The effect of bone marrow mesenchymal stromal cell exosomes on acute myeloid leukemia's biological functions: a focus on the potential role of LncRNAs

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تعداد نتایج: ۱۲ | نوع: همه | مجله | کتاب | راهنما | دسترسی: همه | مشترک | رایگان | غیرمشترک | تصویر جلد:

No.	Title	Subject Category	Publisher/ Holder	IF	IF Quartile	CiteScore	CiteScore Quartile	H-Index	Indexed in	Details
1	Clinical and Experimental Medicine ISSN/ISBN: 1591-8890, 1591-9528	General Medicine Genetics + 2 more ...	Springer, ProQuest	3.200	Q2	4.80	Q2	55	ISI, Scopus, PubMed, Embase	

Clinical and Experimental Medicine (2024) 24:108
https://doi.org/10.1007/s10238-024-01364-6

RESEARCH

The effect of bone marrow mesenchymal stromal cell exosomes on acute myeloid leukemia's biological functions: a focus on the potential role of LncRNAs

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Received: 12 March 2024 / Accepted: 29 April 2024
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Introduction

Acute Myeloid Leukemia (AML) is a malignant hematological disease characterized by the accumulation of immature blood cells (blasts) in the bone marrow, leading to impaired blood production. It is also one of the most prevalent types of malignant hematological disorders. Despite recent therapeutic advances, AML management remains challenging,
with a notably high mortality rate.

Bone marrow mesenchymal stromal cells (BM-MSCs) and their exosomes have emerged as potential modulators of tumor progression and treatment response. These effects are primarily attributed to their ability to regulate cell signaling through molecules such as long non-coding RNAs (lncRNAs).

Introduction

LncRNAs are RNA molecules longer than 200 nucleotides with limited or no protein-coding potential. Despite this, they are crucial in regulating gene expression, cell signaling, and chromatin remodeling. Dysregulation of lncRNAs is often associated with cancer progression and drug resistance.

- H19
- Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1),
- HOX Transcript Antisense RNA (HOTAIR)
- Taurine-Upregulated Gene 1 (TUG1)

are considered as poor-prognosis lncRNAs whose aberrant overexpression has proliferative and anti-apoptotic efects on AML cells and is correlated with higher BM blast counts, poor chemotherapy response, and shorter overall survival (OS) in AML patients

Introduction

This study aims to investigate how BM-MSC-derived exosomes influence the biological functions of AML cells, with a particular focus on their impact on lncRNAs such as MALAT1, HOTAIR, TUG1, and H19, which are linked to poor prognosis and drug resistance.

BM-MSCs

Bone marrow mesenchymal stromal cells were obtained from a healthy 26-year-old male donor. They were cultured in serum-free alpha-MEM medium and validated by flow cytometry for markers CD90, CD105, and CD73

HL-60 AML Cells

HL-60 cells, known for high expression of MALAT1, HOTAIR,TUG1 and H19 lncRNAs, were cultured in RPMI-1640 medium with 15% FBS

Isolation of BM-MSC Exosomes

BM-MSC-derived exosomes were isolated from a conditioned medium using a precipitation method and characterized by the following tests:

TEM (Transmission Electron Microscopy)	DLS (Dynamic Light Scattering)	Flow Cytometry
Visualized spherical exosome morphology.	Measured exosome size, ranging between 80–100 nm.	Confirmed the presence of exosomal markers CD63, CD9, and CD81.

Experimental Assays

Cell Viability

The metabolic activity of HL-60 cells treated with BM-MSC exosomes (50 µg/mL and 100 µg/mL) was assessed using the MTT assay.

Cell Cycle Analysis

Propidium iodide (PI) staining was performed to assess the distribution of HL-60 cells across different cell cycle phases after exosome treatment

Apoptosis Assay

Annexin V/PI staining and flow cytometry were used to quantify early and late apoptotic cells

Reactive Oxygen Species (ROS) Assay

ROS levels were evaluated using DCFH-DA staining and flow cytometry.

Experimental Assays

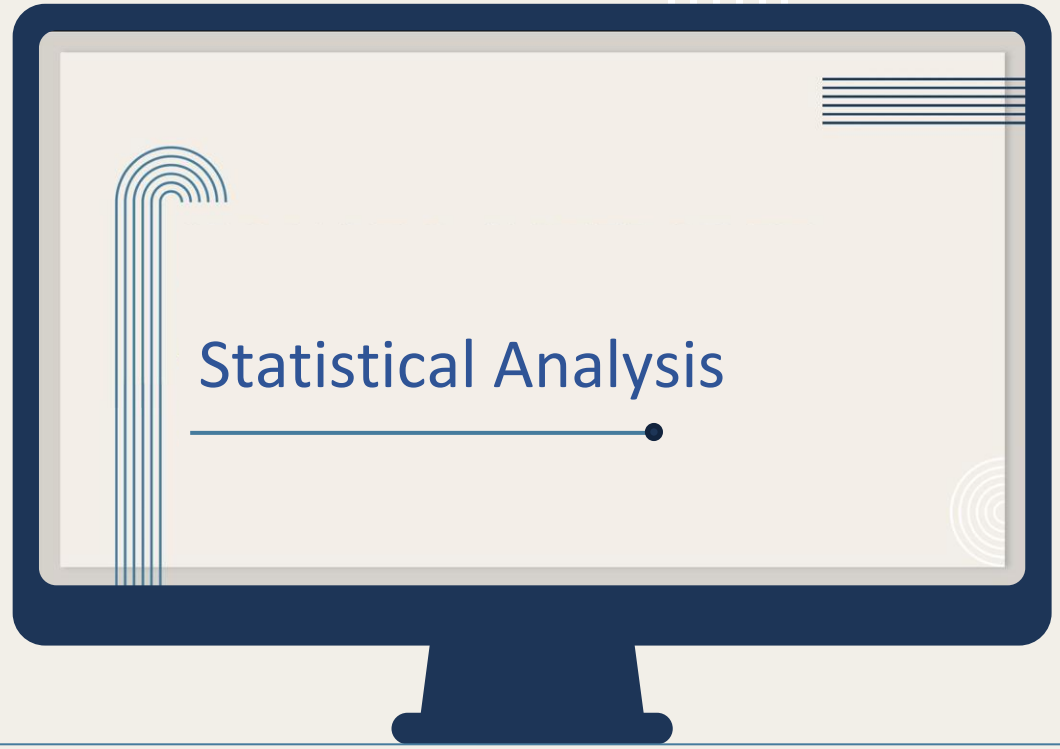
Ki-67 Cell Proliferation Assay:

- HL-60 cells treated with 100 µg/mL BM-MSC exosomes for 24 hours were analyzed for Ki-67 protein expression using flow cytometry.
- After fixation, cells were incubated with a Ki-67-specific antibody and Perm/Wash solution, followed by fluorescence analysis to measure cell proliferation levels

Gene Expression:

- RNA was extracted from treated HL-60 cells, reverse-transcribed, and analyzed using RT-qPCR.
- Expression levels of lncRNAs (MALAT1, HOTAIR, H19) and apoptosis-related genes (BAX, BCL2, p53, p21) were measured.

Data were analyzed using GraphPad Prism software. Paired t-tests were used for two-group comparisons, while ANOVA was applied for multi-group analyses. Statistical significance was set at $p < 0.05$



Results

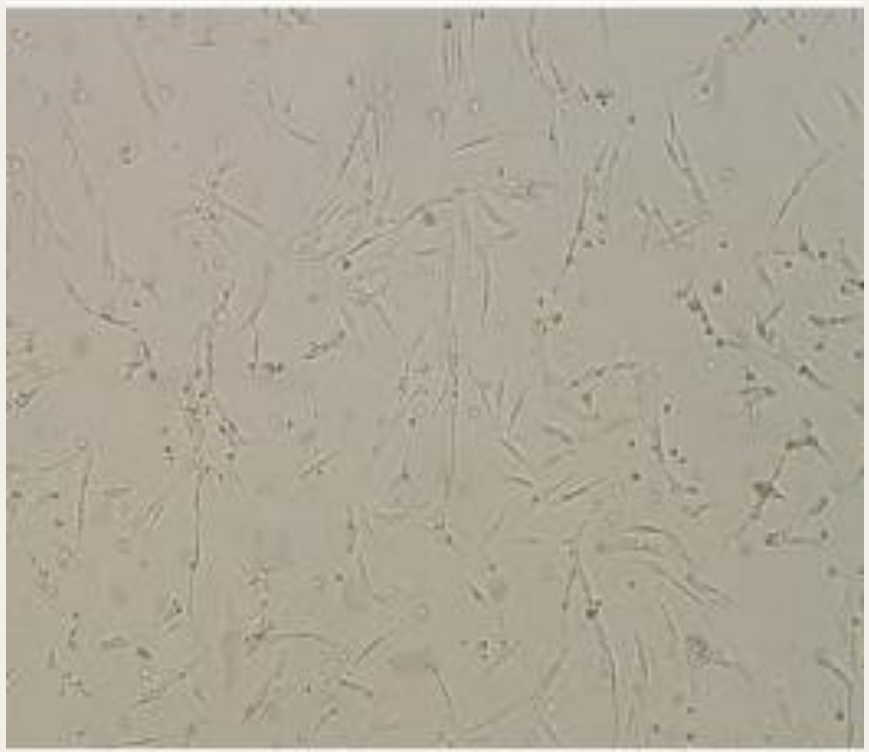
BM-MCS

MORPHOLOGY

Exosomes

HL-60 cells

BM-MSCs exhibited the fbroblast-like morphology under an inverted microscope



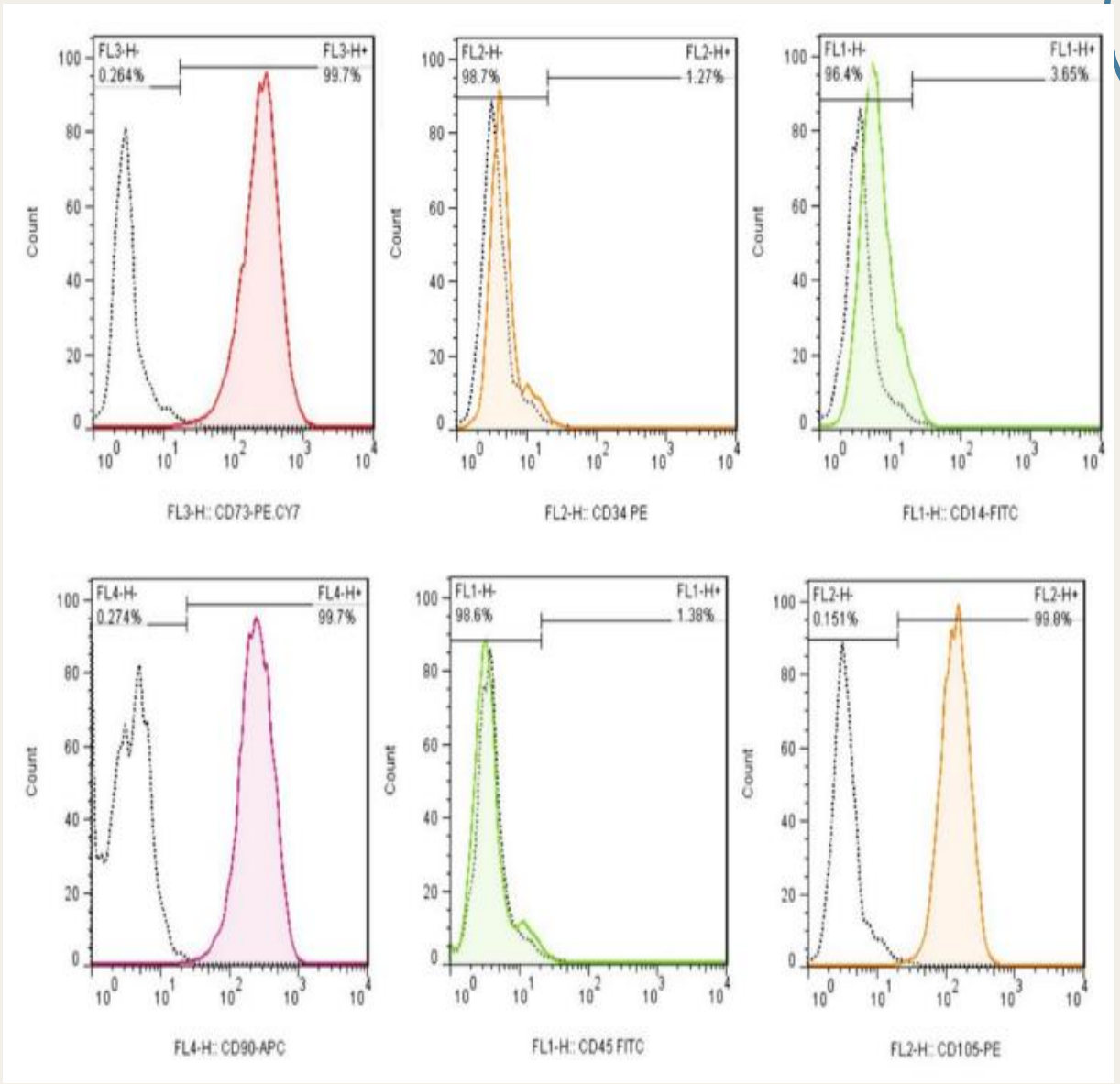
BM-MCS

Immuno-phenotyping

Exosomes

HL-60 cells

Flow cytometry analysis confirmed the expression of surface markers CD90, CD105, and CD73, while CD14, CD34, and CD45 were not expressed, validating the mesenchymal nature of the cells



BM-MCS

Exosomes

TEM

DLS

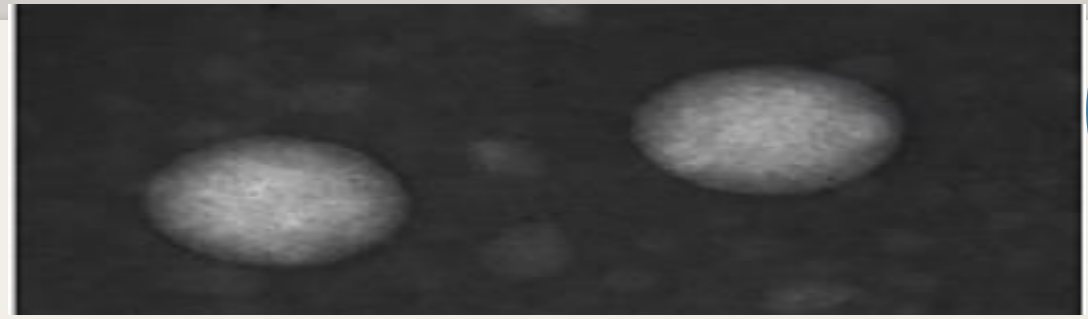
Flow Cytometry

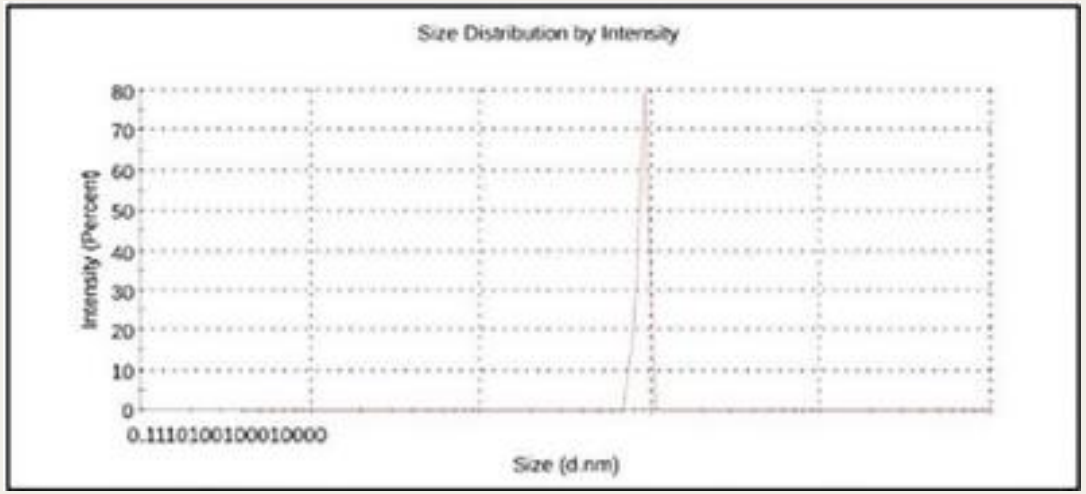
HL-60 cells

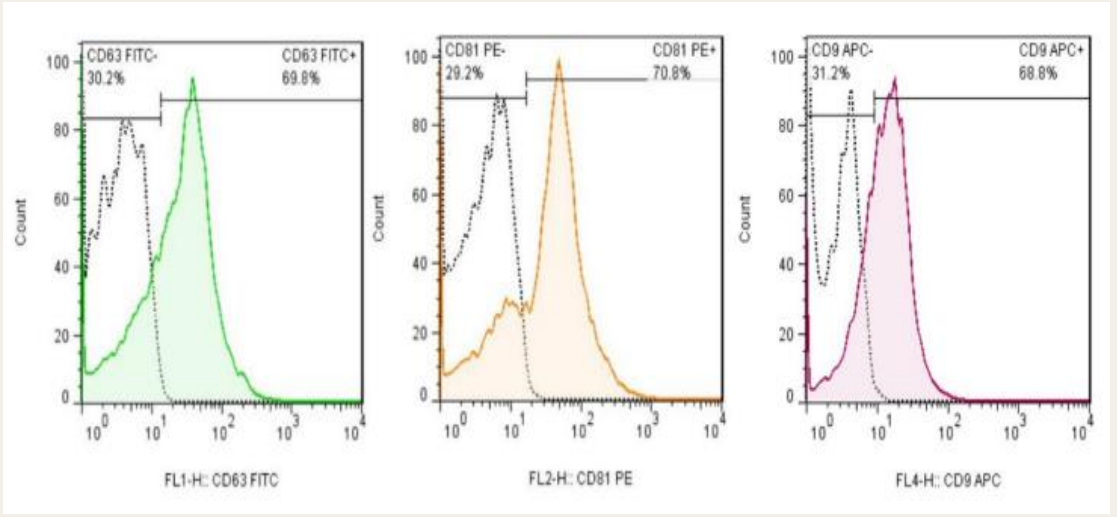
TEM: Revealed their spherical morphology

DLS: Measured their size as ranging between 80–100 nm

Flow Cytometry: Confirmed the presence of exosomal surface markers CD63, CD9, and CD8







BM-MCS

Exosomes

HL-60 cells

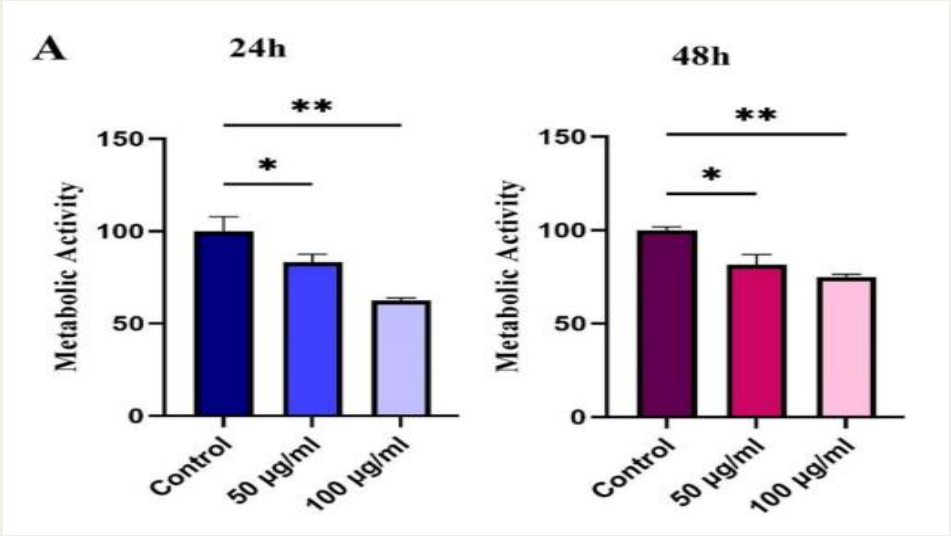
Cell Viability

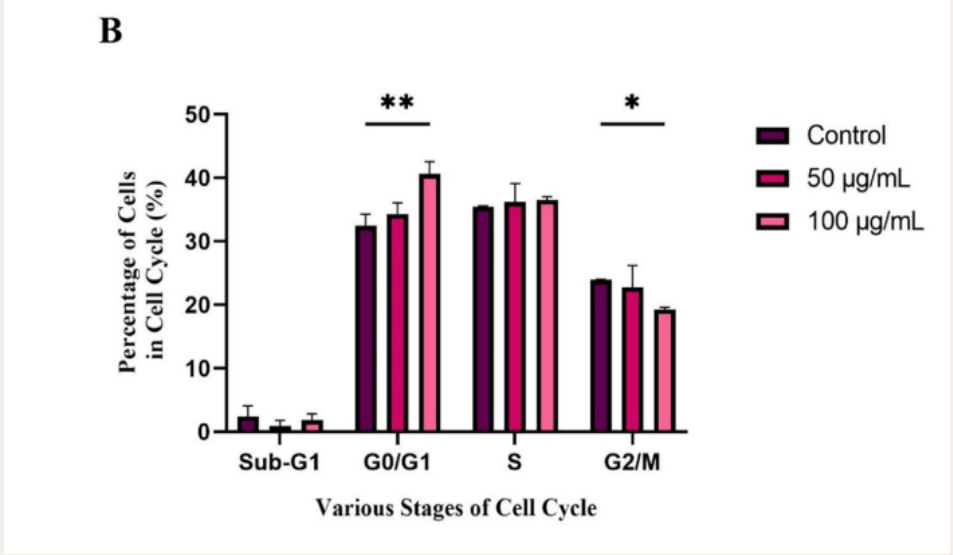
Cell Cycle Arrest

MTT assay showed a significant reduction in HL-60 cell metabolic activity following treatment with BM-MSC exosomes at 50 µg/mL and 100 µg/mL.

The inhibitory effect was stronger at 100 µg/mL and 24 hours of treatment

PI staining revealed that BM-MSC exosomes caused cell cycle arrest in the G0/G1 phase, particularly at 100 µg/mL





BM-MCS

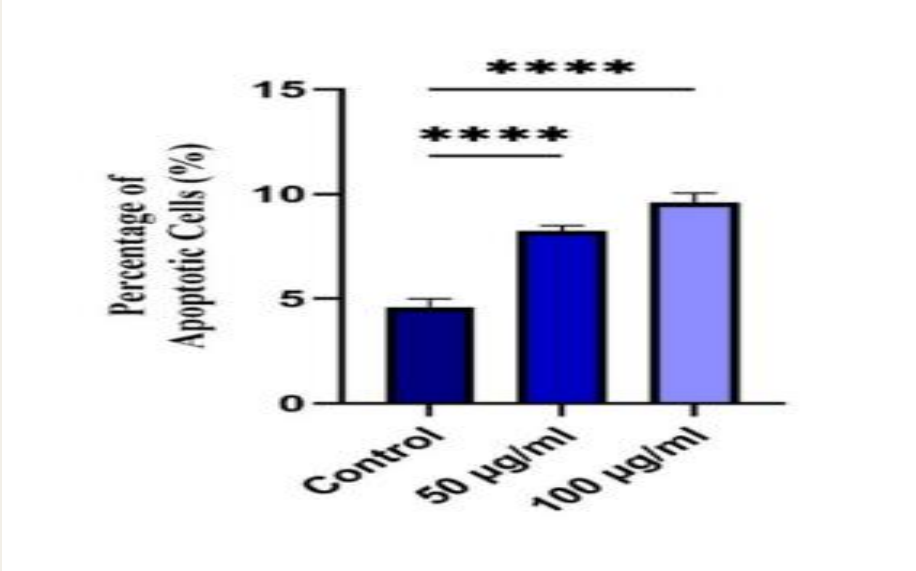
Exosomes

HL-60 cells

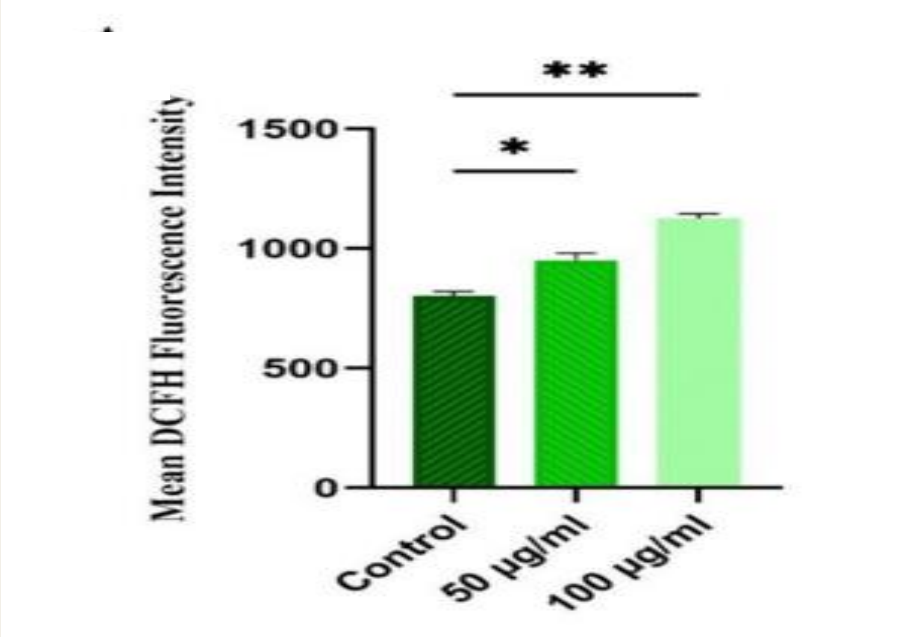
Apoptosis Induction

ROS level

Annexin V/PI staining demonstrated that BM-MSC exosomes significantly increased both early and late apoptosis in HL-60 cells in a dose-dependent manner



ROS levels were significantly elevated in HL-60 cells treated with BM-MSC exosomes, indicating increased oxidative stress



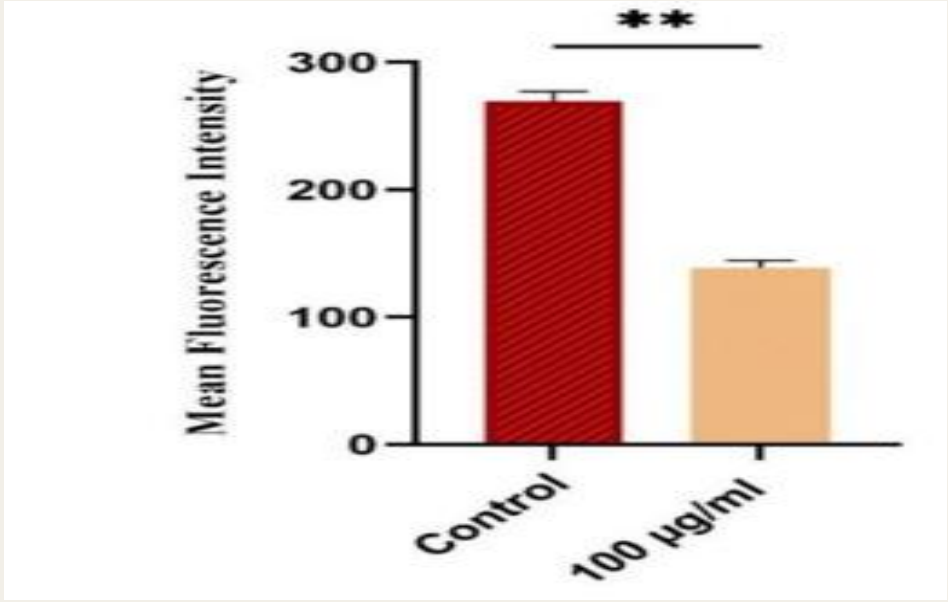
BM-MCS

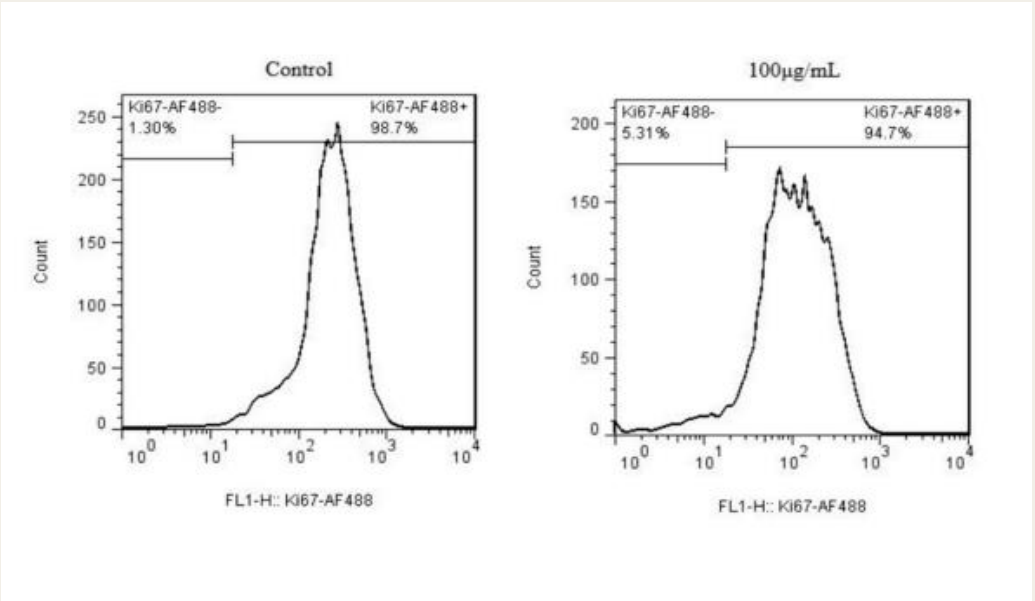
Exosomes

HL-60 cells

Ki-67 Expression

Flow cytometry analysis showed a significant reduction in Ki-67 expression in HL-60 cells treated with 100 µg/mL BM-MSC exosomes, suggesting reduced proliferation





BM-MCS

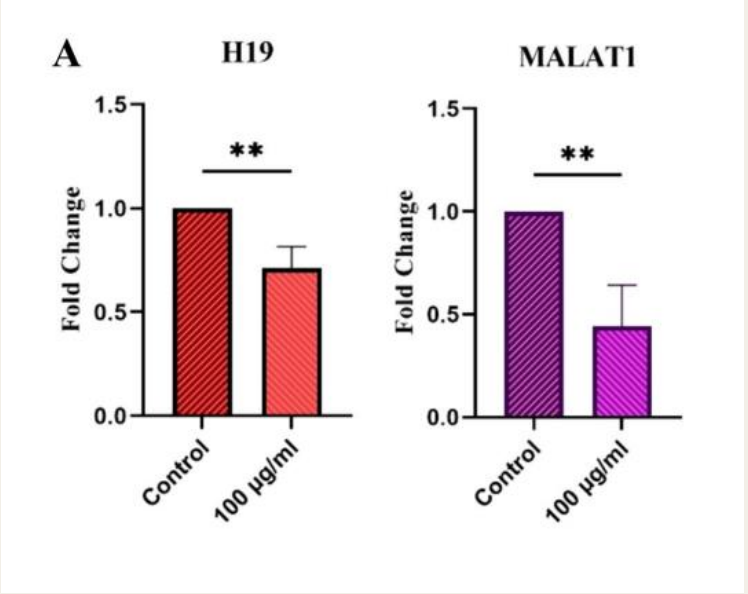
Exosomes

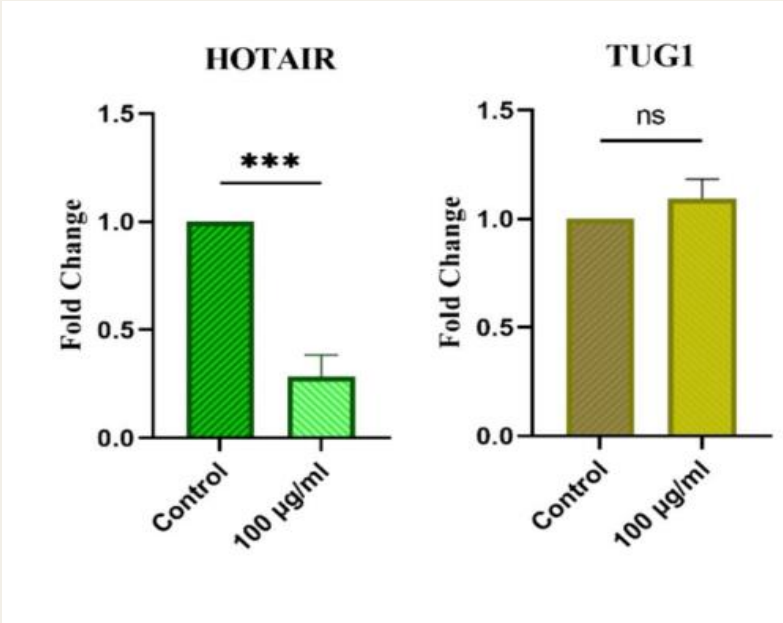
HL-60 cells

Gene Expression

RT-qPCR analysis revealed a reduction in the expression of oncogenic lncRNAs (MALAT1, HOTAIR, and H19).

but BM-MSC exosomes did not have a significant effect on TUG1 expression





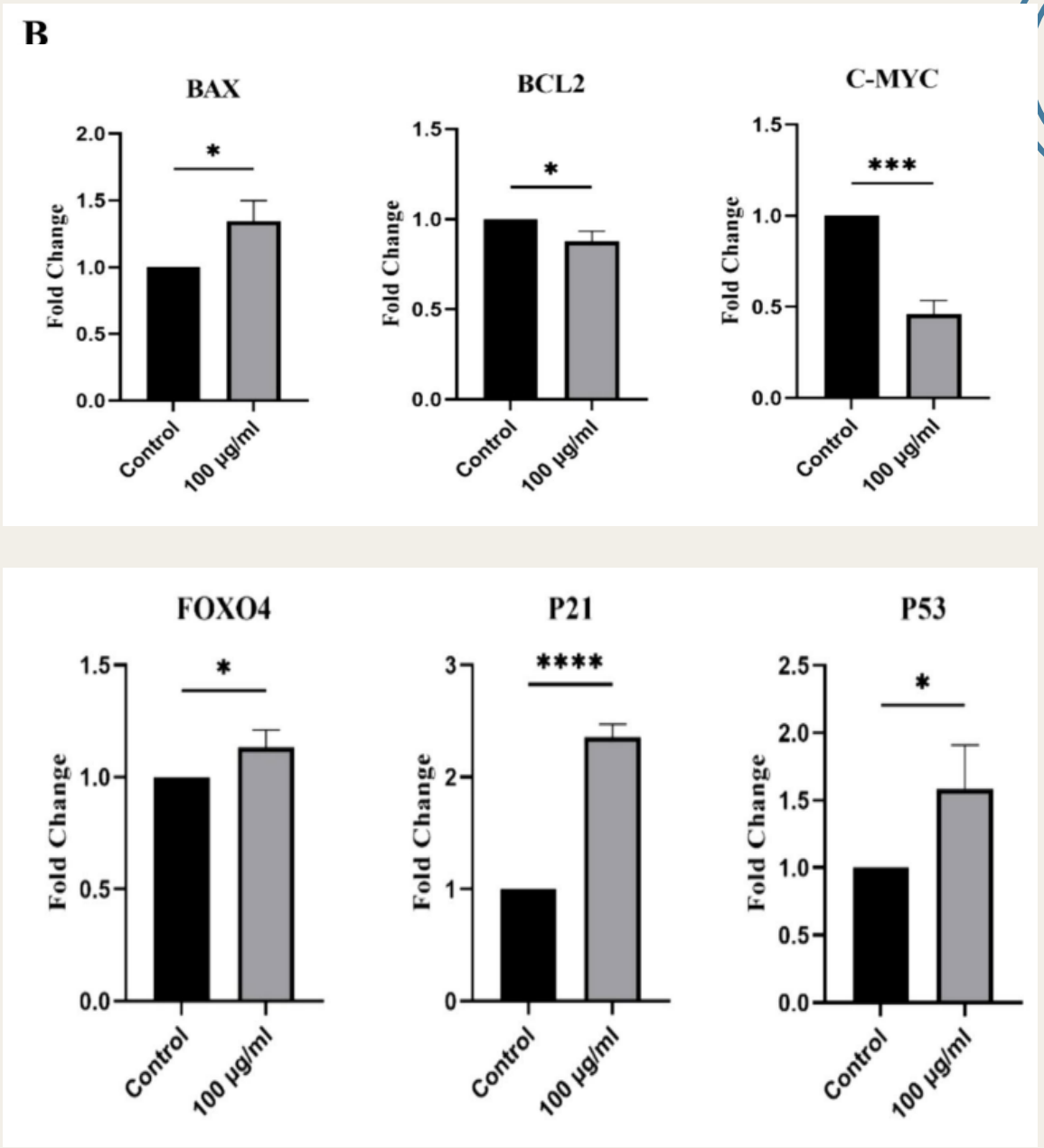
BM-MCS

Exosomes

HL-60 cells

Gene Expression

Pro-apoptotic genes (BAX, p53, and p21) were upregulated, while anti-apoptotic genes (BCL2 and c-Myc) were downregulated



Discussion

Exosome Anti-Leukemic Effects

- **Therapeutic Potential**:
  - BM-MSC-derived exosomes mimic the anti-tumor effects of MSCs without the risks of direct cell therapy.
  - Show significant potential as supportive treatments for AML by suppressing leukemic cell growth and inducing apoptosis.
- **Mechanisms of Action**:
  - **Apoptosis Induction**:
    - Exosomes elevate ROS, leading to DNA damage and programmed cell death.
    - Upregulation of pro-apoptotic genes (e.g., BAX, p53) and suppression of anti-apoptotic genes (e.g., BCL2).

Exosome Anti-Leukemic Effects

- **LncRNA Modulation:**
  - **Downregulation of oncogenic lncRNAs:**
  - **MALAT1: Involved in AML progression via PI3K/AKT and Wnt/β-catenin pathways; its suppression reduces proliferation and enhances apoptosis.**
  - **HOTAIR: Promotes leukemia stem cell renewal; its downregulation impairs growth and survival of leukemic cells.**
  - **H19: Drives anti-apoptotic and proliferative functions; suppression enhances apoptosis and may involve p53 regulation.**
- **No significant change in TUG1 expression, suggesting selective lncRNA modulation by exosomes**

Comparison with Previous Studies

- The findings align with prior in vitro and in vivo studies showing that BM-MSC exosomes can induce apoptosis and inhibit proliferation in leukemic models.

- Results confirm the anti-proliferative and pro-apoptotic effects observed in KG-1a and THP-1 cells and animal models of AML.

- Exosome-treated AML cells exhibit reduced tumor load and Ki-67 expression, consistent with earlier observations in splenomegaly models

Clinical Implications

- BM-MSC exosomes can be explored as adjunct therapies to enhance the efficacy of chemotherapeutics in AML, especially for overcoming drug resistance.
- Downregulation of lncRNAs like MALAT1, HOTAIR, and H19 offers a targeted approach to control AML progression.
- Exosomes provide a safer alternative to MSC transplantation by leveraging their paracrine effects.

Limitations and Future Directions

- **Limitations:**
  - **The study was conducted on a single AML cell line (HL-60), which limits its generalizability.**
  - **In vitro design may not fully replicate in vivo tumor microenvironment complexities.**
- **Future Directions:**
  - **Validation in diverse AML cell lines and animal models.**
  - **Exploration of exosome effects on additional cellular functions.**

Development of clinical trials to assess therapeutic efficacy and integration into existing AML treatment regimens

Conclusion

This study demonstrated that **BM-MSC-derived exosomes** exhibit significant anti-leukemic effects on HL-60 AML cells.

These exosomes suppress cell proliferation and metabolic activity, induce apoptosis, and increase ROS levels in AML cells

Summary of study

The findings suggest that BM-MSC exosomes could serve as a promising supportive therapy for AML, with the potential to enhance treatment efficacy and overcome drug resistance

Clinical Implications

The study revealed that BM-MSC exosomes regulate the expression of critical lncRNAs (MALAT1, HOTAIR, H19) and apoptosis-related genes (BAX, BCL2, p53, and c-Myc), highlighting their role in targeting poor-prognosis markers in AML

Key Molecular Insights

Future Potential

Further research is required to validate these findings in other AML models and clinical settings to establish their safety and therapeutic value

Thanks for your attention