Original Article

ANATOLIAN CURRENT MEDICAL

DOI: 10.38053/acmj.1359905

*Anatolian Curr Med J.* 2023;5(4):503-510

# Conditioned media of tonsil derived mesenchymal stem cells shows different rates of cytotoxicity on solid cancer cells

**Melek Yüce[1], Esra Albayrak[1], Çağrı Gümüşkaptan[2]**

[1]Stem Cell Research & Application Center, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey
[2]Department of Molecular Medicine, Institute of Health Sciences, Ondokuz Mayıs University, Samsun, Turkey

**Cite this article as:** Yüce M, Albayrak E, Gümüşkaptan Ç. Conditioned media of tonsil derived mesenchymal stem cells shows different rates of cytotoxicity on solid cancer cells. *Anatolian Curr Med J.* 2023;5(4):503-510.

**Received**: 13.09.2023 ◆ **Accepted**: 18.10.2023 ◆ **Published**: 27.10.2023

## ABSTRACT

**Aims**: Mesenchymal stem cells (MSCs) are the apple of the eye of cancer studies. It was indicated that the secreted factors, especially released by MSCs, have tumoral or anti-tumoral effects on tumor progression. MSCs obtained from different sources show different anti-tumoral effects, while MSCs originating from the same source also show different tumoral effects in different cancer cells. Here, we investigated the anti-tumor effects of soluble factors secreted from palatine tonsil MSCs (TMSC) as a new source of MSC on human lung carcinoma (A549) and pancreatic cancer (PANC-1) cell lines.

**Methods**: Conditioned medium (CM) was obtained from TMSCs isolated from palatine tonsil tissue, and the cytotoxic effect of CM on the growth of A549 and PANC-1 in a dose-dependent manner was demonstrated by MTT analysis. In addition, the function of CM treatment on the cell cycle status of cancer cells and the apoptosis process were investigated through cell cycle analysis with propidium iodide (PI) and Annexin-V/PI detection method by flow cytometry analysis, respectively.

**Results**: We demonstrated that TMSC-CM treatment significantly decreased the viability of A549 and PANC-1 cell lines in a dose-dependent manner post-48 hours. In addition, CM treatment differentially induced the apoptosis on A549 and PANC-1 cells and also, caused G2/M arrest in the cells.

**Conclusion**: In light of these findings, our study is the first to show that TMSC-CM has an anti-tumoral effect by stimulating apoptosis on A549 and PANC-1 cells. These findings reveal that the usage of CM has a cell-free cellular therapeutic potential.

**Keywords**: Cancer, cellular therapy, cytotoxicity, mesenchymal stem cell, palatine tonsil

## INTRODUCTION

Cancer remains a serious health problem with considerable morbidity and mortality rates worldwide despite the rapid progress in diagnostic and therapeutic research.[1] Mesenchymal stem cells (MSCs) have important curative potential because of their self-renewal and multilineage differentiation properties and are broadly investigated in tissue engineering and regenerative medicine.[2] The use of stem cells in regenerative medicine has many advantages; however, the usage also brings with it a series of problems, such as a low survival rate after transplantation, immunological responses after administration, and a decrease in regenerative potential.[3] Recent research has shown that the therapeutic potential of MSCs is due to the different paracrine effects that they secrete. Based on the principle of paracrine signaling mechanism, MSC conditioned medium (MSC-CM) has intensive paracrine factors containing a variety of cytokines, chemokines, growth factors and nano-scale cellular vesicles exosomes.[4] The conditioned medium (CM) as a cell-free cellular therapy has an important advantage due to no requirement of donor-recipient compatibility. Besides, MSC-CMs are more accessible and applicable because of the requirement of less controlled conditions for application procedures than stem cells, which require special production and application conditions. In addition, they can be easily packaged and commercialized by freeze-dried or lyophilized, and they can be accessed and transported more easily as they eliminate the cryopreservation requirement compared to cell-based therapy.[4] Based on all these properties, CMs are promising candidates for biological pharmaceuticals, but many unknowns remain unclear.[5]

Stem cells from different sources have been shown to have the potential to secrete different paracrine factors to treat a variety of degenerative diseases. The paracrine factors released from cells vary according to the age of the cells, culture status, and tissue. Therefore, tissue selection plays a main role in therapeutic applications using stem cells.[3,4]

**Corresponding Author**: Melek Yüce, melek.yuce@omu.edu.tr

Tonsil tissue provided as waste tissue after tonsillectomy is recommended as an important source of MSC because of no need for unnecessary surgical procedures compared to bone marrow and its relatively high proliferation rate and low immunogenicity properties.[6] TMSCs not only show the expression of MSC-specific cell surface antigens, including CD29, CD44, CD73, CD90, and CD105, but also have their distinctive markers, such as CD106, CD108, and CD166, associated with adhesion, migration, and immunomodulation.[7] Besides, the palatine tonsil is important because it is the secondary lymphoid tissue that provides the formation of an effective immune response against antigens. In addition, TMSCs have faster proliferation and shorter doubling times than other stem cell types, and also, TMSCs from multiple donors can be used together. Its stem cell properties are well preserved and are not damaged after freezing and thawing. These properties make TMSCs important candidates for stem cell banking and are advantageous for their use in regenerative medicine.[8]

Different studies showing different results have been carried out to determination of the effects of MSC-CMs on cancer cell growth.[9-11] CMs obtained from human lung-derived MSCs suppress tumor cell growth,[12] human Wharton's jelly-derived MSC (WJMSC)-CM did not show any effect on proliferation and apoptotic potential of A549 lung cancer cells,[13] bone marrow-derived MSC (BMMSC)-CM has been shown to suppress the proliferation of lung cancer cells and cause apoptosis on the tumor cells in vitro.[14] Another study, evaluating the anti-cancer effect of the paracrine factors of umbilical cord-derived MSCs on MCF-7 tumor cells, showed a dose-dependent cytotoxic effect of MSC secretome on the breast cancer cell line.[15] The limited studies are showing the growth inhibitory effect on TMSC cancer cells. The study on head and neck squamous cell carcinoma (HNSCC) cell lines has demonstrated that TMSCs can cause notably growth inhibitory effects on HNSCC cell lines through number-dependent increased cell cycle arrest and apoptosis.[16] In the studies of the authors investigating the effect of TMSC-CM on hematological cancer cell lines, it was shown that CM induces cell death in hematological cancers after heat induction of TMSCs.[17] It causes changes in cancer behavior depending on the variability of the tumor niche in hematological and solid cancers.[18] Studies have supported that different therapeutic responses to the same therapeutic agent occur in hematological and solid cancers.[19] In this direction, in our study, we aimed to determine the concentration-dependent effects of the secretome released by TMSCs on the solid cancer cell line proliferation and further elucidate their anti-cancer properties and mechanism of function.

## METHODS

The study was carried out with the permission of Ondokuz Mayıs University Clinical Researches Ethics Committee (Date: 08.07.2021 Decision No: 2021/347). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Human tonsil derived mesenchymal stem cell isolation

Tonsil tissues were obtained from donors who underwent tonsillectomy with the approval of the donor and the clinical research ethics committee. TMSCs were enzymatically isolated and characterized as described in our previous study,[17] and cultured with DMEM supplemented with 10% Fetal Bovine Serum (FBS, Gibco), and 1% antibiotic-antimycotic (Sigma Aldrich). Characterization was done in the second or third passages. Stem cells from passages four or five were used in the experiments.[17]

### Immunophenotyping of Tonsil Derived Mesenchymal Stem Cells

Characterization of TMSCs was done based on specific cell surface antigens. For that, the cells were incubated with anti-human CD90, CD73, CD166, CD34, and CD45 monoclonal antibodies (Biolegend) according to the Manufacturer's directions. Next, the cells were analyzed by flow cytometry (Cytoflex S, Beckman Coulter).[6]

### Evaluation of Multilineage Differentiation Potential of Tonsil Derived Mesenchymal Stem Cells

The adipogenic and osteogenic differentiation protocol was performed as in our previous study.[17] Briefly, the $10\times10^4$ cells were cultured in the six well plate with the specific differentiation mediums for 3 weeks. The differentiation mediums were changed every two days. Then, the differentiated cells were washed with PBS and fixed in 4% paraformaldehyde (PFA; Sigma Aldrich). After fixation, the cells were stained with 2% Oil Red O (Sigma Aldrich) solution and also, were stained with 2% Alizarin Red S (Sigma Aldrich) at room temperature (RT). Intracellular lipid droplets for adipogenic differentiation, and extracellular matrix calcification for osteogenic differentiation were observed under inverted microscopy.

### Collection of Tonsil Derived Mesenchymal Stem Cells Conditioned Medium

To collect CM from TMSCs in passage four, the cells were cultured until 80-90% confluence. Then the cells were washed with PBS and with serum-free culture medium. After washing, they were incubated for 48 h in a serum-free culture medium at 37°C, and CMs were collected. The cell debris removed with centrifuged at 1500 rpm for 10 min. Afterward, they were passed through a 0.22 µm filter, aliquoted, and stored at -80°C to be used in the next experiments.[20]

### Cell Lines and Cell Cultures

Human lung carcinoma cell line A549 and pancreatic carcinoma cell line PANC-1 cells were used in this study. The cells were cultured with DMEM supplemented with 10% FBS (FBS; Gibco), and 1% antibiotic-antimycotic (Sigma Aldrich) at 37°C in 5% CO2.

### MTT Proliferation Assay on Cancer Cells

The Methylthiazolediphenyl-Tetrazolium Bromide (MTT-Sigma-Aldrich) assay was performed to determine the effect of TMSC-CM treatment on cancer cell proliferation. For that, the cancer cells were seeded on 96-well plates at a density of $10\times10^3$ cells/well. The medium was exchanged for each well with 100 µl of TMSC-CM (at a ratio of 1:1, 1:2) and incubated for 48 h. Then, 10 µL of MTT (5 µg/ml stock solution) was added to each well. After 4 h of incubation, the formazan crystals were dissolved by adding 100 µl of solvent solution (0.01 N HCl with 10% SDS; Sigma-Aldrich) and incubated at 37°C for 16 h. The absorbance values were measured at a wavelength of 570 nm in a plate reader (BioTek Epoch). The absorbance data were analyzed using Prism 7.00 (GraphPad Software, Inc) program compared to untreated control cells.[17] All experiments were performed in triplicate.

### Annexin-V/PI Apoptosis Detection Assay

Apoptotic cell death was performed with FITC-Annexin-V/ PI detection kits. $5\times10^4$ cells were seeded in 24-well plates and after a day treated with CM at a ratio of 1:1. Normal culture medium was added to the control group. After 48 h incubation, the cells were trypsinized and stained with FITC-Annexin-V and PI for apoptosis analysis (Invitrogen) according to the manufacturer's instructions. The stained cells were analyzed by flow cytometry.[17]

### Cell Cycle Assay

Cell cycle analysis was performed with flow cytometry following PI staining. After 48 h post-treatment of $5\times10^4$ A549 and PANC-1 cells with TMSC-CM, the cells were suspended in cold 75% ethanol at -20°C 1 h. After fixation, cells were washed with PBS and stained with 50 µg/ml propidium iodide (PI) and 50 µg/ml RNase A dissolved in 0.5 µmL PBS. The stained cells were analyzed by flow cytometry after incubation at 37°C for 30 min. in the dark.[17]

### Statistical Analysis

The results are expressed as mean ± SEM. "2-tailed Student's t-test" was used to determine the level of significance. If the values had $p<0.05$, the results were considered statistically significant.

## RESULTS

### Tonsil Derived Mesenchymal Stem Cells Have Stem Cell Characteristics

MSCs successfully isolated from palatine tonsil tissues exhibited typical standard fibroblastic cell morphology (**Figure 1A**). One of the characteristic features of MSCs is their multi-lineage differentiation capacity. The adipogenic and osteogenic differentiation potentials of the isolated TMSCs were evaluated by Oil red O and alizarin red S staining, demonstrating the ability to differentiate into adipocytes and osteocytes (**Figure 1A**). In addition, specific cell surface antigens of sub-cultured TMSCs up to passage three were determined by flow cytometry. More than 90% of the cells showed positive expression levels for the typical MSC antigens CD73, CD90, and CD166, while markedly low expression levels were detected for the hematopoietic stem cell markers CD34 and CD45 (**Figure 1B**). These results confirmed the mesenchymal stem cell characteristic of cells isolated from palatine tonsil tissue.[17]

### TMSC-CM Showed the Anti-proliferative Effect on A549 and PANC-1 Cancer Cells

To determine the cytotoxic effect of TMSC-CMs on cancer cell lines, we treated them with CM at two different concentrations (1:1 and 1:2). Accordingly, the cytotoxic effects of CMs applied at two different concentrations after 48 h were evaluated by MTT analysis. The cell viability was significantly reduced in cancer cells post the treatment of a 1:1 ratio compared to a 1:2 ratio. For A549 cells, the cell viability was decreased at 2.6-fold post-treatment at a ratio of 1:1 compared to the control and 1:2 ratio CM treatment ($p<0.001$). Besides, the cell viability declined approximately 4-fold in PANC-1 cells post-treatment at a ratio of 1:1 compared to control ($p<0.001$). We did not find any significant decrease in PANC-1 cell viability post-treatment of a 1:2 ratio (**Figure 2**). However, It was observed that the application at a ratio of 1:2 was statistically more effective in A549 cells. These findings demonstrate the dose-dependent growth inhibitory effect of TMSC-CM on cancer cells.

### TMSC-CM Highly Induced Apoptosis in A549 and PANC-1 Cancer Cells

After indicating the cytotoxic effect of 1:1 CM concentration on cancer cells by MTT analysis, we performed the apoptosis assay to understand the mechanism of the anti-proliferative effect of TMSC-CM on A549 and PANC-1 cancer cells at this concentration. After the treatment with CM, no significant differences were found for the post-apoptotic and necrotic cell populations in A549 cells, while pre-apoptotic cells increased approximately 2-fold post-TMSC-CM treatment ($P<0.05$) (**Figure 3A, B**).

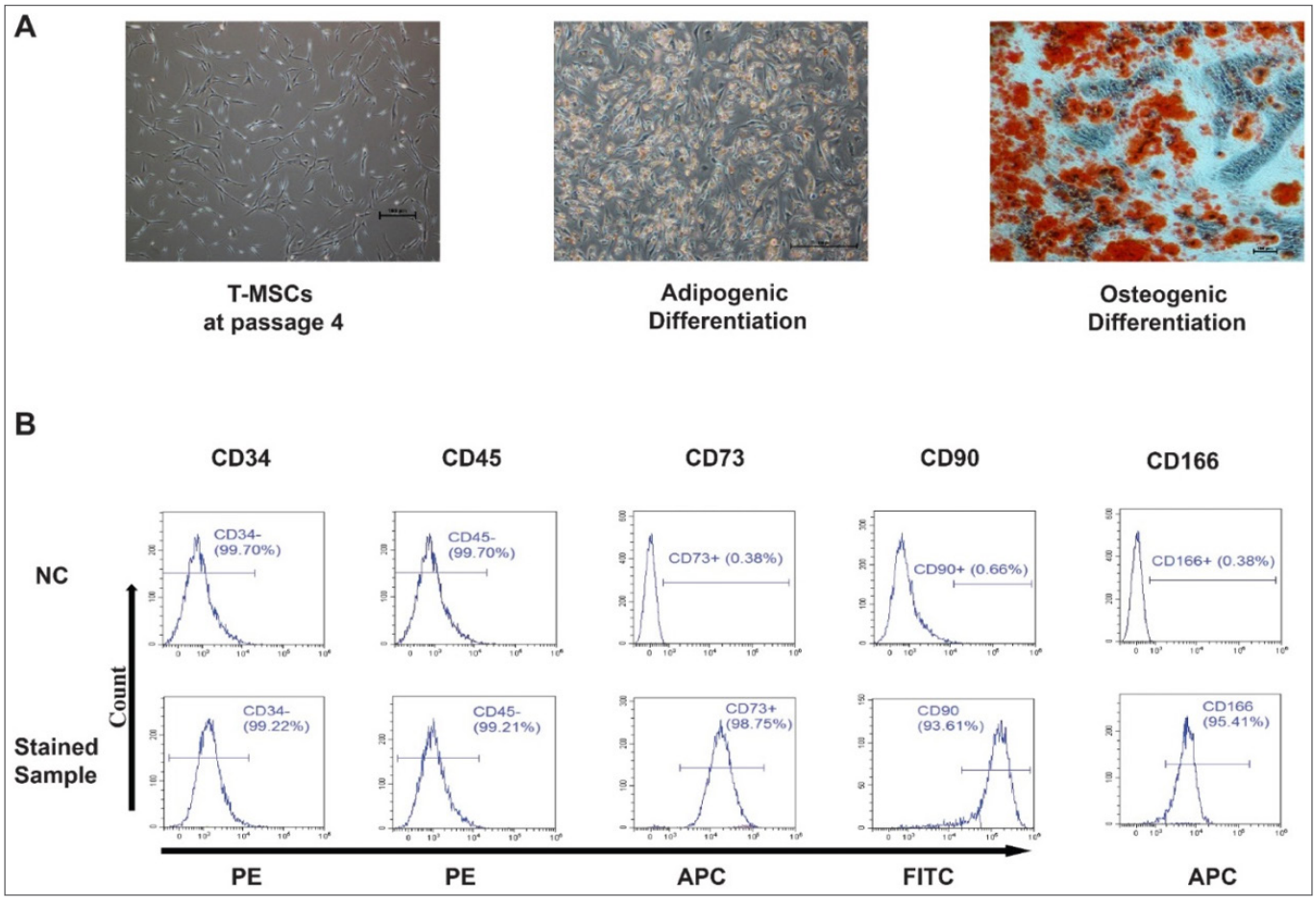


**Figure1.** Characterization of T-MSCs by determination of the multi-lineage potential and cell surface CD markers (A) the microscopic views of multi-lineage differentiation post specific staining to differentiation types and (scale bar 100 µm) (B) the percentage of T-MSCs carrying CD markers on the cell surface. PE: Phycoerythrin; APC: Allophycocyanin; FITC: Fluorescein isothiocyanate.

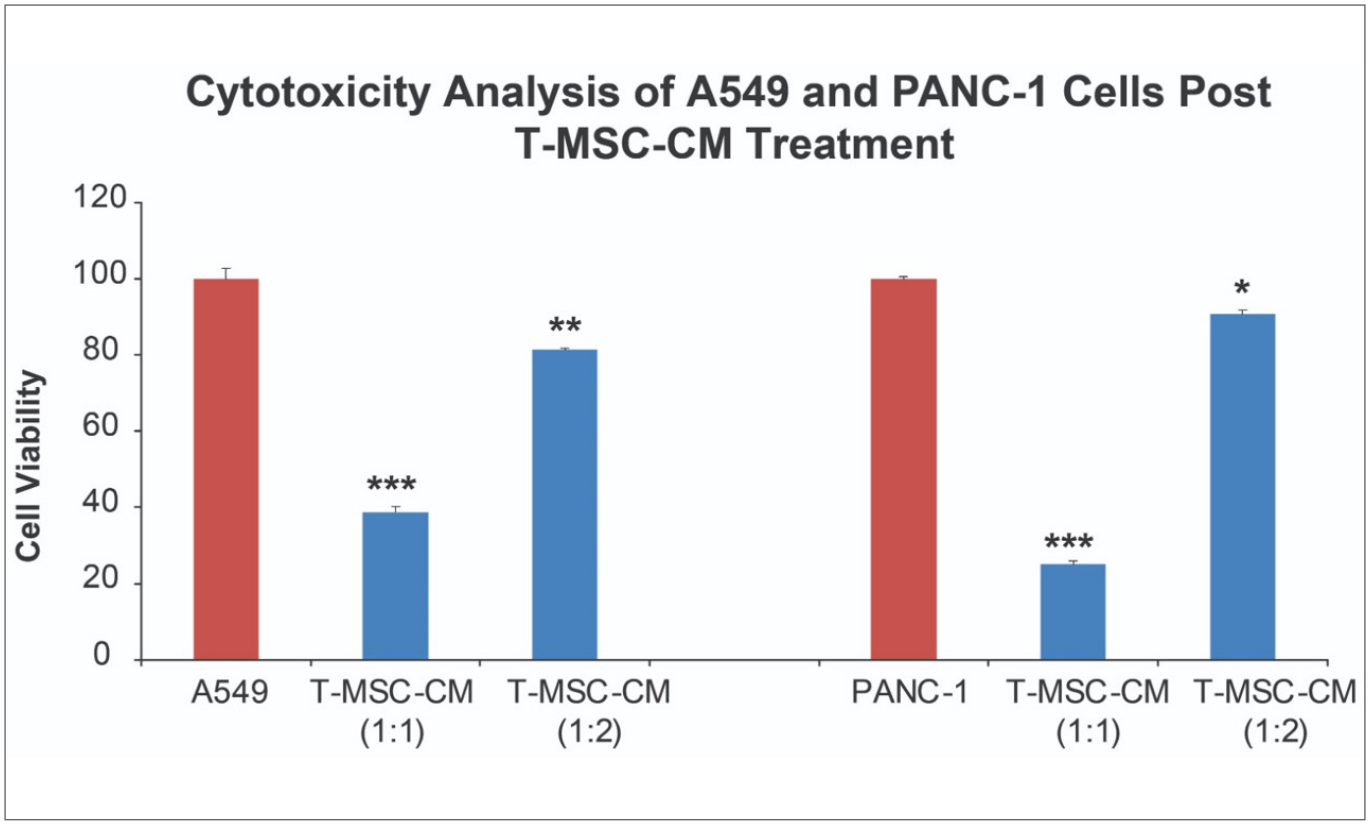


**Figure 2.** Cytotoxic effect of T-MSC-CM treatment on cancer cell lines. 48 h administration of 1:1 ratio T-MSC-CM significantly reduced cell viability in A549 and PANC-1 cell lines. n=3. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

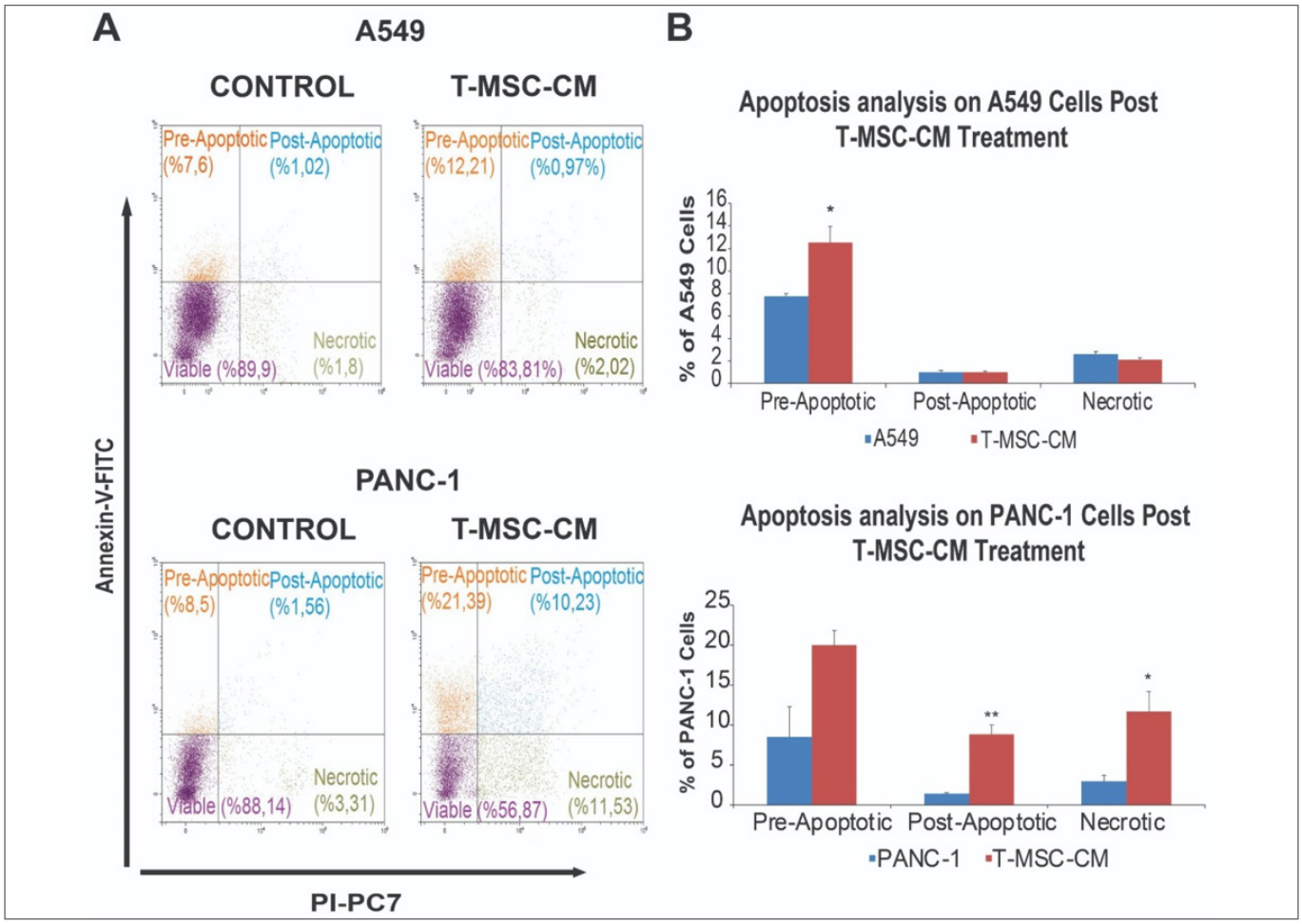


**Figure 3.** The apoptotic effect of T-MSC-CM administration on A549 and PANC-1 cells was determined by flow cytometry after Annexin-V-PI staining. The flow plots (A) and the quantification results as a graph (B) were shown. T-MSC-CM applied at a ratio of 1:1 induced apoptosis on cancer cells at different rates. n=3, * p<0.05 and ** p<0.01.

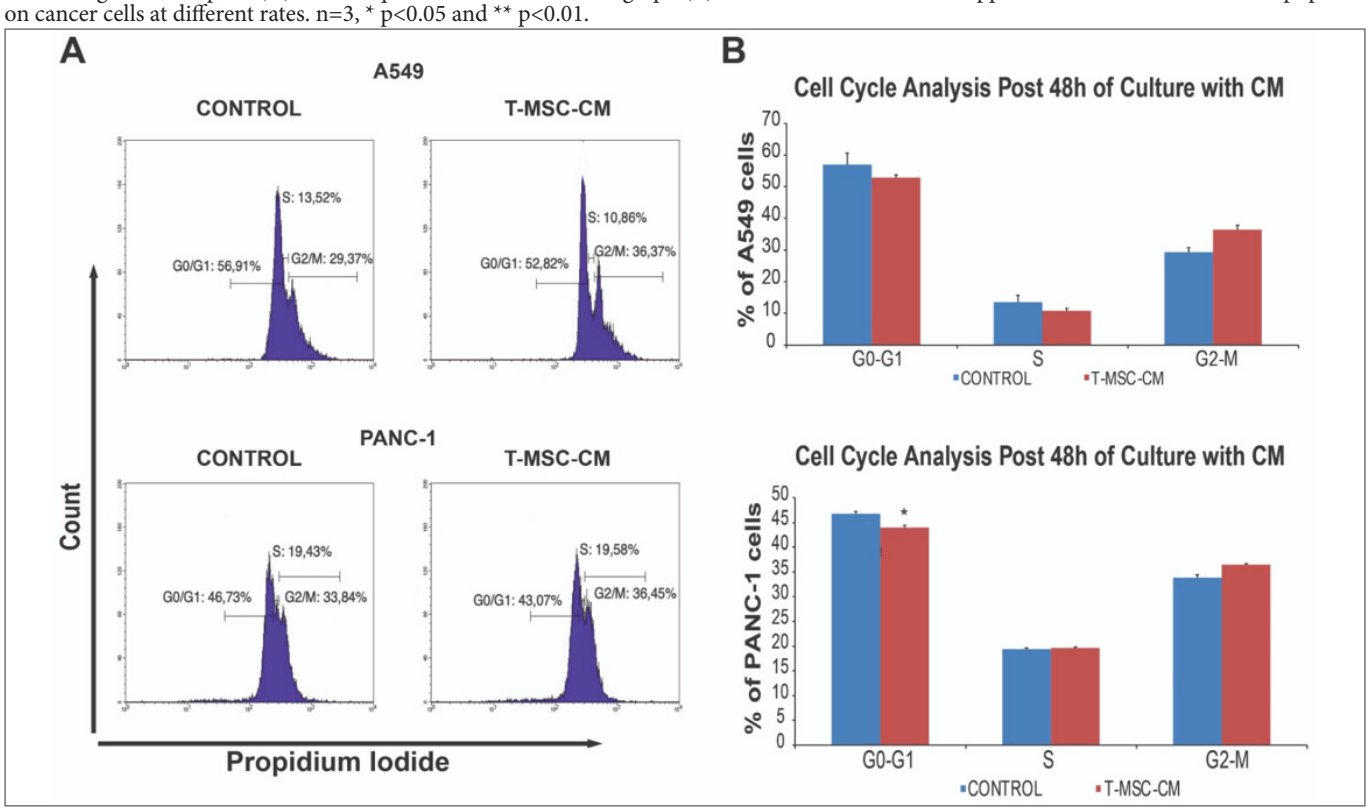


**Figure 4.** Cell cycle analysis with PI staining on A549 and PANC-1 cells post T-MSC-CM treatment. The cell cycle arrest of TMSC-CM on A549 and PANC-1 cancer cells was determined by flow cytometry. The flow plots (A) and the quantification results as a graph (B) were shown. T-MSC-CM applied at a ratio of 1:1 induced G2/M arrest of the cell cycle on cancer cells. n=3, * p<0.05.

When the apoptotic effect of TMSC-CM treatment on PANC-1 cells was examined, a significant increase (approximately 6.5-fold) in the post-apoptotic cell ratio ($P<0.01$) was observed compared to the control group. In line with this finding, a 28% reduction in PANC-1 viable cell rate ($P<0.01$) was detected after TMSC-CM treatment (**Figure 3A, B**). We also found that the treatment of TMSC-CM increased the pre-apoptotic and necrotic PANC-1 cell ratio at 2.5-fold and 3.8-fold, respectively (**Figure 3A, B**). These findings reveal that TMSC-CM induces apoptosis at different rates in A549 and PANC-1 cancer cells and is more efficient in PANC-1 cells.

### A549 and PANC-1 Cells were Arrested in the G2/M Phase of the Cell Cycle Post-TMSC-CM Treatment

Representative images of cell cycle analysis results for TMSC-CM are summarized in Figure 4. After treating A549 cells with TMSC-CM, it was determined that the cells in the G0/G1 and S phases decreased, and cell cycle arrest occurred in the G2/M phase. While 29% of the cells in the control group were in the G2/M phase, this increased to 36% of the rate cells after treatment. A similar cell cycle profile was observed in PANC-1 cells as with A549. After the treatment, a statistically significant decrease was observed in the number of cells in the G0/G1 phase. Similarly, there was G2/M cell cycle arrest, but it was not statistically significant.

## DISCUSSION

MSCs have been shown to have anti-tumor effects in vitro and in vivo cancer models.[21,22] The therapeutic potential of MSCs is mediated by direct interaction with paracrine factors released from MSCs.[23] The latest research has indicated that the anti-tumor properties of MSCs on different cancer cells are related to the various factors released by MSCs.[24] CMs from MSC cultures have been shown to have an inhibitory effect on the proliferation of hepatoma cells. According to the study results, further treatment of liver cancer cell lines with an MSC-conditioned medium resulted in decreased expression of β-catenin, Bcl-2, c-Myc, PCNA, and survivin genes.[21] In the study reported by Eiró et al., the effects of MSCs derived from the human uterine cervix (hUCMSCs) on different cell types in tumor tissue, including cancer cells, fibroblasts, and macrophages, were investigated. The researchers demonstrated that hUCMSCs stimulated apoptosis and reduced cell proliferation in human breast cancer cells. In addition, the lyophilized hUCMSCs-CMs were applied to the MDA-MB-231 cell line, which exhibits aggressive cancer properties, and as a result, a dose-dependent inhibition of cell proliferation in these cells has been demonstrated. Besides, it is known that cancer-associated fibroblasts are very effective in cancer development and aggressiveness. Therefore, the administration of hUCMSCs-CM to cancer-associated fibroblasts has resulted in induced apoptosis and reduction of cell proliferation and migration abilities.[25]

It was indicated that the anti-tumoral function of MSCs from different sources was variable as their pro- or anti-tumor potential both in vitro and in vivo due to the MSC sources. These different tumoral effects have been associated with the heterogeneity of MSCs depending on the used source. Accordingly, it is important to find alternative MSC sources, which have anti-tumor effects, for cancer therapeutics.[25] The anti-tumoral effects of MSCs and their CMs from different sources have been studied in divergent solid and hematological cancers. In the study investigating the effect of human WJ-MSC secretome on the proliferation of lung cancer cells, it was determined that WJ-MSC secretome did not have any important results on tumor cell proliferation and apoptosis.[13]

We applied TMSC-CM on A549 and PANC-1 cells to determine the tumoral effects of paracrine factors of MSCs derived from the tonsil, which is a new source of MSCs and has a high proliferation potential, in our study. In contrast to the research carried out by Hendijani et al.[13] we found that TMSC-CMs reduced cancer cell viability through the induction of apoptosis in a concentration-dependent manner. In our study, TMSC-CMs were treated at a ratio of 1:1 and 1:2 with cancer cells, and a significant reduction in cancer cell viability was found at 1:1 concentration treatments by MTT analysis. These results are similar to the results of Pan et al.[26] In addition, we found that TMSC-CM treatments induced apoptosis in A549 cells. The TMSC-CM treatment also significantly induced post-apoptotic cell ratio in treated PANC-1 cells compared to control groups. Interestingly, this induction found for PANC-1 cells is higher than the apoptotic induction found for A549 cells. Consistently with our findings, the study reported by Bagheri et al. in 2021 showed that CMs obtained from bone marrow and amniotic membrane MSCs (AMMSCs) reduced the cell viability and the proliferation of squamous carcinoma cells (SCC) at different rates depending on the source. Also, CMs induced apoptosis in SCC cell lines depending on the treatment time.[23] AMMSCs downregulated the expression of cell cycles progression-related genes such as cyclin and cyclin-dependent kinase in some solid and hematological cancer cell lines, and thus, they have shown an anti-proliferative effect through cell cycle arrest in the G0/G1 phase.[27] In our study, cancer cells were arrested at the G2/M phase, although there was no statistically significant difference after treatment with CM. In a study using the amnion as the source of MSC (AMSC), the anti-tumoral potential of AMSC-CMs was tested

on prostate cancer cell lines, and it was reported that a time-dependent decrease in cancer cell proliferation was achieved by preventing its progression through the cell cycle.[28] Limited studies are showing the growth inhibitory effect on TMSC cancer cells. The study on HNSCC cell lines has shown that TMSCs can stimulate the growth inhibitory effects on HNSCC cell lines through number-dependent increased cell cycle arrest and apoptosis.[16] On the other hand, no other study was found reporting the effect of TMSC-CMs on cancer proliferation. In our study, we showed that CMs obtained from tonsillar tissue, as a new source of MSC, induced apoptosis at different rates in A549 and PANC-1 cell lines depending on the concentration. Our findings are consistent with the previously carried out studies on TMSC and cancer cells. Besides, our study shows consistent results with the study performed by Li et al. on 2 different lung cancer cell lines. Researchers reported that BMMSC-CMs decreased the proliferation in cancer cells due to some secretable factors and induced apoptosis in vitro. In addition, in vivo, studies on BALB/c nude mice showed a lower incidence of tumors when tumor cells were injected into mice after administration with MSC-CMs compared to control groups.[14]

We suggest that tonsillar tissue is more advantageous as a source of MSC because bone marrow supply requires a more invasive and painful procedure. The donor age is an important parameter in MSC isolation, and the numbers of MSCs collected from bone marrow are significantly decreased with donor age.[29] However, the potential importance of TMSCs for obtaining more cells before clinical applications were emphasized due to higher proliferation capacity and the presence of immune modulatory properties.[30]

## CONCLUSION

Lung and pancreatic cancers are known as the major cause of cancer death worldwide. The 5-year survival rate is still low considering the standard therapies applied in the clinic. Besides, the standard treatments have very important side effects. Therefore, new therapeutical strategies, which have fewer side effects and are better tolerated, are needed. TMSC-CMs, which are easily accessible as waste tissue and show a high proliferation potential, showed anti-proliferative properties by inducing apoptosis in some solid cancers in our study. The various paracrine factors released from TMSC-CMs, such as several chemokines, cytokines, growth factors, and exosomes as nanometer-sized vesicle, play a key role in the emergence of this effect. Therefore, the relation between this therapeutical function and paracrine factors needs to be investigated in detail.

## ETHICAL DECLARATIONS

**Ethics Committee Approval:** The study was carried out with the permission of the Ondokuz Mayıs University Clinical Researches Ethics Committee (Date: 08.07.2021, Decision No: 2021/347).

**Informed Consent:** All patients signed the free and informed consent form.

**Referee Evaluation Process:** Externally peer-reviewed.

**Conflict of Interest Statement:** The authors have no conflicts of interest to declare.

**Financial Disclosure**: This study was funded by the Scientific Research Project Office Department of Ondokuz Mayıs University, Turkey.

**Author Contributions:** All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

**Acknowledgments:** We would like to thank Assoc. Dr. Fatih Kocabaş, Yeditepe University Department of Genetics and Bioengineering faculty member for generously granting the cancer cell lines.

## REFERENCES

1. Chu D-T, Nguyen TT, Tien NLB, et al. Recent progress of stem cell therapy in cancer treatment: molecular mechanisms and potential applications. *Cells*. 2020;9(3):563.
2. Raj AT, Kheur S, Bhonde R, Gupta AA, Patil S. Assessing the effect of human mesenchymal stem cell-derived conditioned media on human cancer cell lines: a systematic review. *Tissue Cell.* 2021;71:101505.
3. Bhang SH, Lee S, Shin J-Y, Lee T-J, Jang H-K, Kim B-S. Efficacious and clinically relevant conditioned medium of human adipose-derived stem cells for therapeutic angiogenesis. *Molecul Ther.* 2014;22(4):862-872.
4. Gunawardena TNA, Rahman MT, Abdullah BJJ, Abu Kasim NH. Conditioned media derived from mesenchymal stem cell cultures: The next generation for regenerative medicine. *J Tissue Eng Regen Med.* 2019;13(4):569-586.
5. Pawitan JA. Prospect of stem cell conditioned medium in regenerative medicine. *BioMed Res Int.* 2014;2014
6. Oh S-Y, Choi YM, Kim HY, et al. Application of tonsil-derived mesenchymal stem cells in tissue regeneration: concise review. *Stem Cells.* 2019;37(10):1252-1260.
7. Cho K-A, Park M, Kim Y-H, Woo S-Y, Ryu K-H. RNA sequencing reveals a transcriptomic portrait of human mesenchymal stem cells from bone marrow, adipose tissue, and palatine tonsils. *Scientific Rep.* 2017;7(1):17114.
8. Cho K-A, Lee HJ, Jeong H, et al. Tonsil-derived stem cells as a new source of adult stem cells. *World J Stem Cells*. 2019;11(8):506.
9. Gauthaman K, Yee FC, Cheyyatraivendran S, Biswas A, Choolani M, Bongso A. Human umbilical cord Wharton's jelly stem cell (hWJSC) extracts inhibit cancer cell growth in vitro. *J Cell Biochem.* 2012;113(6):2027-2039.
10. Lin DH, Biswas A, Choolani M, Fong C-Y, Bongso A. Induction of immunogenic cell death in lymphoma cells by Wharton's jelly mesenchymal stem cell conditioned medium. *Stem Cell Rev Rep.* 2017;13:801-816.

11. Onzi GR, Ledur PF, Hainzenreder LD, et al. Analysis of the safety of mesenchymal stromal cells secretome for glioblastoma treatment. *Cytotherapy.* 2016;18(7):828-837.
12. Cortes-Dericks L, Froment L, Kocher G, Schmid RA. Human lung-derived mesenchymal stem cell-conditioned medium exerts in vitro antitumor effects in malignant pleural mesothelioma cell lines. *Stem Cell Res Ther.* 2016;7:1-8.
13. Hendijani F, Javanmard SH, Rafiee L, Sadeghi-Aliabadi H. Effect of human Wharton's jelly mesenchymal stem cell secretome on proliferation, apoptosis and drug resistance of lung cancer cells. *Res Pharmaceutical Sci.* 2015;10(2):134.
14. Li L, Tian H, Chen Z, Yue W, Li S, Li W. Inhibition of lung cancer cell proliferation mediated by human mesenchymal stem cells. *Acta Biochim Biophys Sin.* 2011;43(2):143-148.
15. Mirabdollahi M, Haghjooyjavanmard S, Sadeghi-Aliabadi H. An anticancer effect of umbilical cord-derived mesenchymal stem cell secretome on the breast cancer cell line. *Cell Tissue Banking.* 2019;20:423-434.
16. Lim Y-S, Lee J-C, Lee YS, Lee B-J, Wang S-G. Growth inhibitory effect of palatine tonsil-derived mesenchymal stem cells on head and neck squamous cell carcinoma cells. *Clin Exp Otorhinolaryngol.* 2012;5(2):86-93.
17. Yüce M, Albayrak E. Hyperthermia-stimulated tonsil-mesenchymal stromal cells suppress hematological cancer cells through downregulation of IL-6. *J Cell Biochem.* 2022;123(12):1966-1979.
18. Mancini SJ, Balabanian K, Corre I, et al. Deciphering tumor niches: Lessons from solid and hematological malignancies. *Front Immunol.* 2021;12:766275.
19. Slingerland M, Guchelaar H-J, Gelderblom H. Histone deacetylase inhibitors: an overview of the clinical studies in solid tumors. *Anti-Cancer Drugs.* 2014;25(2):140-149.
20. Dhiman N, Shagaghi N, Bhave M, Sumer H, Kingshott P, Rath SN. Indirect co-culture of lung carcinoma cells with hyperthermia-treated mesenchymal stem cells influences tumor spheroid growth in a collagen-based 3-dimensional microfluidic model. *Cytotherapy.* 2021;23(1):25-36.
21. Qiao L, Xu Z, Zhao T, et al. Suppression of tumorigenesis by human mesenchymal stem cells in a hepatoma model. *Cell Res.* 2008;18(4):500-507.
22. Khakoo AY, Pati S, Anderson SA, et al. Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi's sarcoma. *J Exp Med.* 2006;203(5):1235-1247.
23. Bagheri R, Bitazar R, Talebi S, Alaeddini M, Etemad-Moghadam S, Eini L. Conditioned media derived from mesenchymal stem cells induces apoptosis and decreases cell viability and proliferation in squamous carcinoma cell lines. *Gene.* 2021;782:145542.
24. Shah K. Mesenchymal stem cells engineered for cancer therapy. *Adv Drug Delivery Rev.* 2012;64(8):739-748.
25. Eiró N, Sendon-Lago J, Seoane S, 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.
26. Pan M, Hou L, Zhang J, et al. Inhibitory effect and molecular mechanism of mesenchymal stem cells on NSCLC cells. *Molecul Cell Biochem.* 2018;441:63-76.
27. Magatti M, De Munari S, Vertua E, Parolini O. Amniotic membrane-derived cells inhibit proliferation of cancer cell lines by inducing cell cycle arrest. *J Cell Molecul Med.* 2012;16(9):2208-2218.
28. Rolfo A, Giuffrida D, Giuffrida MC, Todros T, Calogero AE. New perspectives for prostate cancer treatment: in vitro inhibition of LNCaP and PC3 cell proliferation by amnion-derived mesenchymal stromal cells conditioned media. *Aging Male.* 2014;17(2):94-101.
29. Cho K, Lee H, Jeong H, Kim M, Jung S, Park H. Tonsil-derived stem cells as a new source of adult stem cells. *World J Stem Cells.* 2019;11(8):506-518.
30. Ryu K-H, Cho K-A, Park HS, et al. Tonsil-derived mesenchymal stromal cells: evaluation of biologic, immunologic and genetic factors for successful banking. *Cytotherapy.* 2012;14(10):1193-1202.