CellR4 2023; 11: e3419 DOI: 10.32113/cellr4_202312_3419

Investigation of the effects of B16F10 derived exosomes in induction of immunosuppressive phenotype in the hematopoietic stem cells

M.R. Atashzar[1], A. Ghanbari Asad [2], M.M. Mokhtari Tabar[3], S. Niknia[3], Z. Shokrolahi[1], N. Jooyan[4], M.R. Ataollahi[1]

[1]Department of Immunology, School of Medicine, Fasa University of Medical Sciences, Fasa, Iran
[2]Department of Medical Biotechnology, School of Medicine, Fasa University of Medical Sciences, Fasa, Iran
[3]Department of Clinical Biochemistry, Faculty of Medicine, Fasa University of Medical Sciences, Fasa, Iran
[4]Medical Physics & Medical Engineering Department, Shiraz University of Medical Sciences, Shiraz, Iran

Corresponding Author: M.R. Ataollahi, MD; e-mail: ataollahimr@gmail.com

Keywords: Myeloid-derived suppressor cells, Tumor cell-derived exosomes, Differentiation, Melanoma, Hematopoietic stem cells.

Abstract

Objective: **This study aimed to elucidate the effects of melanoma-derived exosomes on modulating the differentiation of hematopoietic stem cells (HSCs) towards immunosuppressive myeloid-derived suppressor cells (MDSCs).**

Materials and Methods: **Exosomes were isolated via ultracentrifugation from conditioned media of the B16F10 murine melanoma cell line after adaptation to exosome-free culture conditions. HSCs were extracted from the bone marrow of adult C57BL/6 mice through density gradient separation and MACS column isolation of CD133+ and CD34+ populations. HSCs were cultured with or without B16F10 exosomes for 24 hours. Flow cytometry analyzed the expression of canonical MDSC surface markers CD11b, Ly6G, and Ly6C. Levels of the immunosuppressive cytokines interleukin-10 (IL-10) and tumor necrosis factor beta (TGF-β) in HSC culture supernatants were quantified by ELISA.**

Results: **Compared to untreated controls, HSCs treated with B16F10 exosomes displayed significantly increased percentages of CD11b+Ly6G+ granulocytic MDSCs and CD11b+Ly6C+ monocytic MDSCs, with a notable predominance of the Ly6G+ granulocytic subtype. Additionally, exosome-treated HSCs secreted markedly higher levels of the cytokines IL-10 and TGF-β, which are involved in MDSC-mediated immunosuppression.**

Conclusions: **Our findings demonstrate that melanoma-derived exosomes can orchestrate the differentiation of HSCs into MDSCs with an immunosuppressive phenotype, as evidenced by the upregulation of MDSC surface markers and secreted cytokines. This supports a role for tumor-derived exosomes in driving the systemic expansion and accumulation of immunosuppressive MDSCs through the reprogramming of HSC fate. Elucidating the exosome contents and HSC signaling pathways involved could reveal therapeutic strategies to block this pathway and enhance anti-tumor immunity.**

Introduction

With a high occurrence rate, melanoma is a deadly skin cancer with a low patient survival rate and quickly spreads to other organs[1]. Despite recent immunotherapeutic strategies, including programmed death-ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitors, Bacille Calmette-Guerin (BCG) vaccine, immune checkpoint inhibitors, and interleukin-2 (IL-2) therapy, melanoma is still a growing problem due to tumor immune evasion[2]. One of the

biggest obstacles to receiving effective treatment is the tumor's immunosuppressive microenvironment, in which myeloid-derived suppressor cells (MDSC) play a crucial role in the maintenance and growth of the tumor. MDSC accumulation in the microenvironment of mouse and human melanoma tumors promotes tumor growth by impairing the function of T cells, NK cells, dendritic cells, and macrophages[3]. Granulocytic MDSCs (G-MDSCs) and monocytic MDSCs (Mo-MDSCs) are the two primary subtypes of MDSCs. In contrast to mice, human G-MDSCs and Mo-MDSCs are identified as Ly-6G+Ly-6ClowCD11b+ (F4-80+CD115+CD49d+) and Ly-6G/lowLy-6ChiCD11b+ (F4/80+CD115+CD49d+), respectively. Human G-MDSCs and Mo-MDSCs are distinguished by the expression of CD15+/CD66b+ and CD14+HLA-DR[4]. MDSCs employ a set of mediators and mechanisms to suppress and regulate immune response, including catalyzing the immune cells' essential amino acid, tryptophan, by the expression of indoleamine 2,3-dioxygenase (IDO), the inhibition of the expression of chemokines and proinflammatory cytokines *via* prostaglandin E2 (PGE2) upregulation, the downregulation of ζ-chain of the T cell receptor (TCR) complex *via* increased expression of Arginase-1 (ARG1), and the secretion of such anti-inflammatory cytokines as interleukin (IL)-10 and Transforming growth factor beta (TGF-β) that subsequently leads to the induction of regulatory T cell development[5,6]. The origin of MDSCs is the subject of some theories. Immature myeloid cells (IMC) are prevented from developing during emergency myelopoiesis under inflammation, which ultimately results in the development of functionally active MDSCs. IMCs may also expand extramedullary and differentiate into MDSCs in lymphoid organs. It has also been proved that cytokines, growth factors, and secreted tumor mediators play a crucial role in the formation of MDSCs. One of these mediators is exosomes, which can help the development of MDSCs by affecting various cells[7,8]. Exosomes, ranging in diameter from 35 to 140 nm and are actively discharged by practically all cell types, including leukocytes, stem cells, erythrocytes, and cancer cells, are tiny vesicles wrapped in a phospholipid bilayer. Exosomes are secreted as a result of late endosomes fusing with the plasma membrane. Exosomes carry different cargoes and mostly contain microRNAs, mRNA, lipids, and proteins and thus can be used as mediators for intercellular communication[9]. Exosomes play a pivotal role in signal transduction, intercellular transportation, immunosuppression of tumor microenvironment, and cell differentiation[10,11].

The exact molecular mechanism through which IMCs transform into immunosuppressive MDSCs is still not fully understood. Some studies[12] imply the roles of tumor exosomes in the generation of MDSCs. This study aimed to evaluate the interaction between hematopoietic stem cells (HSCs) and melanoma cell line-derived exosomes in the generation of MDSCs as well as their subtypes.

Materials and Methods

Mice

Five healthy C57BL/6 mice (6-8 weeks) were obtained from the Pasteur Institute of Iran. The Institutional Ethical Committee and Research Advisory Committee of Fasa University of Medical Sciences ethical guidelines for treating and handling animals were followed when maintaining, handling, and raising the animals (IR.FUMS.REC.1399.113). The mice were kept in cages that were free of pathogens and kept at room temperature (25°C) with access to food and water.

Isolation and Purification of Mouse Bone Marrow Stem Cells

C57BL/6 mouse bone marrow (BM) stem cells were collected from the femurs and tibias of pathogen-free laboratory adult mice. Mononuclear cells were obtained from BM aspirates by density gradient separation, and then CD133+ and CD34+ HSCs were isolated using a MACS system isolation kit (Miltenyi Biotec, Germany) according to the manufacturer's instructions.

Cell Lines

The National Cell Bank of Iran (Pasteur Institute, Tehran, Iran) provided the B16F10 cell line. Murine melanoma cell line B16F10 is an adherent, highly invasive cell. This cell line was cultivated at 37°C in a humid atmosphere with 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented

medium plus 10% FBS, 100 units/mL penicillin, and 100 units/mL streptomycin. At seeding densities of 4×10^5 cells/ml and 80% confluency, B16F10 cells were passaged by trypsinization.

Cell Adaptation and Exosome Isolation

The B16F10 cells were sequentially converted to an FBS-free culture in order to address the interference of fetal bovine serum (FBS) with B16F10 exosomes. During the adaption process, the culture medium's FBS concentration was gradually decreased from 10% to 0% over a period of 8 days. Cells having a density of 2.5×10^5 cells/ml and vitality of more than 90% were used to start the treatment. The modified B16F10 cells were grown and maintained in T75 flasks at 37°C for 72 hours. Afterwards, exosome isolation-conditioned media (CM) was collected. Exosomes were isolated from CM in accordance with the manufacturer's instructions using the Exocib kit (Cibzist, Tehran, Iran), and thereafter preserved at -70°C. Cell debris was successfully removed by centrifuging at 300 g for 10 minutes at 4°C. As directed by the manufacturer, the samples were then run through a 0.22-m filter. Later, CM was incubated with Exocib kit buffer for an entire night at 8°C. After 1 hour of centrifugation at 5,000 g at 4°C, the exosome pellets were visible. Both the size of the exosomes and the amount of protein were determined using the exosomes suspended in PBS.

Isolated Exosome Characterization

A protein quantification kit (BCA) made by DNAbioTech (Tehran, Iran), was used to determine the exosome content. A total of 6×10^7 cells in 250 ml produced an average exosome yield of 2,000 µg. The morphology of the exosomes has been examined employing transmission electron microscopy. Exosomes were washed with PBS after being temporarily fixed for an hour in 2% paraformaldehyde. The fixed pelts were then put onto the UV-treated TEM grids. The exosomes were fixed with 2.5% glutaraldehyde for 15 minutes in preparation for scanning electron microscopy (SEM). The exosomes were then washed with PBS and dehydrated. The samples were subsequently put through a SEM examination. The dynamic light scattering (DLS) method was used to examine the size distribution of isolated exosomes. In a nutshell, 10 L of exosomes and 1 mL of PBS were combined, and the solution was periodically agitated at 4°C for 15 minutes. The data was analyzed using Zetasizer software, version 7.11 (Malvern Corp, Malvern, UK).

Cell Viability Assay

Using the MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) test (Sigma-Aldrich, Irvine, UK), cytotoxicity viability was assessed. Hematopoietic stem cells were first seeded into 96-well plates (Nunc, Denmark) for 24 hours at 37°C at a density of 3×10^3 cells per well in 100 µL of the medium. TEX (Tumor exosomes extracted from the B16F10 cell line) was then applied to the cells for 24 hours at five different concentrations (10, 15, 20, 25, and 100 µg/ml). An ELISA reader (BioTek® ELx800, USA) was used to read the triplicate data and calculate the wall's optical density (OD) at 540 nm.

Co-Culture Experiments

The effects of B16F10 melanoma cell-derived exosomes were evaluated through co-incubation with hematopoietic stem cells. This study consisted of two groups: a control group with no exosomes, and a group with exosome-treated hematopoietic stem cells. For this purpose, 1×10^6 hematopoietic stem cells were seeded in each cell, and the test group was incubated with exosomes at 37°C for 24 h in an FBS-free medium. Eventually, supernatant from different groups was collected.

Cytokine Profile by Enzyme-Linked Immunosorbent Assay

Cytokines released from exosomes-treated stem cells were measured in two different groups. According to the manufacturer's instructions, an ELISA assay kit (Karmaniapars gene, Iran) was employed to determine the levels of IL-10 and TGF-β, and the results were reported as an absorbance at 450 nm. Every experiment was carried out in triplicate. The Wilcoxon test was used by SPSS soft-

ware, version 16.2, (SPSS Inc., Chicago, IL, USA), for analyzing the results.

Flow Cytometry

Hematopoietic stem cells were stained with monoclonal antibodies (mAbs) for 30 min at 4°C. The cells were stained with CD34 mAb [phycoerythrin (PE)- eBioscience, Biotechnology, San Diego, CA USA] and CD133 mAb allophycocyanin (APC)-BioLegend, San Diego, USA) antibodies in a flow cytometry staining buffer. The following monoclonal immunofluorescence antibodies were used to stain the treated cells: Anti-CD11b that has been conjugated with fluorescein isothiocyanate (FITC), PE, or APC is available from BioLegend in San Diego, California, as well as anti-Ly-6G and anti-Ly-6C. The tubes containing 2×10^5 cells received 1 µl of each antibody and were then left in the dark for 30 minutes. The supernatant was taken out of the tubes and thrown away after the tubes had been washed with PBS and centrifuged for 5 minutes at 1000g. The cells were examined using the BD FACSAria cell sorter (Becton-Dickinson (BD) Biosciences, San Jose, CA), and the outcomes were examined using FlowJo 2.7.4. software (FlowJo LLC, Ashland, OR, USA).

Statistical Analysis

GraphPad Prism, Fl. 26. (Boston, MA 02110, USA), was used to carry out the statistical analysis. The information is presented as mean standard deviation (SD). The data for the two groups were analyzed using unpaired, two-tailed Student's *t*-tests. The cut-off for statistical relevance was chosen at $p < 0.05$.

Results

Characterization of B16F10 Cell Line-Derived Exosomes

Sequential adaptation, as seen in Figure 1A, was used to acclimate B16F10 cells to an FBS-free media. The average exosome yield from 250 ml (6×10^7 cells) of B16F10 cell culture supernatant using the BCA technique was 2,000 µg. SEM and TEM analyses revealed that the average size of exosomes produced from B16F10 cells was between 35 and 145 nm (Figures 1B and 1C). In a similar vein, DLS results (shown in Figure 1D) demonstrated that the mean dimension of tumor-derived exosomes was 89 nm.

Evaluation of the Cytotoxic Effect of TEX on Stem Cells

Exosomes generated from the B16F10 cell line were tested for cytotoxicity on stem cells at five different doses using the MTT assay after 24 hours (10, 15, 20, 25, 50, and 100 µg/ml). The cell survival of stem cells treated with TEX at the 24-hour mark revealed that the dose of 50 ± 0.17 µg was the least harmful compared to the control group ($p < 0.05$) (Figure 2).

Flow Cytometric Results

First, hematopoietic stem cells marker, CD34 mAb, and CD133 mAb were positive in more than 70% of isolated cells, (shown in Figure 3A). In the exosome-treated hematopoietic stem cell group compared to the untreated stem cell group, flow cytometry analysis suggests greater percentages of CD11b+Ly6G + and CD11b+Ly6C+ [Mean% ± SD: 25.2 % and 17.1% *vs.* 13.2% and 10.1% respectively, (Figure 3B)]. The data from the analysis of exosome-treated hematopoietic stem cells has shown that the percentage of CD-11b+Ly6G + is higher than CD11b+Ly6C+.

Elevated Cytokine Production in Treated Stem Cells

IL-10 and TGF-β secretion from mice stem cells were assessed. The results revealed a significant increase in IL-10 and TGF-β, a signature cytokine of the myeloid-derived suppressor cells, in the supernatant of exosome-treated hematopoietic stem cell groups compared to the exosomes and untreated stem cell groups. The average concentrations of IL-10 and TGF-β were as follows in the groups of untreated and exosome-treated stem cells: (38.5 ± 1 pg/ml *vs.* 22.5 ± 1.1 pg/ml, $p < 0.05$) and (53.7 ± 1.1 pg/ml *vs.* 32.4 ± 1.2 pg/ml $p < 0.05$) respectively, (Figure 4). These results indicated that tumor exosomes change the phenotype of stem cells to suppressor cells and lead to the suppression of immune systems.

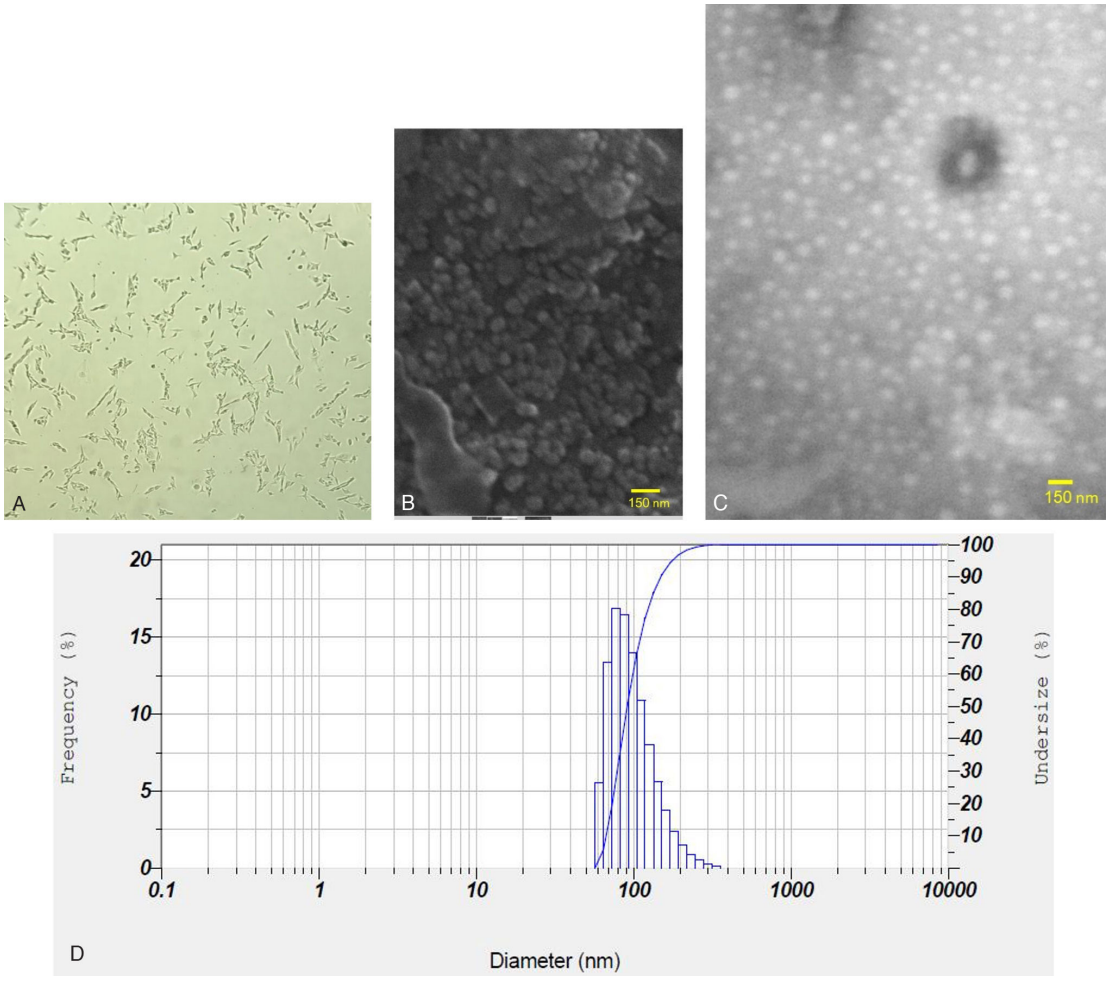

Figure 1. Characterization of B16F10 cell line-derived exosomes. **A**, B16F10 cell morphology under a light microscope in the absence of FBS. **B**, Scanning electron microscopy picture of TEXs obtained from B16F10. **C**, Transmission electron microscopy picture of TEXs obtained from B16F10. **D**, TEX, tumor exosome, size distribution of B16F10-derived TEXs by frequency utilizing dynamic light scattering.

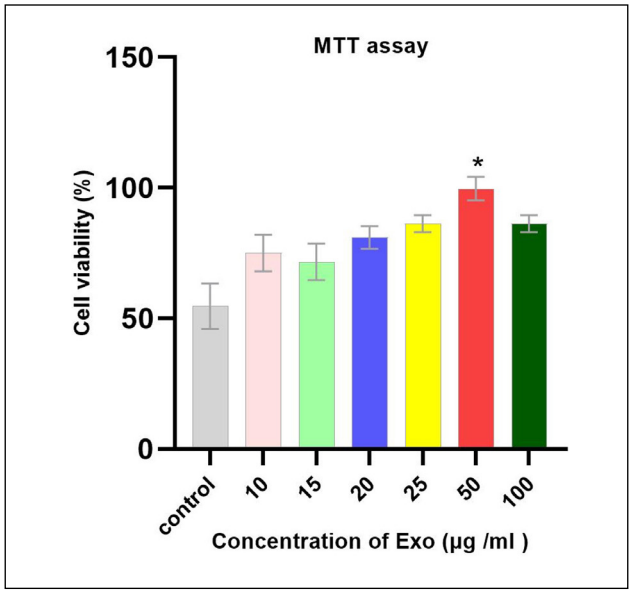

Figure 2. The cytotoxicity of TEX administration on B16F10 cells was studied in a dose-dependent manner. TEX had a substantial viability effect at a dose of 50 ± 0.17 µg. The data are presented as mean standard deviation. *$p < 0.05$ *vs.* untreated or control group.

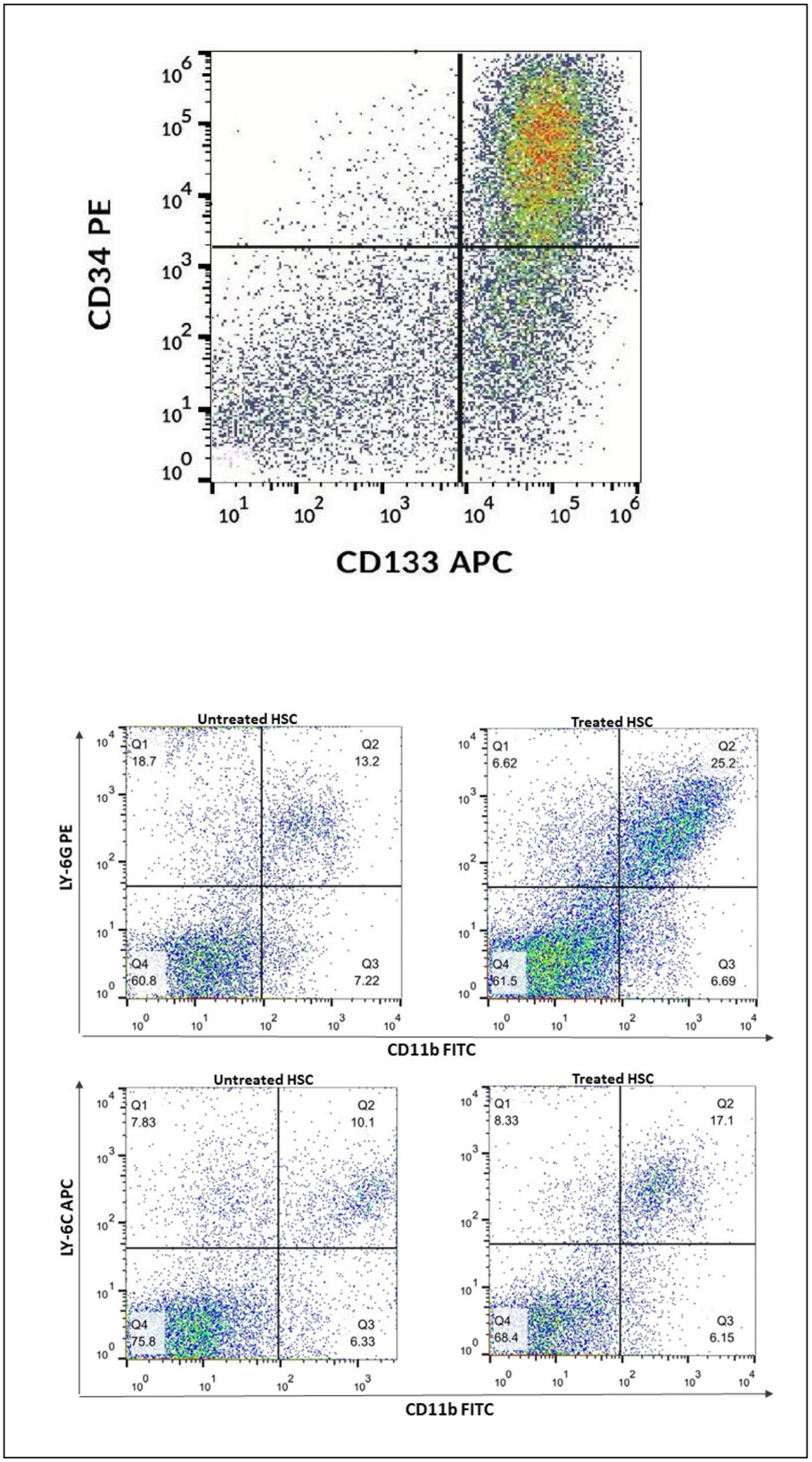

Figure 3. A, The Flow Cytometry graph shows that more than 70% of isolated cells have CD133 + /CD34 + markers. **B**, The analysis reveals that the exosome-treated stem cell group had a larger percentage of CD11b+Ly6G + and CD11b+Ly6+ cells than the untreated stem cell group (Mean% ± SD: 25.2% and 17.1% *vs.* 13.2% and 10.1%, respectively.

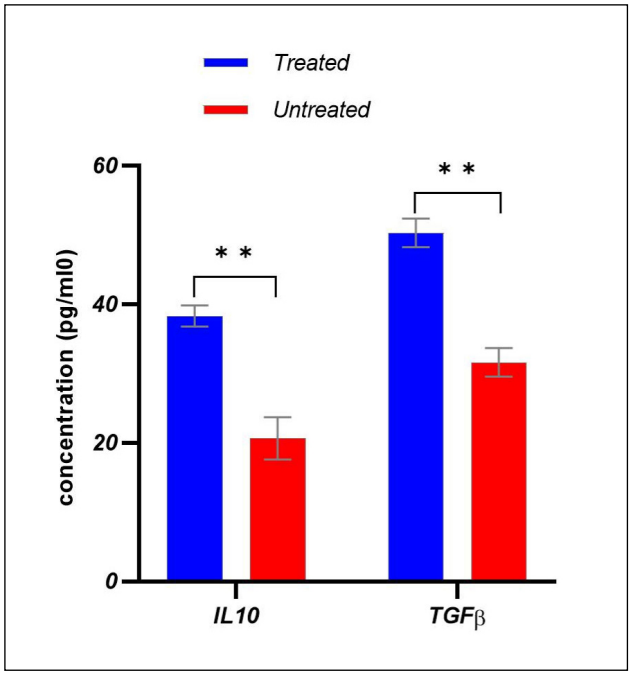

Figure 4. Bar graphs illustrate the concentration of cytokines in the exosome-treated stem cells and untreated stem cells groups as follows: IL-10 (38.5 ± 1 pg/ml *vs.* 22.5 ± 1.1 pg/ml, $p < 0.05$) and TGFβ (53.7 ± 1.1 pg/ml *vs.* 32.4 ± 1.2 pg/ml $p < 0.05$) respectively. Comparatively to the control groups, the cytokine profile revealed a substantial rise in IL-10 and TGF-β, a characteristic cytokine of the MDSC. The outcomes are shown as mean ± SD. The Student's *t*-test with no pairings was used to compare groups. The data are present as mean of standard deviation. ** $p < 0.05$ comparison of treated *vs.* untreated group.

Discussion

One of the major problems of cancer treatment is how to overcome tumor immune evasion. Despite recent advances in cancer immunotherapy of solid cancers (e.g., bladder cancer, non-small cell melanoma, and lung cancer), most patients suffer cancer relapse and recurrence[13,14]. The response to contemporary immunotherapies is restricted by a number of immune-suppressive variables. Regarding immune suppression, MDSCs have been shown to be essential for maintaining and advancing tumors by encouraging angiogenesis and metastasis. The ability of MDSCs to decrease T-cell activity, especially CD8+ T-cell responses, is one of their key functions. The function of NK cells, dendritic cells, and macrophages is also similarly impacted by MDSCs[15,16]. Additionally, it has been noted that immunosuppressive myeloid cells may suppress the immune system by producing cytokines, NO, arginase, and reactive oxygen species (ROS)[17]. The production of MDSCs is an ongoing process that is fed by tumor cells. MDSCs are produced as a typical physiological and pathological response to acute and inflammatory situations, such as malignancy[7]. Finding the new aspects of the origin of MDSCs can lead to a promising treatment for cancer. Tumor-induced activation and expansion of MDSCs can be mediated by soluble factors known as exosomes[18]. Exosomes are constantly released by cells in both healthy and pathological conditions *via* an exocytosis pathway. They are endosome-derived organelles with a dimension range of 35 to 140 nm[19]. While TEXs were described to be immunostimulatory, recent reports have shown that they have a role in MDSC expansion[20].

The current study aimed to determine the impact of exosomes produced from B16F10 on hematopoietic stem cells' immunosuppressive response. In this investigation, we assessed the cytokine production profile and the percentages of CD11b +, Ly6G +, and Ly6+ markers in the exosome-treated hematopoietic stem cells group. Our findings showed that the exosome-treated hematopoietic stem cells group had a considerably larger percentage of CD11b +, Ly6G +, and Ly6+ than the untreated stem cells group. Additionally, more Ly6G + cells than Ly6+ cells were seen. Our findings showed that the supernatant of the exosome-treated hematopoietic stem cells group contained higher levels of IL-10 and TGFβ than did the controls.

Consistent with our findings, the previous studies[8,21] showed an increase of MDSC markers on the surface of stem cells treated by exosomes. They indicated that tumor exosomes boost the suppressive molecules and the activity of MDSCs in tumor models[21]. MDSC has been revealed by Sinha et al[22] to have PGE2 receptors, and E-prostanoid receptor agonists, such as PGE2, can cause bone marrow stem cells to differentiate into CD11b+Gr1+MDSC. Xiang et al[23] cultured bone marrow precursor cells of wild-type MyD88 or TLR2 knockout (KO) mice in the company of GM-CSF (20 ng/ml) and B16 mouse melanoma cells, TS/A cells, 4T-1 mouse breast carcinoma cancer cells derived exosomes for seven days. Exosomes obtained from B16 tumor cells grown *in vitro* were employed as primary exosomes, and exosomes from B16 tumors produced *in vivo* were used as cultured exosomes. They showed that both primary and cultured exosomes induced CD11b Gr-1, IL-6, and phosphorylated Stat3 dependent on MyD88 and both TLR2 and MyD88 dependent, respectively. Exosomes from mel-

anoma cells drive the development of myeloid cells into TGF-β-secreting CD14+HLA-DR- cells while inhibiting the differentiation of myeloid cells into DCs, according to Valenti et al[24]. According to Xiang et al[20], myeloid cells in the bone marrow can take up exosomes from tumor cells. These myeloid cells then displayed the phenotypic and functional traits of MDSCs, including elevated production of Cox2, IL-6, Vascular endothelial growth factor (VEGF), Arg1, and TGF-β. Furthermore, Fleming et al[25] also showed that extracellular vesicles from human melanoma cells activated PD-L1 through the toll-like receptor (TLR)4 and produced immunosuppressive myeloid cells from healthy myeloid cells.

In conclusion, the researchers concentrated on how tumor exosomes, which carry functional components, contribute to the development of MDSCs. As described above, B16F10-derived exosomes can generate MDSC cells with increased surface expression of CD1 1b +, Ly6G +, and Ly6+ and increased IL-10 and TGF-β levels by hematopoietic stem cells. These findings imply the pivotal role of exosome production in the tumor environment. Not only do they promote cancer progression, but they also affect the immune response. Therefore, TEX can be clinically used as a prognostic indicator in the follow-up and treatment of cancers.

Conclusions

This study demonstrates that tumor-derived exosomes can orchestrate the differentiation of hematopoietic stem cells into immunosuppressive MDSCs, unraveling a potential mechanism of MDSC accumulation and immune suppression in the tumor microenvironment. Our findings reveal that exosomes secreted by B16F10 melanoma cells can alter the phenotype and cytokine secretion profile of hematopoietic stem cells isolated from mouse bone marrow. Flow cytometry analysis showed increased surface expression of canonical MDSC markers CD11b, Ly6G, and Ly6C on hematopoietic stem cells after exposure to melanoma exosomes. The specific upregulation of Ly6G over Ly6C suggests polarization towards granulocytic rather than monocytic MDSCs, providing insight into the subtype balance driven by this differentiation program. In addition to inducing an MDSC-like phenotype, melanoma exosome treatment triggered heightened production of the immunosuppressive cytokines IL-10 and TGF-β by hematopoietic stem cells. This aligns with the well-established role of MDSCs in secreting anti-inflammatory mediators to suppress T cell and NK cell function. Our observations indicate that melanoma exosomes can remotely instigate hematopoietic stem cell differentiation into MDSCs with both the suppressive surface receptors and cytokine production armamentarium needed to restrain anti-tumor immunity.

While the exact exosomal components and molecular mechanisms governing this MDSC differentiation remain to be fully elucidated, targeting this pathway could potentially combat melanoma immune evasion and improve immunotherapy efficacy. Inhibition of exosome biogenesis or release and blockade of exosome uptake, specifically in hematopoietic stem cell progenitors, could help attenuate the generation of immunosuppressive MDSCs in the tumor microenvironment. Further research is warranted to fully elucidate the exosome cargo components and signaling pathways responsible for modulating hematopoietic stem cell fate. Inhibition of exosome biogenesis and release from tumor cells could also be explored as a therapeutic strategy. Overall, this study highlights the role of melanoma-derived exosomes in promoting immunosuppression and identifies a novel mechanism of MDSC accumulation mediated through the reprogramming of hematopoietic stem cells.

Authors' Contributions:

MR Ataollahi and MR Atashzar designed the project, analyzed the data, and wrote the paper; MM Mokhtari Tabar, S Niknia, and N Jooyan performed the experiments; A Ghanbari Asad and Z Shokrolahi collaborated in writing the paper. The final manuscript went through review and approval by all authors.

Funding:

This study was financially supported by a grant from Fasa University of Medical Sciences (99086).

Ethics Approval:

The Fasa University of Medical Sciences' Institutional Ethical Committee and Research Advisory Committee reviewed and approved this study protocol with approval number [IR.FUMS.REC.1399.113].

Informed Consent:

Not applicable.

Acknowledgments:

The authors would like to thank colleagues from the Department of Biotechnology, the affiliated Fasa University of Medical Sciences and colleagues from the Department of Biochemistry.

Conflict of Interest:

There are no disclosed conflicts of interest.

ORCID ID:

Mohammad Reza Atashzar: 0000-0002-4135-8178

References

1. Bajetta E, Del Vecchio M, Bernard-Marty C, Vitali M, Buzzoni R, Rixe O, Nova P, Aglione S, Taillibert S, Khayat D. Metastatic melanoma: chemotherapy. Semin Oncol 2022; 9: 427-445.
2. Rotte A, Bhandaru M, Zhou Y, McElwee KJ. Immunotherapy of melanoma: present options and future promises. Cancer Metastasis Rev 2015; 34: 115-128.
3. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 2009; 9: 162-174.
4. Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF, Mandruzzato S, Murray PJ, Ochoa A, Rosenberg SO, Rodriguez PC, Sica A, Umansky V, Vonderheide Di, Gabrilovich DI. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. Nat Commun 2016; 7: 12150.
5. Motallebnezhad M, Jadidi-Niaragh F, Qamsari ES, Bagheri S, Gharibi T, Yousefi M. The immunobiology of myeloid-derived suppressor cells in cancer. Tumor Biol 2016; 37: 1387-1406.
6. Sevko A, Umansky V. Myeloid-derived suppressor cells interact with tumors in terms of myelopoiesis, tumorigenesis and immunosuppression: thick as thieves. J Cancer 2013; 4: 3-11.
7. Millrud CR, Bergenfelz C, Leandersson K. On the origin of myeloid-derived suppressor cells. Oncotarget 2017; 8: 3649-3665.
8. Condamine T, Gabrilovich DI. Molecular mechanisms regulating myeloid-derived suppressor cell differentiation and function. Trends Immunol 2011; 32: 19-25.
9. Pegtel DM, Gould SJ. Exosomes. Annu Rev Biochem 2019; 88: 487-514.
10. Théry C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. Nat Rev Immunol 2002; 2: 569-579.
11. Kalluri R. The biology and function of exosomes in cancer. JCI 2016; 126: 1208-1215.
12. Fleming V, Hu X, Weller C, Weber R, Groth C, Riester Z, Hüser L, Sun Q, Nagibin V, Logo O, Kirschning C, Bronte V, Utikal J, Altevogt P, Umansky V. Melanoma extracellular vesicles generate immunosuppressive myeloid cells by upregulating PD-L1 via TLR4 signaling. Cancer Res 2019; 79: 4715-4728.
13. De Cicco P, Ercolano G, Ianaro A. The new era of cancer immunotherapy: targeting myeloid-derived suppressor cells to overcome immune evasion. Front Immunol 2020; 11: 1680.
14. Yu C, Liu X, Yang J, Zhang M, Jin H, Ma X, Shi H. Combination of immunotherapy with targeted therapy: theory and practice in metastatic melanoma. Front Immunol 2019; 10: 990.
15. Greten TF, Manns MP, Korangy F. Myeloid-derived suppressor cells in human diseases. Int Immunopharmacol 2011; 11: 802-807.
16. Ostrand-Rosenberg S, Sinha P, Beury DW, Clements VK. Cross-talk between myeloid-derived suppressor cells (MDSC), macrophages, and dendritic cells enhances tumor-induced immune suppression. Semin Cancer Biol 2012; 22: 275-281.
17. Talmadge JE. Pathways mediating the expansion and immunosuppressive activity of myeloid-derived suppressor cells and their relevance to cancer therapy. Clin Cancer Res 2007; 13: 5243-5248.
18. Mignot G, Roux S, Thery C, Ségura E, Zitvogel L. Prospects for exosomes in immunotherapy of cancer. J Cell Mol Med 2006; 10: 376-388.
19. Van Niel G, Porto-Carreiro I, Simoes S, Raposo G. Exosomes: a common pathway for a specialized function. J Biochem 2006; 140: 13-21.
20. Xiang X, Poliakov A, Liu C, Liu Y, Deng ZB, Wang J, Cheng Z, Shah SV, Wang GJ, Zhang L, Grizzle WE, Mobley J, Zhang HG. Induction of myeloid-derived suppressor cells by tumor exosomes. IJC 2009; 124: 2621-2633.
21. Tian X, Shen H, Li Z, Wang T, Wang S. Tumor-derived exosomes, myeloid-derived suppressor cells, and tumor microenvironment. J Hematol Oncol 2019; 12: 84.
22. Sinha P, Clements VK, Fulton AM, Ostrand-Rosenberg S. Prostaglandin E2 promotes tumor progression by inducing myeloid-derived suppressor cells. Cancer Res 2007; 67: 4507-4513.
23. Xiang X, Liu Y, Zhuang X, Zhang H, Michalek S, Taylor DD, Grizzle W, Zhang HG. TLR2-mediated expansion of MDSCs is dependent on the source of tumor exosomes. Am J Pathol 2010; 177: 1606-1610.
24. Valenti R, Huber V, Filipazzi P, Pilla L, Sovena G, Villa A, Corbelli A, Fais S, Parmiani G, Rivoltini L. Human tumor-released microvesicles promote the differentiation of myeloid cells with transforming growth factor-β–mediated suppressive activity on T lymphocytes. Cancer Res 2006; 66: 9290-9298.
25. Fleming V, Hu X, Weller C, Weber R, Groth C, Riester Z, Huser L, Sun Q, Nagibin V, Kirschning C, Bronte V, Utikal J, Altevogt P, Umansky V. Melanoma extracellular vesicles generate immunosuppressive myeloid cells by upregulating PD-L1 via TLR4 signaling. Cancer Res 2019; 79: 4715-4728.