



Adipose-Derived Mesenchymal Stem Cells Secretome Induces Apoptosis in Colon Carcinoma HT-29 Cells

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Abstract

Background: Recent studies have demonstrated that adipose mesenchymal stem cells (AMSCs)-derived secretome (AMSC-Se) has anticancer impacts.

Objectives: This study investigated the cytotoxic impacts of AMSC-Se on a colon carcinoma cell (HT-29) line.

Methods: The colon cancer cells were exposed to 50 or 100 $\mu\text{g/mL}$ AMSC-Se for 24 hours. MTT test had used to examine the impacts of AMSC-Se on the survival rates of the cells. Caspase activity, mRNA, and protein expression of Bax and Bcl-2 had evaluated to determine apoptosis.

Results: AMSC-Se could diminish the survival of the cells concentration-dependently. The mRNA and protein expression of Bax was concentration-dependently elevated, while Bcl-2 expression decreased in the AMSC-Se group compared to the control concentration-dependently ($P < 0.05$). The caspase-3 and caspase-9 activities were concentration-dependently enhanced ($P < 0.05$), while the caspase-8 activity did not change with the AMSC-Se.

Conclusions: These findings indicate that AMSC-Se effectively prevents cell growth and induces apoptosis by stimulating the intrinsic apoptotic pathway in these cells.

Keywords: HT-29 Cell Line, Apoptosis, Adipose-Derived Mesenchymal Stem Cells, Secretome

1. Background

Colon cancer is a common type of cancer worldwide. Despite standard treatments, its mortality is high due to drug resistance (1). Therefore, exploring the new anticancer drugs and the involved mechanisms may help to manage this cancer. Mesenchymal stem cells (MSCs) are non-hematopoietic and adherent cells that differentiate into different cell types (2). Furthermore, MSCs secrete soluble factors with various paracrine functions that affect cellular processes such as angiogenesis, apoptosis, and immune response into the microenvironments (3, 4). Adipose tissue, placenta, umbilical cord, and bone marrow are the main sources of MSCs (5).

Adipose tissue is a rich source of stem cells with fewer complications for patients. Furthermore, a small volume of fat tissue is required to isolate MSCs compared to the other origins (6). Adipose-derived mesenchymal

stem cells (AMSCs) release a complex of cytokines and growth factors into the extracellular microenvironment. The combination of these factors is named secretome. The secretome plays a dual role in regulatory mechanisms and can either inhibit or promote the proliferation of malignant cells (7, 8). Several studies have explored the effect of MSCs on various malignant cells or tissues (9, 10). However, their obtained results are controversial. The controversial results may be due to various MSC sources or cancer types (11). Mesenchymal stem cells prevent the growth of some malignancies, including colon cancer, hepatoma, and melanoma (12-14).

2. Objectives

In the current work, the cytotoxic and apoptotic impacts of adipose mesenchymal stem cells

(AMSCs)-adipose mesenchymal stem cells-derived secretome (AMSC-Se) against a colon cancer cell line were explored.

3. Methods

3.1. Adipose Mesenchymal Stem Cell Characterization

The human AMSCs were from Royan Company, Tehran, Iran. The cells have been characterized (in passage 3) using flow cytometry to determine specific markers. Adipogenic and osteogenic potentials were evaluated by oil-red and Alizarin-Red staining (Figure 1).

3.2. Secretome Preparation

Briefly, the AMSCs were harvested in complete media. At 80% confluency, the cells were washed and incubated in a serum-free DMEM overnight. The conditioned media were centrifuged at 4,000 g for 10 min. Then, the conditioned media were re-centrifuged at 4,000 g with an Amicon Ultra-15 centrifugal filter (Sigma) for two hours (15). The protein contents were determined by a BCA kit (Invitrogen, USA) and stored at -80°C.

3.3. Cell Culture

The HT-29 cells (RRID: CVCL_0320) were cultured in McCoy's 5a media with two mM glutamine and FBS (10%) and incubated in the cell culture incubator. At 80% confluency, the media had discarded, and the cells were treated with 0 (control), 50, and 100 µg/mL of AMSC-Se. The optimum concentration and exposure time of AMSC-Se were obtained from the MTT results (Table 1).

Table 1. The Impact of Different Concentrations of AMSC-Se on the Viability of Cancer Cells^{a, b}

Treatment (µg/mL)	12 Hours	24 Hours	48 Hours
25	99.9 ± 1.2	98.4 ± 2.5	97.8 ± 3.9
50	96.7 ± 3.8	73.1 ± 5.3 ^b	69.7 ± 5.3 ^b
100	95.9 ± 4.4	48.7 ± 4.3 ^{**}	28.2 ± 4.1 ^{**}

^a Data are presented as mean ± SD (n = 5).

^b Shows comparison with 12 hours.

3.4. Cell Viability

The 10⁶ cells were harvested in 96-well dishes for one day. The cells exposed to AMSC-Se and 0.5 mg/mL of MTT solution were added to the wells and kept for three hours in an incubator. The supernatants were removed, 100 µL DMSO was added, and the absorbance was read at 570 nm using a BioRad microplate reader. The percentage of cell viability was calculated relative to the control cells.

3.5. Real-time PCR

The RNA was extracted from the cells (10⁷ cells) using an RNeasy kit (Qiagen, Valencia, USA). The quality of extracted RNA was determined by measuring the UV absorption of the samples. A cDNA synthesis kit (Qiagen) was used to synthesize cDNA. The cDNAs were amplified in the PCR reaction solution containing SYBR green master mix and primers. The primer sequences were: Bcl-2: Forward 5'-GGATGCCTTTGTGGAAGTGT-3', Reverse 5'-TCACTTGTGGCCAGATAGG-3'; Bax: Forward 5'-GCTGGACATTGGACTTCCTC-3', Reverse 5'-ACCACTGTGACCTGCTCCA-3' and GAPDH: Forward 5'-GCAAGAGACAAGAGGAAGA-3', Reverse 5'-ACTGTGAGGAGGGGAGATTC-3'.

A 45-cycle program was considered for PCR amplification: initial denaturation (95°C, 10 seconds), denaturation (95°C, 15 seconds), annealing (55°C, 20 seconds), and extension (60°C, 20 seconds).

3.6. Measuring Protein Level of Bax and Bcl-2

The cultured cells were mixed with RIPA lysis buffer plus protease inhibitor. BCA assay kit (Sigma, USA) was used to obtain protein from the cells. The protein level of the Bax and Bcl-2 were measured using ELISA kits (R&D).

3.7. Caspase Activity Assay

The caspase activities were measured by Caspase Assay Kits (Thermo Fisher, USA). The 10⁶ cells were exposed to 50 or 100 µg/mL AMSC-Se for one day. Then, cell lysates were provided and centrifuged at 17,000 rpm for 15 min. The amount of protein was determined by a BCA assay Kit. The cell lysates were exposed to caspase substrate for four hours. The absorbance was read at 405 nm by a Bio-Rad microplate reader.

3.8. Statistical Analysis

Data were analyzed with one-way ANOVA followed by Tukey's post hoc test for multiple pairwise comparisons. The P-values < 0.05 were considered significant.

4. Results

4.1. Adipose Mesenchymal Stem Cells-Derived Secretome Exposure Inhibited the Cell Growth

Adipose mesenchymal stem cells-derived secretome decreased the survival of the cells concentration-dependently. The survival rate (Figure 2) decreased in the 50 µg/mL AMSC-Se-exposed cells compared to the control (P < 0.05). The survival rate of the 100 µg/mL AMSC-Se-exposed cells was reduced compared to the untreated and 50 µg/mL-treated cells (P < 0.05).

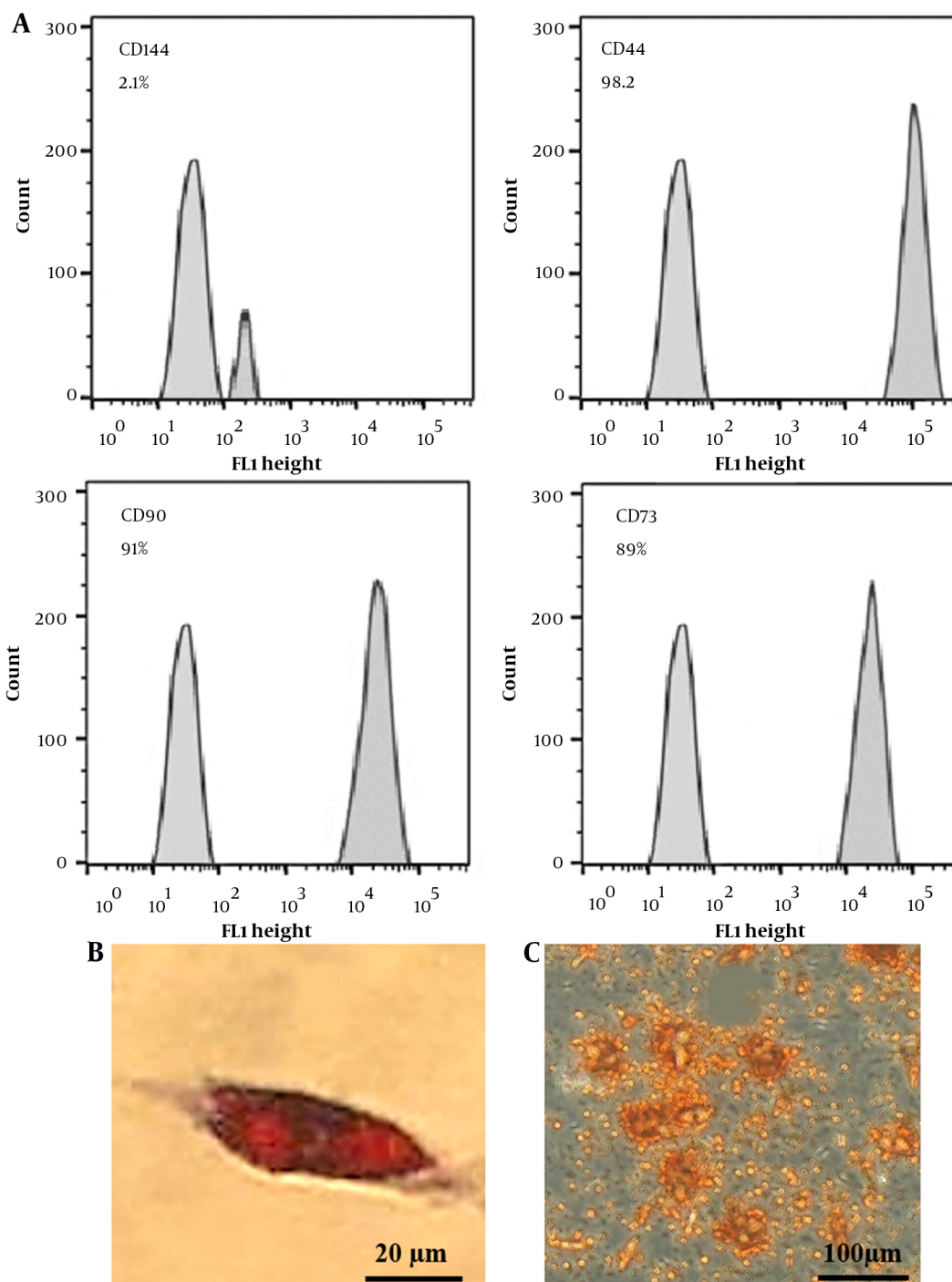


Figure 1. Characterization of AMSCs by flow cytometry (A). AMSCs were found to be positive for MSC-specific markers (CD90, D73, and CD44), and negative for hematopoietic stem cell surface markers (CD144). Adipogenic and osteogenic differentiation potential of AMSCs (B and C). Oil red O (B) and Alizarin red staining (C) positive cells, showing adipogenic and osteogenic differentiation of the AMSCs.

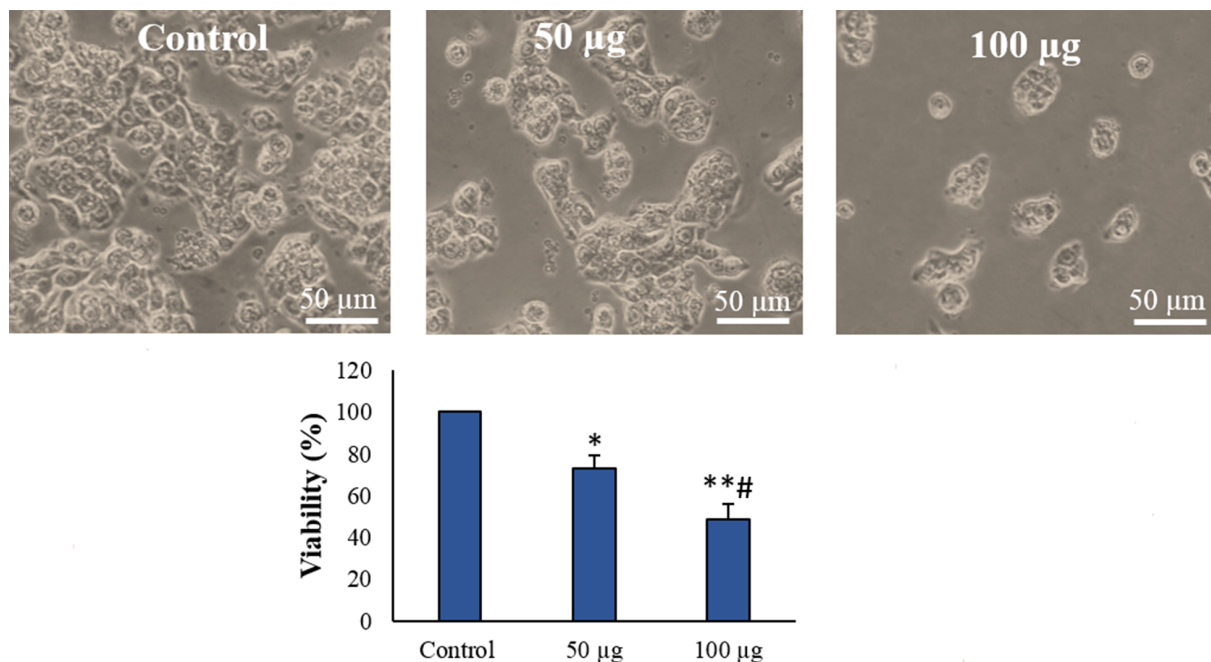


Figure 2. Morphology and viability of the cancer cells (mean \pm SD; n = 6). * and # show comparison with the untreated and 50 μ g/mL AMSC-Se groups, respectively.

4.2. mRNA and Protein Expression of Apoptosis-Related Proteins

Adipose mesenchymal stem cells-derived secretome changed the mRNA and protein expression of Bcl-2 and Bax in the HT-29 cells concentration-dependently. Adipose mesenchymal stem cells-derived secretome at the 50 μ g/mL concentration enhanced the mRNA and protein level of Bax ($P < 0.05$), while the Bcl-2 expression was diminished compared to the control ($P < 0.05$). Adipose mesenchymal stem cells-derived secretome at 100 μ g/mL elevated the mRNA and protein level of Bax and decreased Bcl-2 expression compared to the untreated and 50 μ g/mL AMSC-Se-exposed cells (Figure 3).

4.3. Caspase-3 and Caspase-9 Are Involved in the AMSCs-Se-Induced Apoptosis

The caspase-3 and caspase-9 activities enhanced in the 50 μ g/mL AMSC-Se group in comparison to the control ($P < 0.01$). The activities of caspase-3 and caspase-9 were also significantly enhanced in 100 μ g/mL AMSCs-Se ($P < 0.001$) and 50 μ g/mL ($P < 0.05$) treatments compared to the control. The caspase-8 activity did not change by the AMSCs-Se (Figure 4).

5. Discussion

In this work, the effect of AMSC-Se on HT-29 cell growth has been explored. The results indicated that AMSC-Se significantly diminished proliferation and enhanced apoptosis of HT-29 cells. In line with these results, Zhu et al. revealed that the factors secreted by AMSCs could suppress the survival and proliferation of the K562 leukemia cells (16). Also, Ryu et al. reported that AMSCs conditioned medium prevents breast cancer cell growth (17). Adipose mesenchymal stem cells suppressed the growth of SKBR3 (a breast malignant cell line) cells in direct co-cultures (18). In another investigation, AMSC-Se reduced bladder cancer cell viability and decreased its resistance to ciprofloxacin (7).

In contrast, some reports have demonstrated that secretome from AMSCs promotes the proliferation of breast cancer cells (19, 20). Conditioned medium from AMSCs significantly enhanced hepatocellular carcinoma invasion (21). In another study, AMSC-Se promoted the growth of prostate cancer cells (22). These controversial results may be due to the variability of MSC sources or cancer cell types.

Flow cytometry method was used in this study to explore the relationship of anti-proliferating impact of AMSC-Se with apoptosis. The AMSC-Se-treated cells exhibited more apoptosis than the untreated cancerous

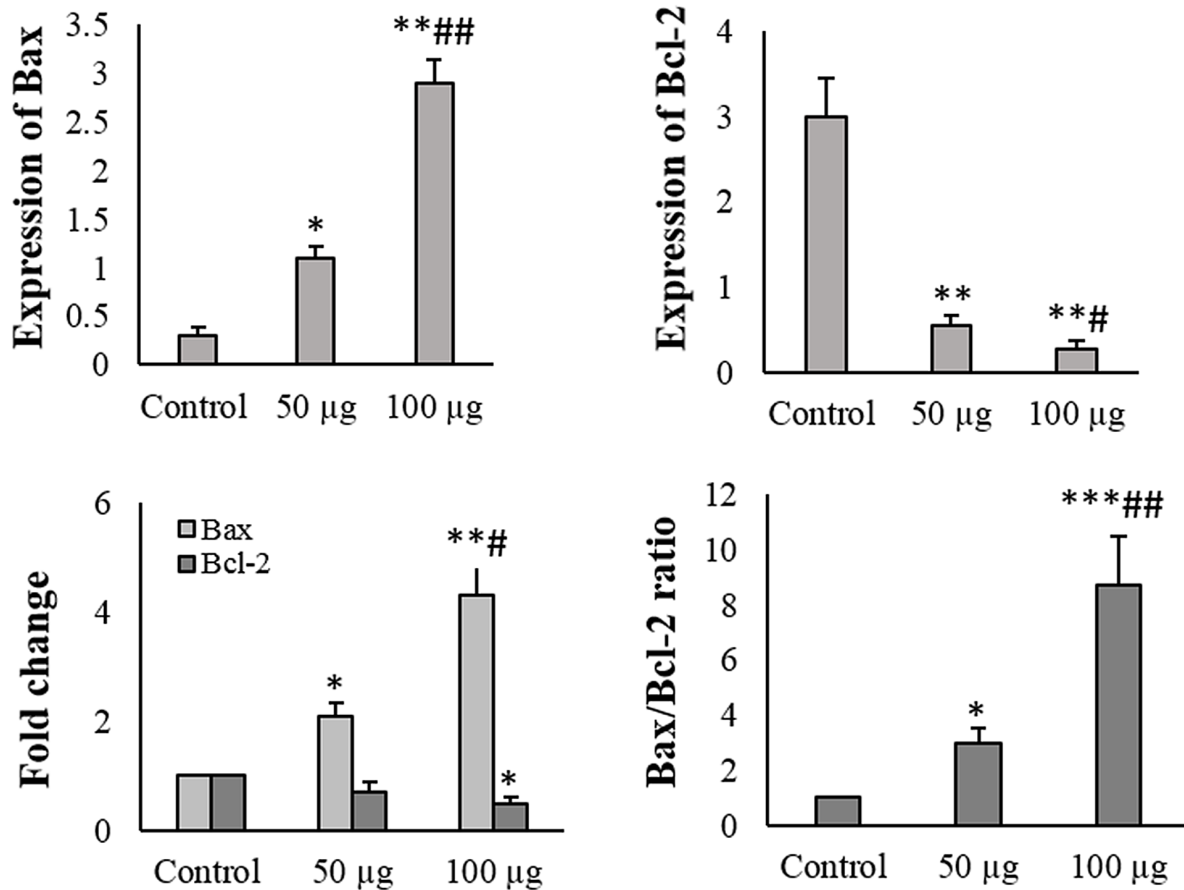


Figure 3. Protein expression (upper panel) and gene expression (lower panel) (mean \pm SD, n = 3). Image J software was applied for quantification of the protein expression, and the cells were normalized to GAPDH band intensity.

cells. Previous studies showed that conditioned medium from AMSCs induces apoptosis in the cancerous cells (23, 24). In this regard, AMSCs increased apoptosis along with proliferation inhibition in hepatocellular carcinoma cell lines (25). Direct co-culture of SKBR3 cancer cells with AMSCs increased chemosensitivity and enhanced apoptosis in response to 5-fluorouracil and doxorubicin (18). Adipose mesenchymal stem cells-derived secretome also prevented melanoma proliferation by inducing apoptosis *in vitro* (24). As shown in flow cytometry results, the necrosis percentage did not change by the AMSC-Se, which revealed a critical role of apoptosis in reducing the survival of the malignant cells of the AMSC-Se.

Protein and mRNA levels of Bcl-2 and Bax, and caspases activity were assessed in the current work to confirm the involvement of apoptosis in AMSC-Se-reduced growth of the cancer cells. Adipose mesenchymal stem cells-derived secretome enhanced the Bax level, while the Bcl-2 level

was reduced within the HT-29 cells. These genes are the main regulators of the intrinsic apoptotic pathway. The enhanced Bax/Bcl-2 ratio expression activates caspase-3 and caspase-9 via cytochrome-c release and eventually induces apoptosis (25). Our results demonstrated the treatment AMSC-Se considerably enhanced the activity of caspase-3 and caspase-9. The activated caspase-9 can activate effector caspases and initiate the intrinsic apoptotic pathway.

Conditioned media from human AMSCs could elevate the activities of caspase-3 and caspase-9 and lead to apoptosis in the human U251 glioma cells (26). Therefore, these results suggest that AMSC-Se stimulates the intrinsic pathway of apoptotic to enhance cancer cell death. The secretome of the MSCs has biological components, such as cytokines, growth factors, angiogenic factors, micro RNAs, anticancer molecules, and exosomes (27-34). A number of studies evaluated the effects of the secreted

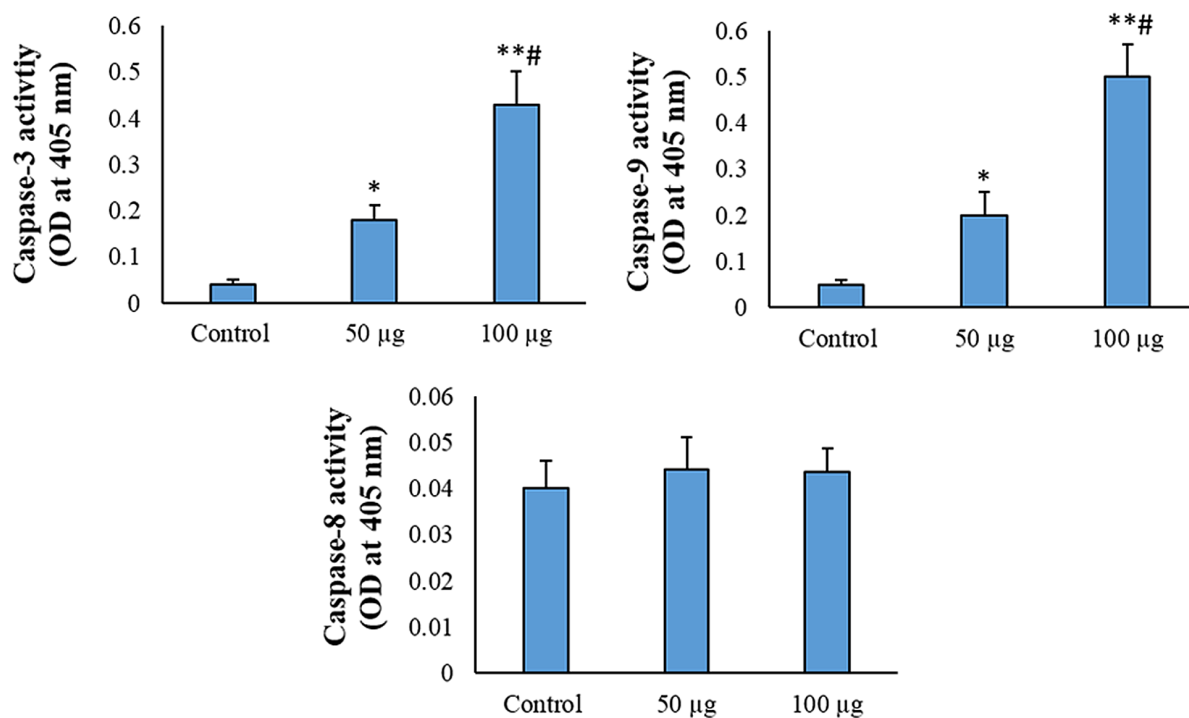


Figure 4. AMSC-Se effects on the caspases activity in different groups (mean \pm SD, n = 6). *: P < 0.01, **: P < 0.001.

molecules from the MSCs. Bone marrow MSCs-derived cytokine-induced neutrophil chemoattractant-1 and tissue inhibitor of metalloproteinases-1 could prevent the proliferation of the myeloid leukemia cells by suppressing the caspase-3 pathway (35). TRAIL can initiate the caspase-8 cascade and promote the extrinsic apoptotic pathway (36).

5.1. Conclusions

The present study has revealed the cytotoxic impacts of AMSC-Se on the HT-29 cells. These findings clearly indicated that the inhibitory effect of AMSC-Se against HT-29 cell growth is modulated by the stimulating intrinsic apoptotic signaling pathway.

Footnotes

Authors' Contribution: MJK: Supervision and reviewing of the manuscript; HA and FR: Sampling and data statistical analysis; AS: Conceived the study.

Conflict of Interests: There is no conflict of interest.

Ethical Approval: This study was approved by the AJUMS Animal Ethics Committee (IR.AJUMS.ABHC.REC.1398.219).

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References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*. 2015;**136**(5):E359-86. [PubMed ID: 25220842]. <https://doi.org/10.1002/ijc.29210>.
2. Kupcova Skalnikova H. Proteomic techniques for characterisation of mesenchymal stem cell secretome. *Biochimie*. 2013;**95**(12):2196-211. [PubMed ID: 23880644]. <https://doi.org/10.1016/j.biochi.2013.07.015>.
3. Ranganath SH, Levy O, Inamdar MS, Karp JM. Harnessing the mesenchymal stem cell secretome for the treatment of cardiovascular disease. *Cell Stem Cell*. 2012;**10**(3):244-58. [PubMed ID: 22385653]. [PubMed Central ID: PMC3294273]. <https://doi.org/10.1016/j.stem.2012.02.005>.
4. Cunningham CJ, Redondo-Castro E, Allan SM. The therapeutic potential of the mesenchymal stem cell secretome in ischaemic stroke. *J Cereb Blood Flow Metab*. 2018;**38**(8):1276-92. [PubMed ID: 29768965]. [PubMed Central ID: PMC6077926]. <https://doi.org/10.1177/0271678X18776802>.
5. Berebichez-Fridman R, Montero-Olvera PR. Sources and Clinical Applications of Mesenchymal Stem Cells: State-of-the-art review. *Sultan Qaboos Univ Med J*. 2018;**18**(3):e264-77. [PubMed ID: 30607265]. [PubMed Central ID: PMC6307657]. <https://doi.org/10.18295/squmj.2018.18.03.002>.
6. Si Z, Wang X, Sun C, Kang Y, Xu J, Wang X, et al. Adipose-derived stem cells: Sources, potency, and implications for regenerative therapies. *Biomed Pharmacother*. 2019;**114**:108765. [PubMed ID: 30921703]. <https://doi.org/10.1016/j.biopha.2019.108765>.

7. Maj M, Kokocha A, Bajek A, Drewa T. The interplay between adipose-derived stem cells and bladder cancer cells. *Sci Rep*. 2018;**8**(1):15118. [PubMed ID: 3031011]. [PubMed Central ID: PMC6181926]. <https://doi.org/10.1038/s41598-018-33397-9>.
8. Zimmerlin L, Park TS, Zambidis ET, Donnenberg VS, Donnenberg AD. Mesenchymal stem cell secretome and regenerative therapy after cancer. *Biochimie*. 2013;**95**(12):2235–45. [PubMed ID: 23747841]. [PubMed Central ID: PMC3825748]. <https://doi.org/10.1016/j.biochi.2013.05.010>.
9. Koellensperger E, Bonnert LC, Zoernig I, Marme F, Sandmann S, Germann G, et al. The impact of human adipose tissue-derived stem cells on breast cancer cells: implications for cell-assisted lipotransfers in breast reconstruction. *Stem Cell Res Ther*. 2017;**8**(1):121. [PubMed ID: 28545495]. [PubMed Central ID: PMC5445287]. <https://doi.org/10.1186/s13287-017-0579-1>.
10. Purnamawati P, Pawitan JA, Rachman A, Wanandi SI. Effects of umbilical cord- and adipose-derived stem cell secretomes on ALDH1A3 expression and autocrine TGF- β 1 signaling in human breast cancer stem cells. *F1000Research*. 2018;**7**:249. <https://doi.org/10.12688/f1000research.13609.1>.
11. Serhal R, Saliba N, Hilal G, Moussa M, Hassan GS, El Atat O, et al. Effect of adipose-derived mesenchymal stem cells on hepatocellular carcinoma: In vitro inhibition of carcinogenesis. *World J Gastroenterol*. 2019;**25**(5):567–83. [PubMed ID: 30774272]. [PubMed Central ID: PMC6371009]. <https://doi.org/10.3748/wjg.v25.i5.567>.
12. Ohlsson LB, Varas L, Kjellman C, Edvardsen K, Lindvall M. Mesenchymal progenitor cell-mediated inhibition of tumor growth in vivo and in vitro in gelatin matrix. *Exp Mol Pathol*. 2003;**75**(3):248–55. [PubMed ID: 14611816]. <https://doi.org/10.1016/j.yexmp.2003.06.001>.
13. Qiao L, Xu Z, Zhao T, Zhao Z, Shi M, Zhao RC, et al. Suppression of tumorigenesis by human mesenchymal stem cells in a hepatoma model. *Cell Res*. 2008;**18**(4):500–7. [PubMed ID: 18364678]. <https://doi.org/10.1038/cr.2008.40>.
14. Eiro N, Sendon-Lago J, Seoane S, Bermudez MA, Lamelas ML, Garcia-Caballero T, et al. Potential therapeutic effect of the secretome from human uterine cervical stem cells against both cancer and stromal cells compared with adipose tissue stem cells. *Oncotarget*. 2014;**5**(21):10692–708. [PubMed ID: 25296979]. [PubMed Central ID: PMC4279403]. <https://doi.org/10.18632/oncotarget.2530>.
15. Dubey NK, Mishra VK, Dubey R, Deng YH, Tsai FC, Deng WP. Revisiting the Advances in Isolation, Characterization and Secretome of Adipose-Derived Stromal/Stem Cells. *Int J Mol Sci*. 2018;**19**(8):2200. [PubMed ID: 30060511]. [PubMed Central ID: PMC6121360]. <https://doi.org/10.3390/ijms19082200>.
16. Zhu Y, Sun Z, Han Q, Liao L, Wang J, Bian C, et al. Human mesenchymal stem cells inhibit cancer cell proliferation by secreting DKK-1. *Leukemia*. 2009;**23**(5):925–33. [PubMed ID: 19148141]. <https://doi.org/10.1038/leu.2008.384>.
17. Ryu H, Oh JE, Rhee KJ, Baik SK, Kim J, Kang SJ, et al. Adipose tissue-derived mesenchymal stem cells cultured at high density express IFN-beta and suppress the growth of MCF-7 human breast cancer cells. *Cancer Lett*. 2014;**352**(2):220–7. [PubMed ID: 25016057]. <https://doi.org/10.1016/j.canlet.2014.06.018>.
18. Kucerova L, Skolekova S, Matuskova M, Bohac M, Kozovska Z. Altered features and increased chemosensitivity of human breast cancer cells mediated by adipose tissue-derived mesenchymal stromal cells. *BMC Cancer*. 2013;**13**:535. [PubMed ID: 24209831]. [PubMed Central ID: PMC3829110]. <https://doi.org/10.1186/1471-2407-13-535>.
19. Muehlberg FL, Song YH, Krohn A, Pinilla SP, Droll LH, Leng X, et al. Tissue-resident stem cells promote breast cancer growth and metastasis. *Carcinogenesis*. 2009;**30**(4):589–97. [PubMed ID: 19181699]. <https://doi.org/10.1093/carcin/bgp036>.
20. Razmkhah M, Jaberipour M, Hosseini A, Safaei A, Khalatbari B, Ghaderi A. Expression profile of IL-8 and growth factors in breast cancer cells and adipose-derived stem cells (ASCs) isolated from breast carcinoma. *Cell Immunol*. 2010;**265**(1):80–5. [PubMed ID: 20705284]. <https://doi.org/10.1016/j.cellimm.2010.07.006>.
21. Teshima T, Matsumoto H, Koyama H. Soluble factors from adipose tissue-derived mesenchymal stem cells promote canine hepatocellular carcinoma cell proliferation and invasion. *PLoS One*. 2018;**13**(1). e0191539. [PubMed ID: 29346427]. [PubMed Central ID: PMC5773216]. <https://doi.org/10.1371/journal.pone.0191539>.
22. Lin G, Yang R, Banie L, Wang G, Ning H, Li LC, et al. Effects of transplantation of adipose tissue-derived stem cells on prostate tumor. *Prostate*. 2010;**70**(10):1066–73. [PubMed ID: 20232361]. [PubMed Central ID: PMC2877148]. <https://doi.org/10.1002/pros.21140>.
23. Zhao W, Ren G, Zhang L, Zhang Z, Liu J, Kuang P, et al. Efficacy of mesenchymal stem cells derived from human adipose tissue in inhibition of hepatocellular carcinoma cells in vitro. *Cancer Biother Radiopharm*. 2012;**27**(9):606–13. [PubMed ID: 22917212]. <https://doi.org/10.1089/cbr.2011.1150>.
24. Zhao J, Zhang Z, Cui Q, Zhao L, Hu Y, Zhao S. Human adipose-derived mesenchymal stem cells inhibit proliferation and induce apoptosis of human gastric cancer HGC-27 cells. *3 Biotech*. 2020;**10**(3):129. [PubMed ID: 32154042]. [PubMed Central ID: PMC7035394]. <https://doi.org/10.1007/s13205-020-2090-0>.
25. Jain AK, Thanki K, Jain S. Novel self-nanoemulsifying formulation of quercetin: Implications of pro-oxidant activity on the anticancer efficacy. *Nanomedicine*. 2014;**10**(5):959–69. [PubMed ID: 24407148]. <https://doi.org/10.1016/j.nano.2013.12.010>.
26. Yang C, Lei D, Ouyang W, Ren J, Li H, Hu J, et al. Conditioned media from human adipose tissue-derived mesenchymal stem cells and umbilical cord-derived mesenchymal stem cells efficiently induced the apoptosis and differentiation in human glioma cell lines in vitro. *Biomed Res Int*. 2014;**2014**:109389. [PubMed ID: 24971310]. [PubMed Central ID: PMC4058294]. <https://doi.org/10.1155/2014/109389>.
27. Phelps J, Sanati-Nezhad A, Ungrin M, Duncan NA, Sen A. Bioprocessing of Mesenchymal Stem Cells and Their Derivatives: Toward Cell-Free Therapeutics. *Stem Cells Int*. 2018;**2018**:9415367. [PubMed ID: 30275839]. [PubMed Central ID: PMC6157150]. <https://doi.org/10.1155/2018/9415367>.
28. Kyurkchiev D, Bochev I, Ivanova-Todorova E, Mourdjeva M, Oreshkova T, Belemezova K, et al. Secretion of immunoregulatory cytokines by mesenchymal stem cells. *World J Stem Cells*. 2014;**6**(5):552–70. [PubMed ID: 25426252]. [PubMed Central ID: PMC4178255]. <https://doi.org/10.4252/wjsc.v6.i5.552>.
29. Ferreira JR, Teixeira GQ, Santos SG, Barbosa MA, Almeida-Porada G, Goncalves RM. Mesenchymal Stromal Cell Secretome: Influencing Therapeutic Potential by Cellular Pre-conditioning. *Front Immunol*. 2018;**9**:2837. [PubMed ID: 30564236]. [PubMed Central ID: PMC6288292]. <https://doi.org/10.3389/fimmu.2018.02837>.
30. Bartosh TJ, Ylostalo JH, Mohammadipour A, Bazhanov N, Coble K, Claypool K, et al. Aggregation of human mesenchymal stromal cells (MSCs) into 3D spheroids enhances their antiinflammatory properties. *Proc Natl Acad Sci U S A*. 2010;**107**(31):13724–9. [PubMed ID: 20643923]. [PubMed Central ID: PMC2922230]. <https://doi.org/10.1073/pnas.1008117107>.
31. Madrigal M, Rao KS, Riordan NH. A review of therapeutic effects of mesenchymal stem cell secretions and induction of secretory modification by different culture methods. *J Transl Med*. 2014;**12**:260. [PubMed ID: 25304688]. [PubMed Central ID: PMC4197270]. <https://doi.org/10.1186/s12967-014-0260-8>.
32. Meiliana A, Wijaya A. Brown Adipose Tissue: A New Target for Antiobesity Therapy. *Indones Biomed J*. 2010;**2**(2):4. <https://doi.org/10.18585/inabj.v2i2.i15>.
33. Lombardi F, Palumbo P, Augello FR, Cifone MG, Cinque B, Giuliani M. Secretome of Adipose Tissue-Derived Stem Cells (ASCs) as a Novel

- Trend in Chronic Non-Healing Wounds: An Overview of Experimental In Vitro and In Vivo Studies and Methodological Variables. *Int J Mol Sci.* 2019;**20**(15):3721. [PubMed ID: 31366040]. [PubMed Central ID: PMC6696601]. <https://doi.org/10.3390/ijms20153721>.
34. Pokrovskaya IA, Zubareva EV, Nadezhdin SV, Lysenko AS, Litovkina TL. Biological activity of mesenchymal stem cells secretome as a basis for cell-free therapeutic approach. *Res Results Pharmacol.* 2020;**6**(1):57-68. <https://doi.org/10.3897/rpharmacology.6.49413>.
35. Fathi E, Farahzadi R, Valipour B, Sanaat Z. Cytokines secreted from bone marrow derived mesenchymal stem cells promote apoptosis and change cell cycle distribution of K562 cell line as clinical agent in cell transplantation. *PLoS One.* 2019;**14**(4). e0215678. [PubMed ID: 31009502]. [PubMed Central ID: PMC6476492]. <https://doi.org/10.1371/journal.pone.0215678>.
36. Tummers B, Green DR. Caspase-8: regulating life and death. *Immunol Rev.* 2017;**277**(1):76-89. [PubMed ID: 28462525]. [PubMed Central ID: PMC5417704]. <https://doi.org/10.1111/imr.12541>.