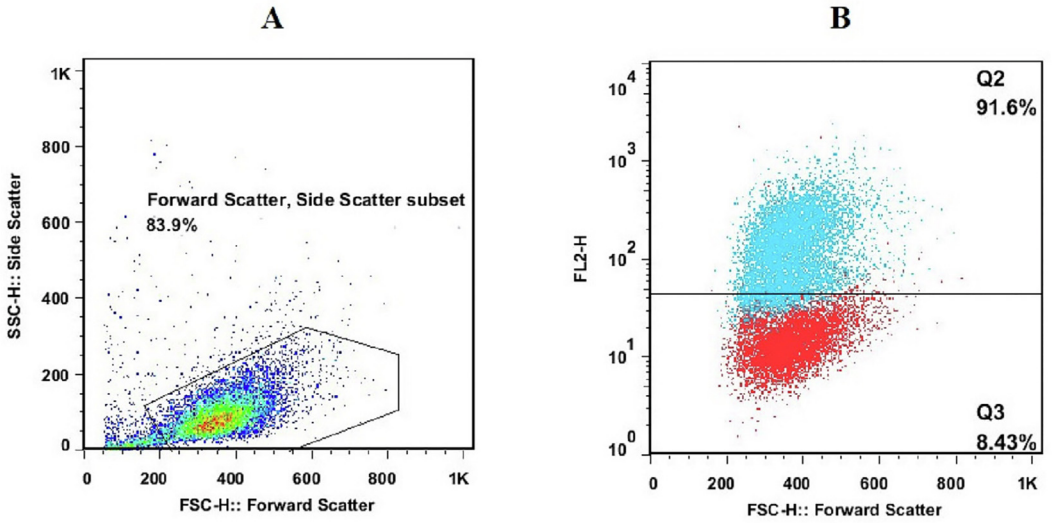

Fig. 2. Characterization of CD34$^+$ LSCs by flow cytometry. (A) Total population of cells used for CD34 evaluation; (B) The expression of CD34$^+$ enriched by magnetic activated cell sorting (MACS) method; 91.6% cells are positive for CD34 marker.

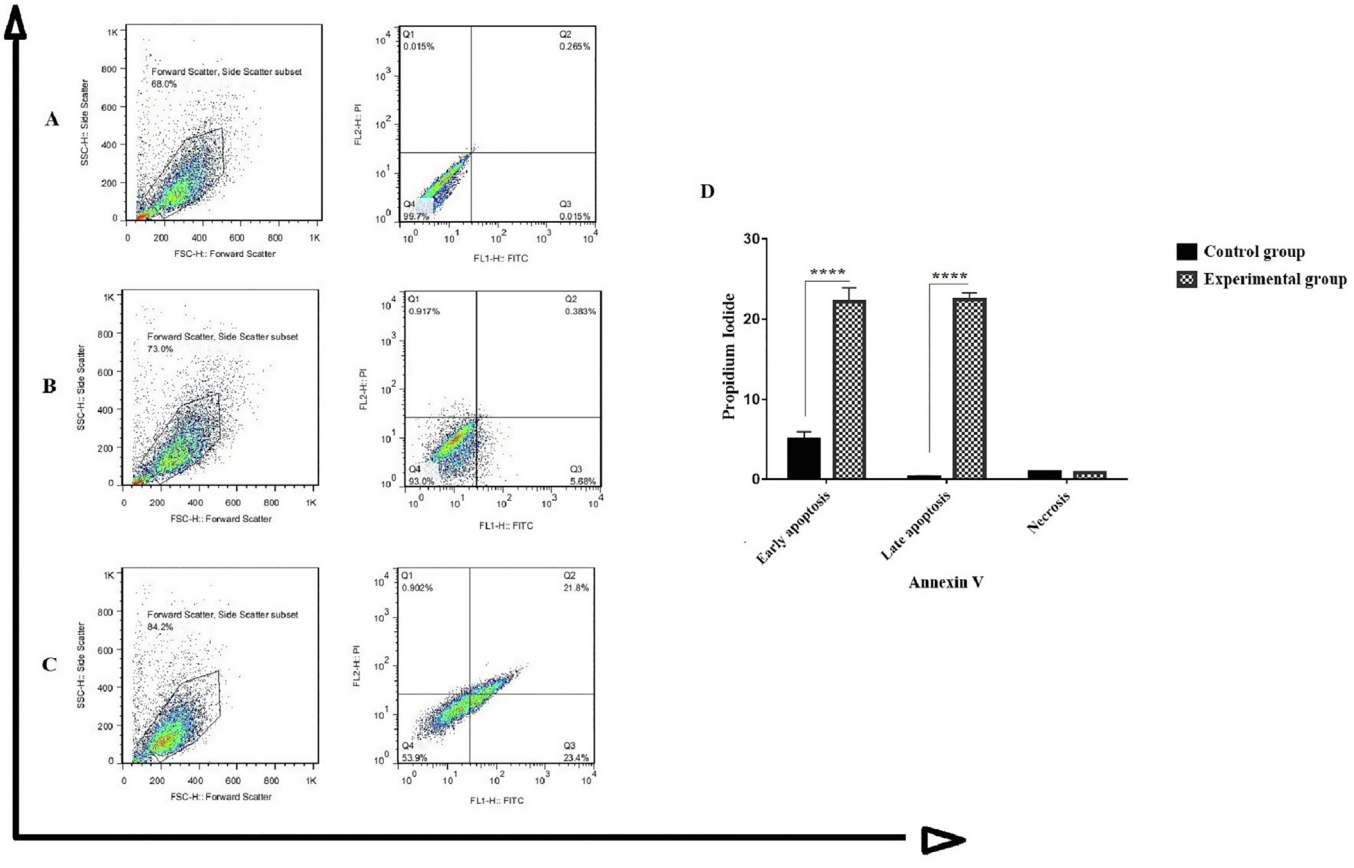

Fig. 3. Investigation of apoptosis percentage of CD34$^+$ LSCs co-cultured with BM-MSCs.A shift from bottom-right quadrant panel (early apoptosis) to top-right quadrant panel (late apoptosis) and top-left quadrant panel (necrosis) was observed. (A) Unstained cells, (B) Control group and (C) Experimental group. The quantification of apoptosis was shown in part D (****$p < 0.0001$).

impact CD34$^+$ LSCs. Cytokines present in the cultured media (IL-4, IL-2, and IL-15) from both groups were analyzed using the ELISA sandwich technique (Fig. 5). The outcomes showed a significant elevation in the secretion of IL-2 and IL-4 in the experimental group than in the controls (****$p < 0.0001$ and ***$p < 0.001$, respectively). Specifically, there was a 6.63-fold elevation in IL-2 and a 1.76-fold increase in IL-4 observed in the culture media of the experimental group in comparison to the controls.

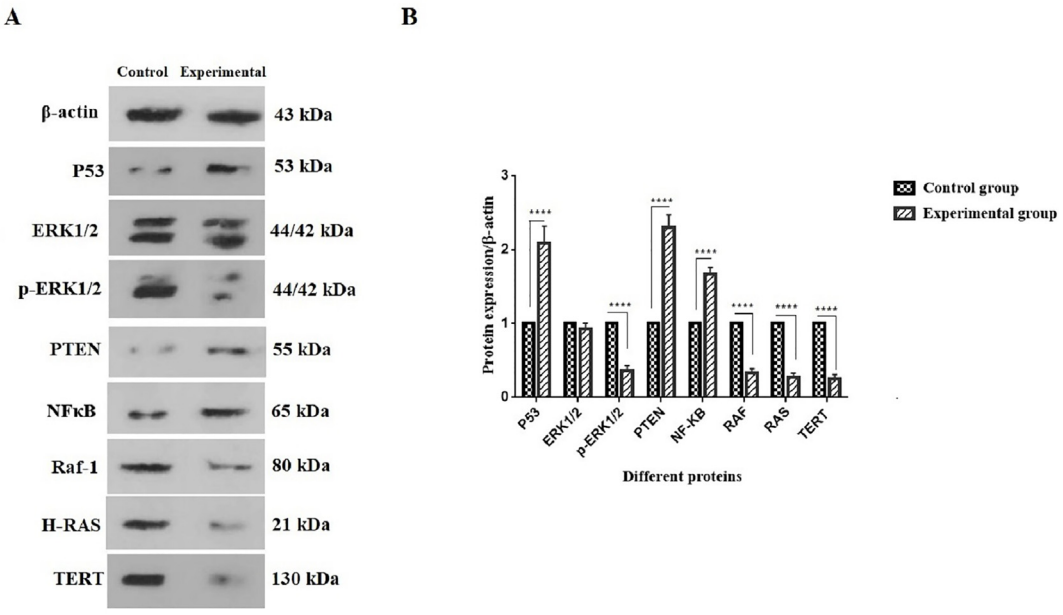

Fig. 4. Effect of BM-MSCs on protein expression of $CD34^+$ LSCs. 1×10^6 cells/well from the two groups of cells (control and experimental) were collected. Following the isolation of whole protein, western blotting was performed as described before. Values are mean ± SD from independent experiments (****$p < 0.0001$).

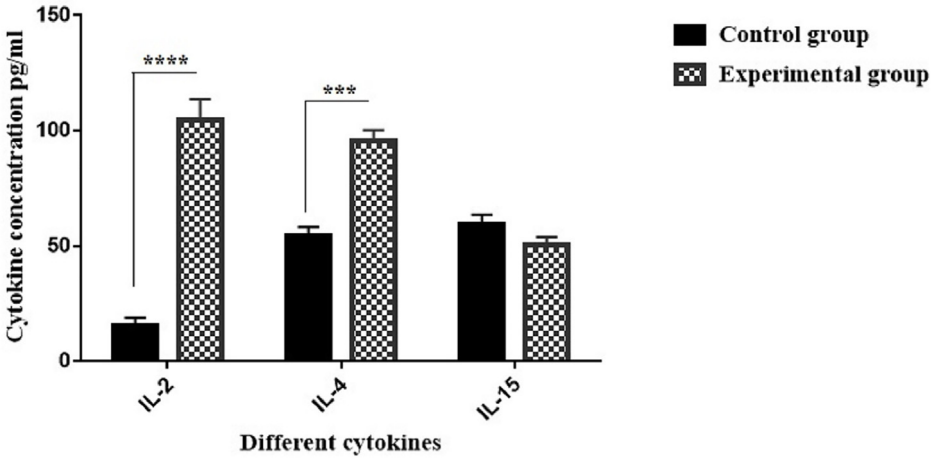

Fig. 5. The secretion levels of cytokines IL-2, IL-4 and IL-15 from two control and experimental groups (****$p < 0.0001$ and ***$p < 0.001$).

4. Discussion

Given that cancer stands as a significant global cause of mortality, research endeavors are delving into more intricate details. Various therapeutic approaches like chemotherapy, radiotherapy, and surgery exist; however, they face limitations such as drug resistance, metastatic recurrence, and associated complications. Stem cell therapy has emerged as a promising tool attracting attention from researchers and clinicians due to these challenges [21]. Stem cells represent a distinct population characterized by their self-renewal capacity and ability for multilineage differentiation. Stem cells are broadly classified into embryonic stem cells and adult stem cells (ASCs), with ASCs further categorized into hematopoietic stem cells (HSCs) and MSCs. MSCs sourced from diverse tissues are utilized in innovative therapies within regenerative medicine [22]. Diseases considered suitable for stem cell therapy encompass heart failure, stroke, neurodegenerative disorders, liver ailments, diabetes, and inflammatory bowel disease, among others [3,23]. Various studies have shown that stem cells secrete some bioactive factors which affect tumors in preclinical animal models. Engineered stem cells that consistently express diverse bioactive factors reduce tumor sizes in preclinical animals [24]. Extensive evidence supports the notion that various types of MSCs can impede tumor development both in laboratory settings and in live animal models. For instance, a study demonstrated that matrix stem cells of umbilical cord effectively suppressed rat mammary adenocarcinoma without any signs of recurrence or metastasis [25]. The anti-tumor and anti-proliferative properties of adipose tissue-derived MSCs (ADSCs) have also been documented. Cousin et al. (2009) illustrated that the intra-tumoral administration of ADSCs in a pancreatic adenocarcinoma model hindered tumor progression [26]. Furthermore, research indicated that ADSCs restrained the proliferation of human U251 glioma cells *in vitro* [27]. Subsequently, Yang et al. (2014) revealed that ADSCs inhibited the breast cancer cell line MCF-7

and rectal cancer cell line HT29 growth, lung cancer cell line A549 [27]. While MSCs have shown inhibitory effects on tumor cells, conflicting data has also emerged. Studies have indicated that MSCs sourced from various connective tissues and BM create a conducive microenvironment supporting the differentiation, growth, and survival of both leukemic and normal hematopoietic cells [28,29]. Additionally, it has been noted that the BM-MSC population plays a significant role in leukemogenesis and contributes to chemoresistance by releasing specific soluble mediators [30,31]. Another study highlighted the involvement of BM-MSCs in the proliferation and tumor angiogenesis of melanoma cells [32].

AML originates from small populations of LSCs causing disease relapse, perpetuation, and resistance against chemotherapy [33]. LSCs ($CD38^-$, $CD34^+$) possess an unlimited self-renewal capacity due to the activation of oncogenes and the inactivation of tumor suppressor genes. Targeting LSCs has been proposed as a potential strategy to enhance the long-term survival of patients with AML [34]. Therefore, understanding the biological characteristics that aid in identifying LSCs is crucial for drug development, targeted therapies, and a deeper comprehension of the disease's molecular mechanisms [35,36]. The identification of LSCs in AML holds particular significance for disease diagnosis, monitoring, prognosis, and drug screening. This process relies on transcription factors, membrane markers, and other specific mechanisms to selectively eradicate LSCs while preserving normal Hematopoietic Stem Cells (HSCs) [37].

Previous studies have explored the impact of stromal cells on primary human AML cells [38,39], yet none of these studies specifically delved into the cytokine-mediated communication between AML cells and MSCs. Our present study is in the following of other studies regarding the effect of MSCs and leukemia cell line. In our previous study, it was shown that BM-MSCs significantly induced apoptosis in KG1-AML cells. MSC-secreted cytokines appear to inhibit tumor cell proliferation through caspase-3 and BAX/BCL2 cascades. However, the relevant cytokines have not been measured [9]. In another study, the effects of secreted cytokines profile from BM-MSCs on the CML (K562) cell line were reported [12]. Research revealed that BM-MSCs significantly triggered cell cycle arrest and apoptosis in a CML cell line. The cell cycle arrest was achieved by halting K562 cells' progression in the G0/G1 stage. Growth factors and cytokines, particularly cytokine-induced neutrophil chemoattractant-1 (CINC-1) and tissue inhibitor of metalloproteinases-1 (TIMP-1) can cause the anti-tumor effects facilitated by BM-MSCs [12]. Another study conducted by our team demonstrated that MSCs, when co-cultured with Molt-4 cells, an acute lymphoblastic leukemia cell line, could potentially enhance Molt-4 cell apoptosis through the expression of caspase-3, BAD, and P53. Furthermore, a reduction in telomere length was observed as an additional impact of MSCs on Molt-4 leukemic cells [40].

An array of cytokines and growth factors can establish a cytokine network that imparts stability and adaptability to cells, facilitating a swift and amplified response to specific stimuli. While it is established that numerous growth factors released by adjacent MSC populations play a significant role in this cytokine network, the specific molecular components and individual functions are still not clearly delineated [41].

MSCs-derived growth factors and cytokines play a crucial role in modulating cancer cells. According to Li et al. (2012), MSCs generate prostaglandin E2 (PGE2) following the IL-1a and IL-1b stimulation, released by colon cancer cells. This interaction triggers MSCs to release IL-6, subsequently enhancing the stemness effects of colon cancer cells [42]. Another study highlighted that the conditioned media from MSC cultures contain IL-6, capable of inducing the Oct4 and Sox2 expression as pluripotent markers in colorectal cancer stem cells (CSCs) [43]. Both studies indicated that cytokines released by MSCs lead to a significant expansion of CSCs, promoting the proliferation and invasion of cancer cell lines. These discoveries oppose the hypothesis of our research. Among the various cytokines secreted by MSCs are IL4, IL10, IL8, CXCL1, IL17b, CXCL5, 6, and 7, as well as EGF. The profile of chemokines and cytokines released by MSCs is heavily influenced by the types of tumor cells and their respective environments [44]. In our current investigation, a notable rise in IL-2 and IL-4 levels was observed in both the experimental and control groups. IL-2 is one of the most important cytokines which was used for the treatment of cancer [45]. Since MSCs have the ability to migrate toward tumor sites, the use of MSCs may be a strategy that can reduce toxicity and ensure targeted delivery of IL-2 like maintain high levels of IL-2 in the tumor microenvironment [46,47]. It has been reported that IL-2 regulates effector T-cells as we as regulatory T-cells, which can inhibit an anti-tumor immune response [48]. Also, it has been demonstrated the IL-4 treatment of MSCs could lead to a greater production of those compounds which improve the anti-inflammatory effects of the MSCs on macrophages stimulated by lipopolysaccharide [49]. With these explanations, we determined the effect of BM-MSCs cocultured with $CD34^+$ LSCs enriched from KG1-a cell line using an apoptosis assay as well as some protein expression related to signaling pathways. As indicated in prior research, the fluorescent probe Annexin V/PI is generally utilized to quantify apoptosis by binding to phosphatidylserine exposed on the surface of apoptotic cells. Conversely, understanding and addressing the mechanism of cell damage or death triggered by growth factors and cytokines released by MSCs are crucial for evaluating the biological response to cell therapy. Consistent with the Annexin V/PI findings, it was established that the proportion of early and late apoptotic cells significantly increased, with early-apoptotic cells rising from 5.68% in the control group to 23.4% after co-culturing with BM-MSCs. Furthermore, the percentage of late apoptotic cells notably surged from 0.38% to 21.8% seven days post-co-culturing.

The role of telomerase as well as telomere length in cancer progression has been reported by researchers and clinicians. In certain types of cancer, telomerase is activated to preserve telomere length, while in others, telomere length is extended through mechanisms known as alternative lengthening of telomeres. Consequently, diminishing telomerase activity and telomere length can serve as therapeutic strategies to combat cancer [50]. Therefore, measuring the TERT expression as the major limiting agent for telomerase activity is supportive. Our result shows that the co-culture condition causes to decrease in the TERT expression of $CD34^+$ LSCs. Various studies have been conducted in relation to different signaling pathways and cancer treatment. The P53, PTEN, and NF-κB pathways play a crucial role in cancer treatment targeting [51,52]. The activation of P53, PETN, and NF-κB has also been associated with cell death. Our results are in line with previous research, the protein expression of p53, PTEN, and NF-κB in $CD34^+$ LSCs was significantly upregulated in the experimental group than in the controls. The upregulation of RAF, RAS, and ERK1/2 is seen in tumor induction. Our results show that the co-culture condition with BM-MSCs causes to decrease in the expression of RAF, RAS, and ERK1/2 which this outcome is satisfactory for cancer treatment. We did not find a cytokines array, as reported by our previous study [12]. Surprisingly, we are the first to report the effect of cytokines derived from BM-MSCs on the $CD34^+$ LSCs.

5. Conclusion

There remains a lack of definitive information regarding the impact of MSCs on cancer cells, given the conflicting impacts that could either support or hinder cancer cell growth. The complexity arises from cellular interactions between MSCs and cancer cells,

involving processes like metabolites, membrane fusion, or growth factors that influence the dynamic between MSCs and tumor cells. It is imperative to exercise caution in the realm of cell-based treatment using MSCs in patients with a history of cancer. Essentially, if cancer cells persist post-surgery, they may potentially activate resident MSCs to stimulate tumor angiogenesis, thereby promoting tumor progression. Within this context, the study's findings revealed a notable increase in apoptosis of $CD34^+$ cells enriched from the KG-1a cell line when exposed to BM-MSCs. It is postulated that cytokines and growth factors like IL-2 and IL-4 could play a significant role in the anti-tumor effects facilitated by BMSCs. The substantial induction of apoptosis in the co-culture medium of $CD34^+$ LSCs and BM-MSCs suggests that these cytokines may be involved in signaling pathways such as P53, PTEN, and ERK1/2, among others.

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Ethical approval

Ethical consent was approved by an ethics committee at Tabriz University of Medical Sciences, Tabriz, Iran (Ethic Code No: IR.TBZMED.VCR.REC.1402.836).

Author contributions

R. F as the executive of the project, had the main contribution to conception and design, the performance of experiments, data analysis, and manuscript writing; E. Z and B. V involved in the performance of manuscript writing and supervised the manuscript preparation; S. V involved in the performance of experiments; all authors approved the final version of the article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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