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Tonsil mesenchymal stem cells-derived exosomes differentially effect the cell proliferation depending on different temperature conditions

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ABSTRACT

Objectives: Exosomes are suggested as cellular components with therapeutic and prognostic potential in various disease therapies and exhibit different secretion profiles under cellular stress. Mesenchymal stem cells (MSCs), which play an important role in regenerative medicine, are particularly rich in exosome release compared to other cell types. In this context, the effect of exosomes obtained from palatine tonsil tissue derived MSCs (T-MSC) under different temperature conditions on cell proliferation were investigated *in vitro* on cancer and healthy cells.

Methods: Exosomes were isolated from MSCs under normal temperature conditions and heat stress, and their concentrations and size distribution were determined by nanoparticle tracking analysis. The effects of exosomes obtained under different conditions on cell proliferation in healthy and cancer cell lines were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, while their effects on cell migration were evaluated by wound healing assay.

Results: Exosomes obtained under normal temperature conditions and heat stress did not exhibit very different properties in terms of size distribution, but particles were obtained at approximately 2 times higher concentrations under normal temperature conditions. They showed different effects in terms of cell proliferation. Exosomes obtained under normal conditions significantly increased cell proliferation in keratinocyte cells depending on the concentration. However, exosomes obtained in cancer cells, especially after heat stress, significantly inhibited cell proliferation.

Conclusions: While exosomes obtained under heat stress come to the forefront in inhibiting cell proliferation in cancer cells, exosomes obtained under normal conditions stand out as effective in wound healing by stimulating increased normal cell proliferation.

Keywords: Tonsil derived mesenchymal stem cell, heat stress, exosomes, cell proliferation

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Exosomes, defined as small membrane microvesicles of endosomal origin, have prospered in the latest years as potential therapeutic tools and prognostic biomarkers. Exosomes hold many roles in cellular executions such as intercellular communication via transportation of proteins, lipids, miRNA, mRNA, and DNA; tissue regeneration; immunity responses; signal transduction; antigen presentation. It has been shown with these properties that exosomes hold prognostic and therapeutic potentials regarding chronic inflammation, cardiovascular and renal diseases, neurodegenerative diseases, cancer etc. [1-4]. Exosomes, rich in annexins, tetraspanins (CD63, CD81, CD9) and heat shock proteins (Hsp60, Hsp70), contain low amounts of phosphatidylserine and cell type-specific proteins [5] and exhibit different secretion profile under cellular stress [6]. Oxidative stress alters the RNA contents of exosomes secreted from mast cells of a mouse. The subjection of hypoxia and TNF-a to endothelium cells has caused secreted exosomes to have modulated contents of both mRNA and protein, although high concentrations of glucose or mannose subjected to endothelium cells have no effect on the exosomal profiles of protein or mRNA. These results show the exosomal communication occurs via both protein transfer and RNA. Exosomes are also held responsible for the secretion of heat shock proteins (HSP's) in various cell types including tumor cells, B lymphocytes, astrocytes and endothelium cells. HSP's are found on the surface of exosomes and they hold an important role in the modulation of immunologic responses. Heat shock protein 70 (HSP70) concentration in exosomes derived from peripheral blood mononuclear cells (PBMC) is significantly high [5, 7, 8]. HSP expression in normal cellular conditions increases greatly under the additional conditions of heat shock, toxin exposure, oxidative stress, glucose deficiency, and various other cellular stress factors [9-11].

Mesenchymal stem cells (MSCs) are multipotent cells that hold a crucial role in tissue healing and regenerative medicine due to their ability to differentiate into various cell types and self-renewal. In addition to bone marrow originated MSCs, they can be commercially obtained from various adult and fetal tissues such as fat, muscle, umbilical cord blood, peripheral blood, liver, placenta, skin, amniotic fluid, breast milk, synovial membranes, and dental pulp [12, 13]. MSCs have a significant place in regenerative medicine due to their secretive ability regarding signaling molecules that support tissue regeneration and their low immunogenicity. The therapeutic potentials of MSCs are associated with the paracrine factors they release. They are known to be richer in exosome secretions compared to other cell types. The therapeutic potential of stem cell-derived exosomes has been shown for various disease therapies, can repairing damaged tissues [14]. Exosomes purified from MCSs have been reported to reduce infarct size and mediate cardioprotective paracrine effects in a mouse model of myocardial ischemia/reperfusion injury [15]. It has also been shown that MSC exosomes have positive effects on acute kidney injury [16], induce the proliferation of human and rat hepatocytes *in vitro* [17], and can protect heart tissue from ischemic damage [18].

Although MSC derived exosomes are morphologically and marker expressionally same as other exosomes, they differ with their contents of RNA and proteins. According to various studies, different exosomes of different cellular origins have varying effects of function and generally, MSC exosomes have the potential to alter the capacity of MSC's differentiation and regeneration [1, 19].

It is known that stress conditions and heat stress affect exosome behavior and cause changes in the content of released exosomes. MSC-exosomes obtained from different sources also exhibit different regenerative potential. In this context, the effect of exosomes obtained from palatine tonsil tissue derived MSCs (T-MSC) under normal conditions and heat stress on cell proliferation was investigated *in vitro* on cancer and healthy cells.

METHODS

The tissues used in the study were obtained from patients (age, <15 years; patient signed a consent form) who underwent tonsillectomy at the Samsun Health Sciences University, Training and Research Hospital, the Department of Ear, Nose and Throat Diseases, after being approved by the Ondokuz Mayıs University, Clinical Research Ethics Committee (Ethics committee number: OMÜ KAEK 2022/282; 2023/120).

Enzymatic Isolation of Tonsil Derived Mesenchymal Stem Cells

T-MSCs were obtained by enzymatic isolation with 0.075% collagenase type 1 at 37°C for 30 minutes. Cells were incubated in a humidified incubator at 37°C with 5% CO_2 in high-glucose Dulbecco's Modified Eagle Medium (H-DMEM) supplemented with 10% Fetal Bovine Serum (FBS) as well as 1% antibiotic/antimycotic. After the initial incubation, cells that were unable to adhere to the surface of the culture dish were washed away with PBS, and the adherent cells were cultured under the same conditions as above. When the cell density reached 80-90%, the cells were subcultured by trypsinization with 0.25% trypsin containing 0.02% EDTA. The characterization of T-MSCs was performed at the third passage. T-MSCs at the fifth passage were used for exosome isolation. Approximately 120-150 mL of MSC-conditioned medium was collected for the experiments [20, 21].

Determining of Tonsil Derived Mesenchymal Stem Cells Surface Antigens by Flow Cytometer Analysis

The characterization of third passage T-MSCs by their specific surface antigens have been done with flow cytometer analysis (BD FACSCalibur Cell Analyzer). Following trypsinization, 1×10^5 cells were suspended in 100 µL PBS supplemented with 3% FBS and stained with CD90, CD105, CD73, CD34, and C45 monoclonal antibodies and 4°C for 30 minutes. The cells were then washed with PBS, resuspended in 200 µL of PBS, and analyzed using a flow cytometer [22, 23].

Determination of Tonsil Derived Mesenchymal Stem Cells *in vitro* Adipogenic and Osteogenic Differentiation Potentials

Cells on the third passage were used for determining adipogenic and osteogenic differentiation potentials of T-MSCs. For adipogenic differentiation, a specific media was cultured by adding 10% FBS, 1 µL dexamethasone, 100 µg/mL 3-isobutyl-1 methyxanthine, 5 µg/mL insulin, and 60 µM indomethacin to a basal DMEM growth medium for 3 weeks. Osteogenic differentiation was made possible by the preparation of an osteogenic media made from a basal DMEM growth medium supplemented with 10% FBS 0.1 mM dexamethasone, 10 µM ß-glycerophosphate, and 50 µg/mL ascorbic acid for the duration of three weeks. Culture mediums were changed once every three days. At the end of the third week, T-MSC's were anchored in 4% paraformaldehyde at room temperature for 30 minutes. Following that, T-MSCs were washed with PBS twice; for adipogenic differentiation they were stained with 2% Oil Red O solution in room temperature for 1 hour, and for osteogenic differentiation they were stained with 2% Alizarin Red S solution in room temperature for 15 minutes. The cells were observed under an inverted microscope again after the staining procedure [24, 25].

Obtaining Conditioned Media from Tonsil-Derived Mesenchymal Stem Cells under Normal and Heat Stress Conditions for Exosome Isolation

Media were acquired under normal and heat stress conditions from the cells at their 5th passage. Under normal temperature circumstances, the cells were incubated in a 5% CO_2 environment at 37 oC for 48 hours in order to obtain growth media. After the incubation, the conditioned media was collected and then centrifuged at 1500 rpm for 10 minutes to remove cell debris, followed by filtration through a 0.22 µm sized filter. The cells were washed once with PBS and once with serum-free culture media after the cell concentration reached 80-90% confluence for the obtainment of conditioned media from the heat-stressed T-MSCs. In pursuit of this process, serum-free culture media was added and the cells were then incubated at 43°C for 1 hour in a 5% CO_2 environment for 48 hours. Cell debris was removed after the process of this incubation via centrifugation, and the media was filtered through a 0.22 µm filter. The conditioned media were stored at -80°C until the exosome isolation could proceed.

Isolation of Exosomes of Normal and Heat-Stress Conditions by Sucrose Cushion Method from Conditioned Media

For the isolation of exosomes from T-MSCs media conditioned under normal heat conditions and 43°C temperature have been filtered and centrifuged at 12000 ×g in 4°C for 10 minutes. The media was then put through a 0.22 µm sized filter after collecting the supernatant. The exosomes were released from inside the cells into the media. To purify the mentioned exosomes, sucrose cushion method was used. The collected media was centrifuged at 1000 ×g for 5 minutes

to remove the cell debris. Supernatant was removed again, and the remaining solution was centrifuged at 18000×g for 30 minutes to repel the microvesicle pollution. The 10mL of supernatant was then added into a 1M 1.5 mL sucrose solution with a pasteur pipette to create a phase. The prepared samples were centrifuged at 100000 ×g at 4°C for 1 hour with ultracenrifuge (Beckman CoulterOptima XE100, SW-41 Rotor, USA). After the centrifugation the 1mL of exosome-bearing sucrose solution was pipetted out from the bottom of the test tubes. In order to expel the sucrose molecules from the remaining solution, 100 kDa ultrafiltration colons were used. 13 mL of deionized water was added to a 1 mL of exosome solution for dilution and re-concentration purposes. At each washing process, colons were opened and centrifuged in 3500×g for 10 minutes in rotored centrifuges for re-concentration purposes. After the third process of washing, 200 µL of pure exosome solution was acquired and then kept at -80°C to be used in the later analyses [26, 27].

Nanoparticle Tracking Analysis of Exosomes

Exosomes obtained from T-MSCs cells under normal and heat stress conditions were quantified by nanoparticle tracking analysis. Accordingly, a wavelength of 488 nm laser module from the device Nanosight NS300 was used to minimize the effects of several contaminants such as protein aggregates. Quantification of exosomes was done on 10 video clips each 30 seconds long. Depending on these analyses, both the concentrations and the sizes of exosomes obtained from media were identified [26].

Cell Culture

The cells were cultured with HG-DMEM supplemented with 10% FBS, 2 mM L-glutamine, 1% antibiotic/antimycotic. The cell cultures were incubated at 37°C in a 5% CO_2 atmosphere. After the density of the cells reached 80-90%, they were trypsinized with 0.25% trypsin containing 0.02% EDTA and subcultured for use in subsequent experiments.

Determination of Proliferation of Exosomes of Different Conditions on MIA PaCa-2 and HaCaT Cells via MTT Analysis

After the trypsinization process, 100 µL of growth media that contained 1×10^4 of cell concentration were added to each well of a 96 well culture plate. After 24 hours of incubation, after the cells adhered to the surface, exosomes obtained under different conditions at concentrations of 10^6 particles/mL, 5×10^6 particles/mL, 5×10^7 particles/ml were applied for 24 and 48 hours. The cells were treated with their own culture media as negative control and culture media containing PBS in which the exosomes were suspended was used as vehicle control. At the end of the incubation period, 10 µl MTT solution was added and incubated for 4 hours. At the end of incubation, 100 µL of solvent solution containing 10% SDS in 0.01 M HCl was added to each well and optical density was measured 16 hours later using a microplate reader at 570 nm. Experiments were performed three times for each group. Each concentration was compared against its vehicle control [28].

Determination of the Effects of Exosomes Obtained Under Different Conditions on Cell Migration Using *in vitro* Wound Healing Assay

The wound healing assay is one of the oldest methods developed to study cell migration *in vitro*. It is particularly suitable for studies on the effects of cell matrix and cell-cell interactions on cell migration. This method mimics cell migration. Accordingly, MiaPaCa and HaCaT cells were seeded into a 6 well culture plate at a density of 10×10^5 cells per well, and once they reached 100% confluence, two straight lines were drawn across the well using a 100 µL pipette tip, from 12 o'clock to 6 o'clock and from 9 o'clock to 3 o'clock. Subsequently, the wells were washed several times with PBS to remove cell debris. MSC exosomes were applied at the optimum concentration determined by the MTT assay. After particle application, the cells were incubated for 24 hours in H-DMEM containing 10% FBS. Images of cell migration into the scratch area were captured at 0, 24, and 48-hour intervals using an inverted microscope for each sample. The obtained images were analyzed using ImageJ [29, 30].

Statistical Analysis

The statistical analysis was performed using GraphPad Prism software. The experiments were performed in triplicate and analysis of variance was used to analyse the variance among groups. “2-tailed Student's t-test” was used to determine the significance level. The results were considered statistically significant if the values were $P<0.05$.

RESULTS

Stem Cell Properties of Tonsil Tissue Derived Mesenchymal Stem Cells

Stem cells which were attained from enzymatic isolation from tonsil tissue showed morphologically adherent and fibroblastic properties (Fig. 1a). In order to investigate the multipotent differentiation lineages of T-MSCs, adipogenic and osteogenic differentiation potentials were studied. The formation of lipid droplets and calcium aggregates in T-MSCs was determined by Oil Red O and Alizarin Red S staining after a 3-week induction period. As observed in Figs. 1b and 1c, the cells exhibited a positive staining profile characteristic of MSCs. To evaluate the cell surface antigen phenotypes of T-MSCs, an additional flow cytometry analysis was performed, and it was observed that more than 95% of the cells were positive for the expected MSC markers CD90 and CD73. For the hematopoietic cell surface antigens CD34 and CD45, less than 1% expression was detected (Fig. 1d).

Nanoparticle Tracking Analysis of Exosomes Obtained from T-MSCs under Normal and Heat Stress

Nanoparticle tracking analysis (NTA) was performed to determine the size distribution and average

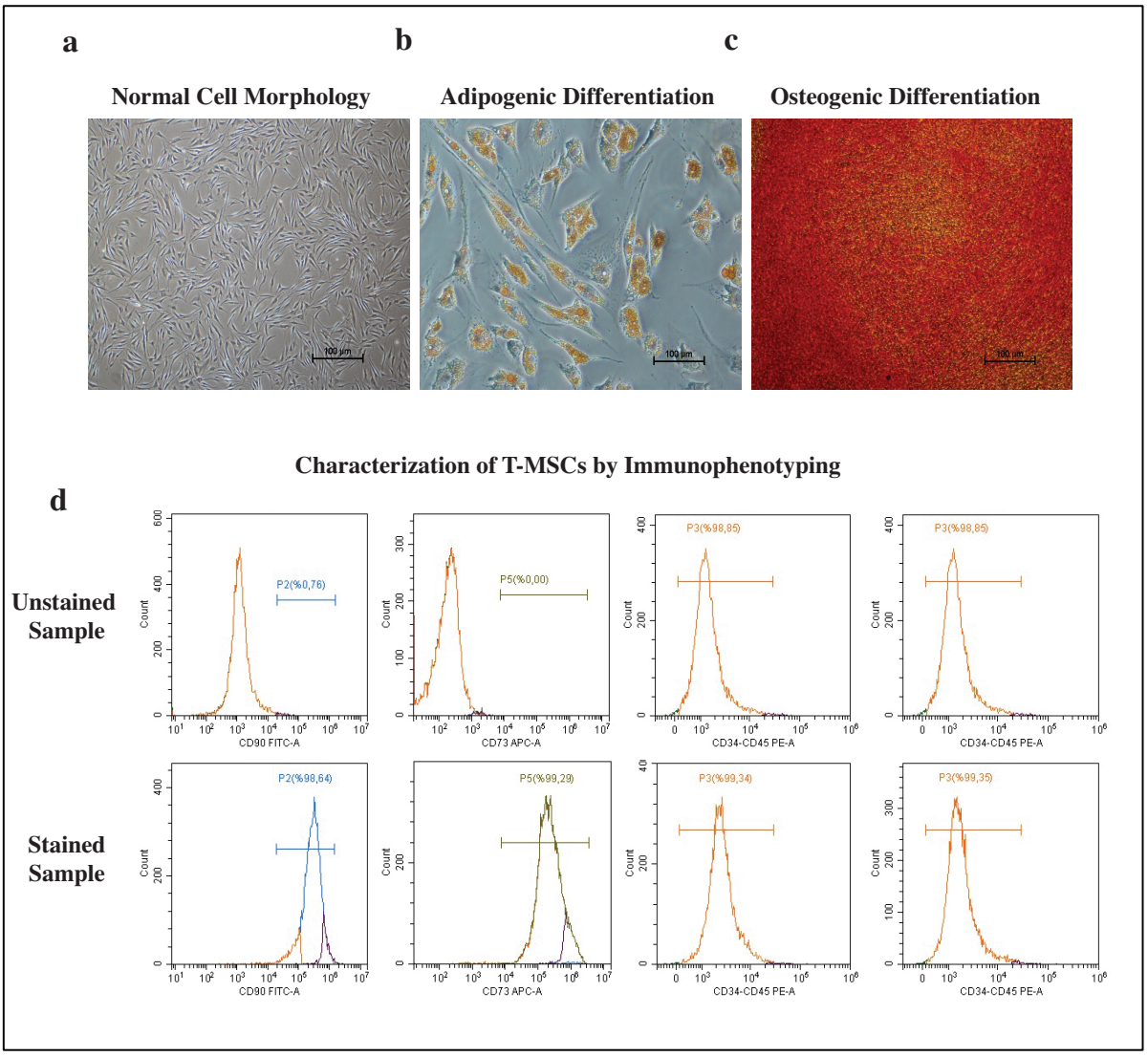

Fig. 1. Characteristic properties of tonsil-derived MSCs. (a) morphology, (b) microscopic images of lipid droplets of adipogenic differentiation potential after staining with Oil Red O, (c) microscopic images of lipid droplets of osteogenic differention potential after staining with Alizarin Red S, and (d) expression of cell surface antigens. PE=Phycoerythrin, APC=Allophycocyanin, FITC=Fluorescein isothiocyanate. Scale bar 100 µm.

size of exosomes obtained from T-MSCs under normal and heat stress. As a result of these analyses, the concentrations and size distributions of exosomes obtained from the medium were determined. Accordingly, the particle concentration of T-MSC exosomes isolated under normal temperature conditions was calculated as approximately 4.28×10^9. This concentration was approximately 2-fold higher compared to exosomes obtained after heat stress. As observed in Fig. 2a, two distinct peaks were observed in the graph, indicating that it contained highly concentrated groups of particles with two different sizes in general, but mostly around 72 nm in size. The peak with the highest concentration has a range around the ~72 nm region. The particle concentration of T-MSC exosomes isolated under heat stress was calculated as $1.98\text{x}10^9$. As observed in Fig. 2b, several peaks were observed in the graph, suggesting that it contains concentrated groups of particles that share more similar particle sizes to each other. The peak with the highest concentration has a range around the ~76 nm region. Afterwards, concentrated exosome groups with sizes of 120 and 162 nm are observed.

Exosomes Obtained under Different Temperature Conditions Induce Different Proliferative Effects in Healthy and Cancer Cells

The effects of T-MSC exosomes obtained under normal conditions and heat stress on cell proliferation were investigated on cancer and healthy cells. The effects of exosomes on cell proliferation on pancreatic cancer cell lines MiaPaCa-2 and immortal keratinocyte cell lines obtained from adult human skin, HaCaT cells, were determined by MTT analysis for

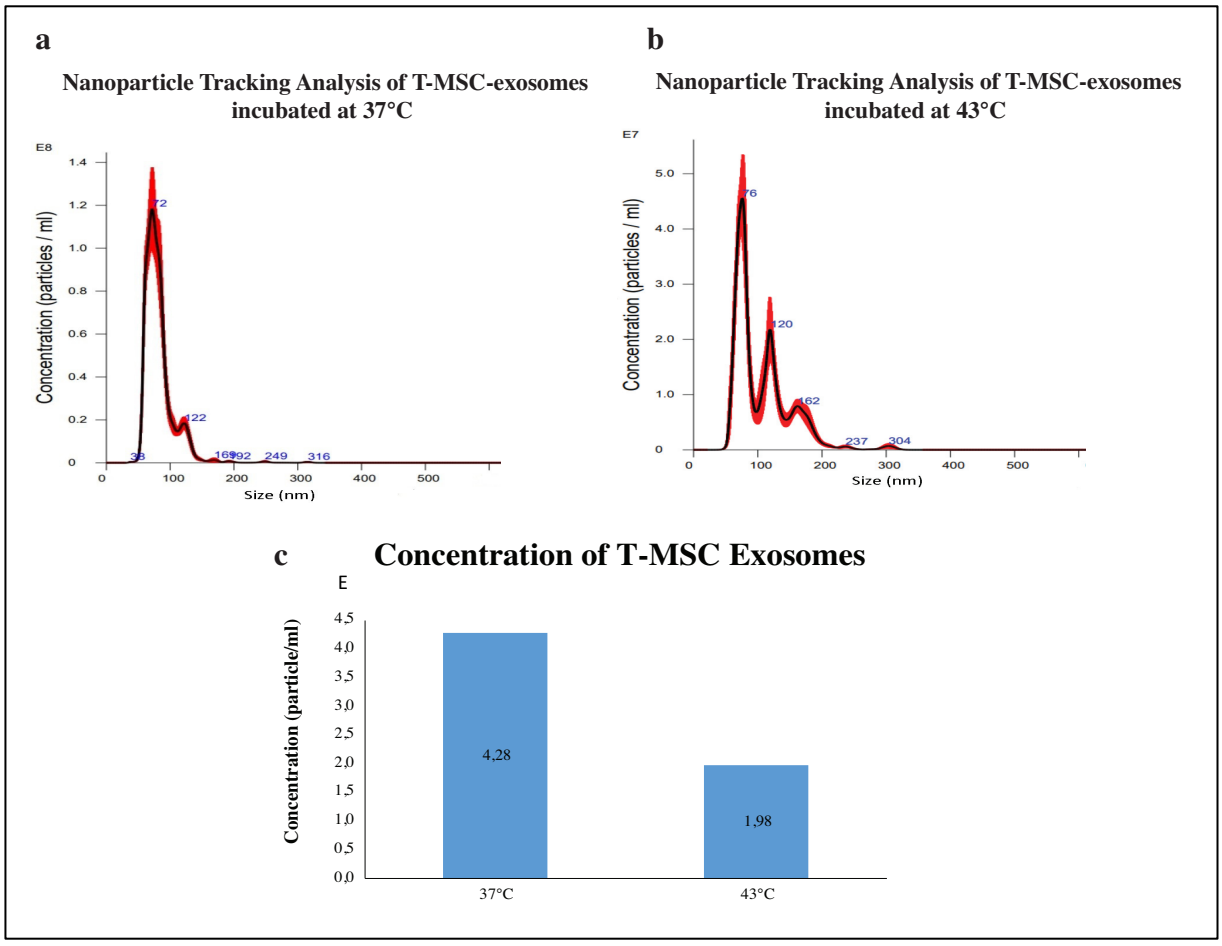

Fig. 2. Nanoparticle tracking analysis profile showing the size distribution of MSC exosomes isolated from tonsil tissue. (a) Nanoparticle tracking analysis graph of exosomes obtained under 37°C temperature, (b) Nanoparticle tracking analysis graph of exosomes obtained under 43°C temperature, and (c) The concentration of T-MSC exosomes obtained under different temperature conditions were also presented.

24 and 48 hours. It was determined that exosomes obtained under different conditions exhibited different proliferative effects on cells. The exosomes obtained at a concentration of 5×10^7 particles/ml under 37°C from the exosomes obtained under two different conditions at concentrations of 106, 5×106 and 5×10^7 particles/ml were found to induce significant cell proliferation in HaCaT cells at the end of 24 and 48 hours of application (27% and 30%, respectively) (***P< 0.001). However, exosomes obtained at the same concentration applied at 43°C stimulated 21% cell proliferation after 48 hours of incubation, but this induction was lower than exosomes obtained at normal temperature conditions (**P<0.01) (Fig. 3a).

The proliferative/non-proliferative effects of exosomes applied at the determined concentrations on MiaPaCa cancer cells showed a different profile than HaCaT cells. It was observed that exosomes obtained under heat stress (43°C) on cancer cells inhibited cell viability by 10% (**P<0.01) (Fig. 3b), but exosomes which were obtained at 37°C and applied at a concentration of 5×10^6 particles/ml for 48 hours caused a decrease in the proliferation of cancer cells by approximately 7% (*P<0.05).

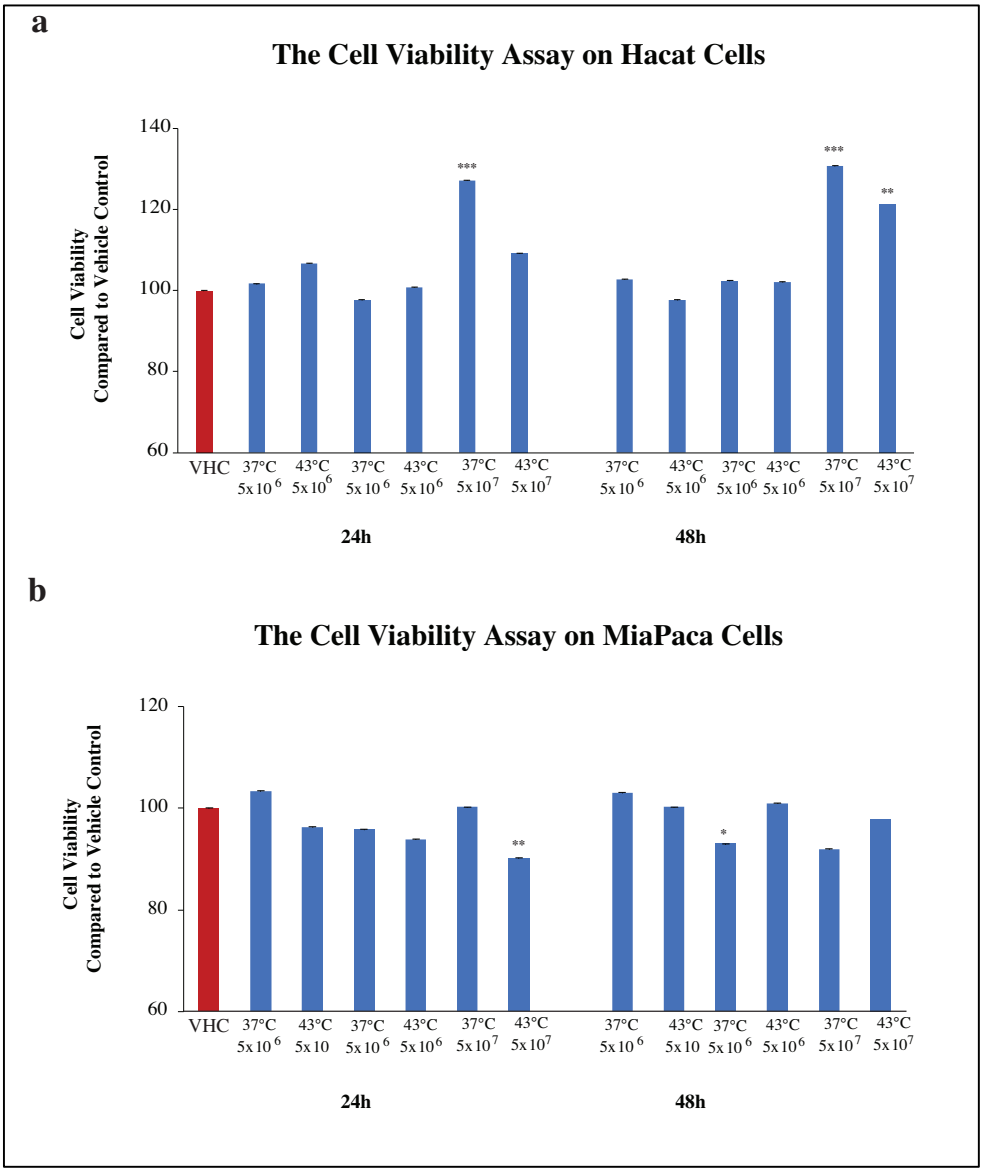

Fig. 3. Effects of T-MSC exosomes obtained under normal conditions and heat stress on healthy (a) and cancer (b) cell proliferation. Each concentration was compared with its own vehicle control. n=3, *P<0.05, **P<0.01, and ***P<0.001.

Exosomes Obtained under Different Conditions Show Different Effects on Cell Migration

After determining the effective concentrations of exosomes obtained under normal and heat stress conditions by MTT assay (5×10^7 particles/mL obtained at 37°C for HaCaT cells and 5×10^7 particles/mL obtained at 43°C for MiaPaCa cells), their effects on cell migration of HaCaT and MiaPaCa cells were determined by wound healing assay. The scratch closure percentages of the groups 24 and 48 hours after treatment were analyzed using ImageJ software. Consistent with the MTT assay, 100% closure was observed in exosomes obtained under normal temperature conditions for HaCaT cells 24 h after treatment (***P<0.001) (Fig. 4a). In cancer cells, exosomes applied at the same concentration obtained under heat stress caused approximately 80% of the scratch closure after 24 hours and 97% at the end of 48 hours (***P<0.001) (Fig. 4b).

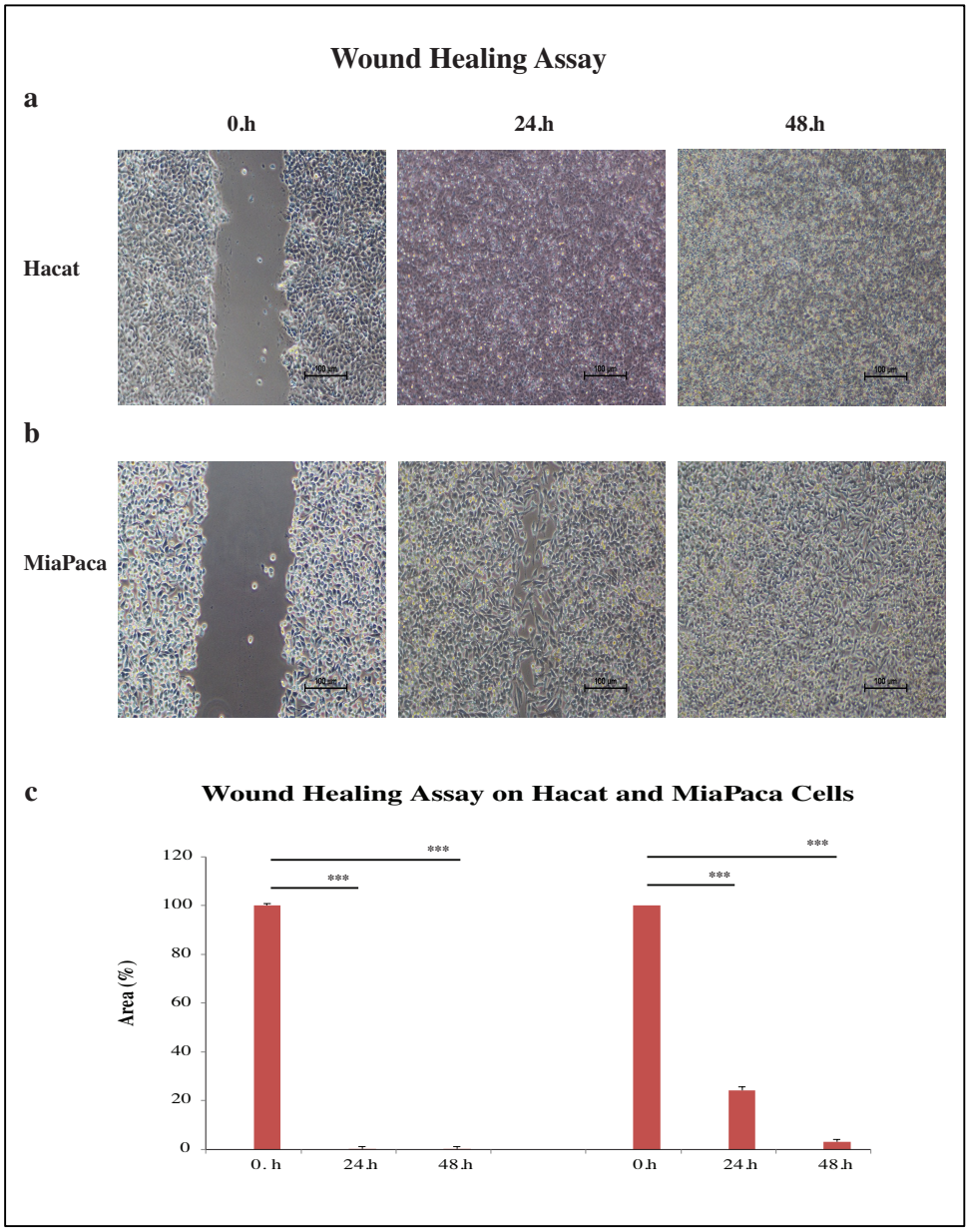

Fig. 4. Effects of T-MSC exosomes obtained under normal conditions on wound healing. (a) Scratch closure micrographs of HaCaT cells at 0, 24 and 48 hours after exosome treatment, (b) Scratch closure micrographs of MiaPaCa cells at 0, 24 and 48 hours after exosome treatment, and (c) Scratch closure rates as %. Scale bar 100 µm. n=3, *P<0.05, **P<0.01, and ***P<0.001.

DISCUSSION

The increasing data of the recent years have highlighted the therapeutic potential of exosomes, which essentially act as mediators of cellular communication [31, 32]. Exosomes derived from stem cells have been reported to have a wide range of therapeutic effects, from healing liver damage [33, 34], to inhibiting cancer cells [35]. The mechanisms by which mesenchymal stem cell exosomes affect wound healing have been explored so far, particularly through the activation of various signaling pathways and the expression of growth factors [29]. The studies have also shown that exosomes can inhibit cancer cells in various types of cancer, depending on different factors [36, 37]. Conversely, some studies suggest that exosomes from different sources can induce the progression of cancerous cells and promote metastasis [38, 39].

It is known that mesenchymal stem cells showcase different secretion profiles depending on the source of the cells which exosomes are derived from [19] and the different stress conditions that they are exposed to [6]. Additionally, the effects of exosomes on cell proliferation and migration can vary depending on the exosomes' source, target cell population, various cellular processes and concentrations [40]. In this context, our study aimed to research the effects of exosomes obtained under different heat conditions on cell proliferation and migration in healthy cells and cancerous cells, and to determine the impact of heat stress on exosome behavior compared to exosomes acquired under normal circumstances. According to our results, exosomes released from tonsil-derived MSCs under normal temperature conditions significantly induced cell proliferation in healthy keratinocyte cells in a concentration-dependent manner. These findings are generally consistent with the literature's information. For instance, bone marrow-derived MSC exosomes were reported to stimulate the proliferation of keratinocyte cell lines and dermal fibroblasts by 54-80% in a concentration-dependent manner after a 3-day application period, with a more pronounced effect in keratinocyte cells [41]. In our study, the cell proliferation was increased by 27-30% in keratinocyte cells. The percentage difference might be related to the duration of application and the different stem cell sources from which the exosomes were obtained. Another study investigating the effects of MSC-derived exosomes from different sources on dermal fibroblast and keratinocyte cell proliferation also reported that exosomes stimulated fibroblast proliferation depending on the MSC source and applied concentration [40]. Interestingly, in our study, exosomes obtained under normal temperature circumstances did not significantly alter the cell proliferation in cancerous cells. The studies have reported both stimulatory and inhibitory effects of exosomes on cancerous cells [36, 38]. In our study, although exosomes at a concentration of 5×10^6 particles/mL had shown some inhibitory profile after 24 hours, it was not statistically significant; but showed a statistically significant inhibition after 48 hours. Higher concentrations of exosomes also inhibited cancer cell proliferation after 48 hours, but this decrease was not statistically significant.

The main focus of our study was to determine the effect of heat stress on exosome behavior. Exosomes obtained after exposure to heat stress did not significantly induce cell proliferation in HaCaT keratinocyte cells compared to exosomes obtained under normal circumstances. But at the highest dose applied, there was a statistically significant increase in cell proliferation after 48 hours of treatment. Importantly, in pancreatic cancer cells (MiaPaCa), exosomes obtained after heat stress inhibited cell proliferation compared to exosomes not subjected to heat stress. Particularly at a concentration of 5×10^7 particles/mL, there was a statistically significant decrease in cell viability after 24 hours of application. The rate of decrease in cell viability slowed as the application duration increased. There are limited studies on the behavior of exosomes derived under heat stress. It has been shown that exosomes released from cancer cells after heat stress inhibit tumor growth by inducing the formation of T helper type 17 (Th17) cells through Heat Shock Protein 70 (HSP70) [42]. Heat shock proteins are known to be crucial in maintaining cellular homeostasis and protecting cells against heat stress. Although hyperthermia does not change the amount of exosomes released from peripheral blood mononuclear cells, it significantly increases the HSP70 content of the exosomes [8]. HSPs are excessively expressed in damaged tissues following injury under the healing process, and delays in their expression can lead to delayed healing in various clinical conditions [8, 43]. HSPs also exhibit anti-apoptotic properties and play a role in protecting tumor cells and maintaining their

survival during malignant progression [44]. In our study, we did not investigate the content of exosomes after heat stress. However, the concentration and size distribution of exosomes were determined using nanoparticle tracking analysis (NTA). Under normal temperature circumstances, approximately 4.28×10^9 particles per mL of exosomes were calculated. The exosomes with the highest concentration had a size range of approximately ~72 nm, followed by the presence of exosomes with a size range of ~122 nm at a much lower concentration. After the application of heat stress, the exosomes with the highest concentration had a size range of approximately ~76 nm, followed by exosomes with size ranges of ~120 and ~162 nm. Approximately 1.98×10^9 particles per mL of exosomes were obtained. The studies have shown that the condition of hyperthermia suppresses the proliferation of cancer cells and also supports exosome secretion [45]. Unlike our study, it was shown that exosomes obtained after hyperthermia in cancer cells were in higher concentration compared to normal heat application [45]. The reason for this difference is thought to be related to the fact that the cells used in our study were not cancer cells but MSCs obtained from tonsil tissue. Indeed, it has also been reported that cellular stress does not significantly affect vesicle size or concentration in healthy endothelial cells [7].

Along with cell proliferation the effect of exosomes obtained under different circumstances on cell migration was also investigated. Consistent with our cell viability results, exosomes obtained under normal conditions rapidly closed the scratch in keratinocyte cells within 24 hours. However, exosomes obtained after heat stress did not cause complete closure of the scratch in pancreatic cancer cells after 24 hours. It is thought that the inhibition of cell proliferation observed in cancer cells after heat stress may be related to the heat shock proteins secreted. To support these findings, it is necessary to comprehensively investigate the biological content of exosomes obtained from stem cells under different heat circumstances especially focusing on heat shock proteins.

Limitations

In the study, determining the effect of exosomes obtained under different temperature conditions on cell proliferation has made an important contribution to the literature. However, there are some limitations of the study, it is important to determine apoptosis-related assays and changes in the gene level, which are indicators of cell death, to support the obtained data more comprehensively. In addition, the study data should be supported by in vivo models.

CONCLUSION

Exosomes obtained under heat stress circumstances are more prominent in inhibiting cell proliferation in cancer cells, while exosomes obtained under normal circumstances are important for stimulating cell proliferation and promoting wound healing. It is necessary to investigate the content of exosomes in more detail according to heat conditions to uncover the underlying mechanisms. The results of this study highlight the importance of the circumstances to which exosomes are exposed in their therapeutic potential. However, these findings need to be supported by further detailed analyses.

Authors' Contribution

Study Conception: MY; Study Design: MY; Supervision: MY, EA; Funding: İŞ, EÇ, MY; Materials: İŞ, EÇ, MY, OKK, PNT, DÖ; Data Collection and/or Processing: MY, EA, OKK; Statistical Analysis and/or Data Interpretation: MY, EA; Literature Review: MY, İŞ; Manuscript Preparation: MY, İŞ, EA, EC, OKK, PNT and Critical Review: MY, EA, PNT, FŞ.

Ethics Approval

Ondokuz Mayıs University, Clinical Research Ethics Committee (Ethics committee number: OMÜ KAEK 2022/282; 2023/120).

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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