Available online at www.medicinescience.org

ORIGINAL ARTICLE

Medicine Science 2022;11(2):507-14

Medicine Science International Medical Journal

The effect of mesenchymal stem cell-lyophilizate on the proliferation of A549 cells by regulation of JAK-STAT3 signaling pathway

Fatma Nilay Tutak[1], Haluk Uluca[2]

[1]Adiyaman University, School of Medicine, Department of Plastic Surgery and Burns, Adiyaman, Turkey
[2]VETAL Animal and Human Health Products and Research-Development Center, Adiyaman, Turkey

Received 06 October 2021; Accepted 29 November 2021
Available online 05.03.2022 with doi: 10.5455/medscience.2021.09.324

Abstract

To examine the effects of Mesenchymal Stem Cell-Lyophilizate (MSC-L) on the proliferation of A549 lung cancer cells and the activities of the JAK-STAT3 signaling pathway in this process. Our study in experimental design utilized A549 lung adenocarcinoma cells as model cells. First, the proliferative effect of the MSC-L cells on the A549 cells was established. Then, the concentration at which the proliferation was most distinct and a lower dilution were selected as the experimental group. In experimental groups, the A549 cells were exposed to the umbilical cord-derived MSC-L cells at two concentrations: $5X10^5$ MSC-L cells/well and $20X10^5$ MSC-L cells/well. In the control group, A549 cells with no exposure (only serum-free DMEM F12 with lyophilization additive used in lyophilization process of MSC-L cells) were used and the proliferation process of all three groups was monitored. The change in the expression levels of p53, c-MYC, p21, and SOCS2 did not statistically significantly different between the experimental group exposed to $5X10^5$ MSC-L cells/well and the control group ($p>0.05$). On the other hand, the expression level of the Bcl-xL gene was statistically higher than in the control group ($p=0.037$). The expression level of the P21 gene was statistically higher at the concentration of $20X10^5$ MSC-L cells/well compared to the control group ($p=0.037$). Similarly, the expression level of the Bcl-xL gene was statistically higher in the experimental group exposed to $20X10^5$ MSC-L cells/well than in the control group ($p=0.034$). There was no statistically significantly different change in the expression levels of P53, c-MYC, and SOCS2 genes compared to the control group ($p>0.05$). Our study revealed that A549 cells exhibited an increased cell proliferation upon MSC-L exposure; however, the JAK-STAT3 pathway was not involved in the A549 cell proliferation, suggesting that there might be other signaling pathways involved.

Keywords: Mesenchymal stem cell-lyophilizate, A549 cells, JAK-STAT3

Introduction

The Janus Kinase (JAK)- Signal Transducer and Activator of Transcription (STAT) signaling cascade are activated by various ligands and the receptors engaged by these ligands. The JAK-STAT pathways are involved in proliferation, apoptosis, angiogenesis, and other biological processes. STAT3, a member of the STAT group, is known to be effective in cell proliferation and tumor formation. Abnormal transduction of the JAK-STAT signaling cascade that is involved in tumorigenesis has been associated with invasion, metastasis, and tumor prognosis [1]. Bcl-xL is a transmembrane protein found in mitochondria and acts as an antiapoptotic protein. Suppressors of cytokine signaling (SOCS) can inhibit tyrosine phosphorylation and transduction of the JAK-STAT pathway, thereby preventing their activity. SOCS2, a member of the SOCS family, functions as a transcription factor and determines the differentiation of adult cells and the direction of cell differentiation. SOCS proteins are negative regulators of cytokine and growth factor signaling. SOCS2 has been reported to suppress carcinogenesis in many types of cancer (liver, lung, breast, and prostate cancers). In this context, it is stated that low SOCS2 protein expression is associated with tumorigenesis. However, SOCS2 is an important anti-inflammatory regulator and is required for the generation of immune response in various pathological processes [2]. The functions of SOCS2 in wound healing are not fully known, but it is believed that it may have negative regulatory and other different roles in the production of proinflammatory cytokines (interleukin (IL)-6, tumor necrosis factor (TNF)-α, and mast

***Corresponding Author:** Fatma Nilay Tutak, Adiyaman University, School of Medicine, Department of Plastic Surgery and Burns, Adiyaman, Turkey
E-mail: nilayprs@gmail.com

cells). According to the common view, SOCS proteins can affect epithelial cell formation and basic components in wound healing.

The p53 protein is a transcription factor that controls the cellular response to deoxyribonucleic acid (DNA) damage. It takes part in cell cycle arrest until the repair of the DNA damage and induces cell apoptosis if the damage cannot be repaired. Initiation of apoptosis or cell cycle arrest after DNA damage has an important role in preventing the formation of new genetically damaged cells. The p53 protein levels increase significantly within minutes in cells with DNA damage. p21 is a cyclin/CDK inhibitor, especially associated with CDK2, and negatively regulates the cell cycle. The p21 protein is one of the main targets of p53 in case of DNA damage, and an increased intracellular concentration of p53 elevates the p21 expression levels, resulting in a negatively regulated cell cycle [3,4].

Researches have shown that c-MYC and Bcl-xL proteins stimulated by the JAK-STAT cascade are effective in carcinogenesis by controlling the cell cycle and preventing apoptosis. It is further believed that the JAK-STAT-mediated signaling may interact with the activation of the MAP kinase pathway in malignant transformation [22]. Several studies have demonstrated that the JAK/STAT3 cascade activation increases the expression levels of Bcl-xL, c-MYC, p21, p53, and SOCS2 genes (Table 1).

Table 1. Serum Hsp 70 and RANKL concentrations (ng/ml; pg/ml) in umbilical cord blood, presented as Median [min-max]

41st Genes upregulated by STAT3	Cell Line	Reference
Bcl-xL	STAT3-C transformed NIH3T3, U266 myeloma cells, Head and neck squamous cell carcinomas	Bromberg et al. (31) Catlett-Falcone et al.(32)
c-MYC	STAT3-C transformed NIH3T3, BAF/B03, murine pro-B cells, MCF-7, HepG2	Bowman et al. (33) Bromberg et al. (31) Kiuchi et al. (34) Zhang et al. (35)
p21	V transformed NIH3T3 cells and BALB/c3T3 cells	Sinibaldi et al. (36)
p53	MCF-7	Zhang et al. (35)
SOCS2	Many human cancers, including hepatocellular carcinoma (HCC), non-small-cell lung cancer, mesothelioma, and head and neck squamous cell carcinoma (HNSCC)	Croker et al. (37)

Mesenchymal Stem Cells (MSCs) have a strong ability to synthesize, undergo self-renewal, and are capable of differentiating into bone, fat, and cartilage [5-8]. Growth factors (such as VEGF) required for angiogenesis and adipogenesis are secreted by MSCs, which also play an active role in maintaining/increasing tissue volume in adipose tissue regeneration [9]. MSC-lyophilized is preferred due to its therapeutic effect, non-toxicity, easy production, and greater safety in applications.

There are important advantages in the use of derived Mesenchymal Stem Cell-Lyophilizate (MSC-L) from maternal sources its availability, including its anti-inflammatory characteristics, growth factors, chemokines, extracellular vesicles, and cytokines [5-9]. Apoptosis, which is defined as the programmed death of the cells to balance proliferation, remove the cells with loss of functions due to damage, and balance homeostasis [10], also represents a normal and physiological state just like proliferation. Exploration of the cellular proliferation-enhancing properties of MSC-L without any change in apoptosis and identification of the ongoing mechanisms may offer promising results for rapid tissue healing. *Our study aimed to examine the effects of MSC-L on the proliferation of A549 lung cancer cells and the activities of the JAK-STAT3 signaling pathway in this process.*

Materials and Methods

Study Design

Our research is an experimental type of laboratory work. All cell cultures studies were performed in the Cell Culture Laboratories of VETAL Animal Health Products Inc. Gene expression analyses were conducted in the Quality Control Laboratories of VETAL Animal Health Products Inc. Our study utilized A549 cells as model cells. Due to the unknown cytotoxic or proliferative effects of MSC-L cells on A549 cells, first, the proliferative or antiproliferative effects of MSC-L cells on A549 cells were analyzed by MTT assay. As a result, an increase was detected in cell proliferation up to certain exposure doses. Then, the concentration at which the proliferation was most distinct and a lower dilution were selected as the experimental group. In experimental groups, the A549 cells were exposed to the umbilical cord-derived MSC-L cells at two concentrations: $5X10^5$ MSC-L cells/well and $20X10^5$ MSC-L cells/well. The lyophilized MSC-L cells were resuspended with serum-free DMEM-F12. In the control group, A549 cells with no exposure and incubated in serum-free DMEM-F12 with lyophilization additive used in lyophilization process of MSC-L cells were used at the same concentration with the experimental group and the proliferation process of all three groups was monitored.

A549 Lung Cancer Cell Cultures

Our study utilized a lung adenocarcinoma (A549) cell line. Cells were cultured using DMEM-F12 medium containing 10% fetal calf serum (FCS), 0.2 mM glutamine, 100 µg/ml streptomycin, and 100 IU/ml penicillin in 25- and 75-cm^2 sterile flasks at 37°C, 5% CO_2, and 1 atm pressure. The culture medium was changed twice a week and the cell number was increased by passaging the cells by trypsinization from 25 cm^2 to 75 cm^2 flasks when the cells reached a sufficient number. Cells were seeded in 24-well plates for the MTT assay and gene expression analyses. For exposure, when the cells reached 70-80% confluency, a medium containing 10% FCS was discharged from the cultures, followed by washing three times with Hanks' Balanced Salt Solution (HBSS) and replacing by SF (serum-free) medium, and then incubated for 24 hours under these conditions.

MSCs and Lyophilization

After obtaining the necessary legal permissions, MSC-L was obtained from the Tissue Typing Laboratories, Istanbul Acıbadem University (the umbilical cord was used as the source to derive MSCs). Growth of MSC cells to make MSC-L was performed at Vetal Inc lyophilization unit.

Stem cells were grown in 75-cm^2 flasks and serum-free Dulbecco's

Modified Eagle's Medium (DMEM), by incubating at 37°C in a 5% carbon dioxide (CO_2) atmosphere. When the cells reached 70–80% confluency, the cells were passaged using trypsin to increase the number of cells.

Passaged cells were detached using trypsin in the final passage and centrifuged at 400 rpm for 10 minutes at +4°C. The supernatant was discarded, and Physiological Saline (PS) was added. The pellet was suspended, centrifuged again at 400 rpm for 10 minutes at +4°C, the supernatant was discarded and SF was added. The cell culture was centrifuged at 400 rpm for 10 minutes at +4°C for the last time, the supernatant was discarded and then suspended in SF. Then, the lyophilization additive was added. The freeze-drying medium was composed of 30% Polyvinylpyrrolidone (Sigma Aldrich, CAS Number: 9003-39-8), 100 mmol/l trehalose (Merck, CAS Number: 6138-23-4), 100 mmol/l sucrose (Sigma Aldrich, CAS Number: 57-50-1), and serum-free DMEM/F-12 (Gibco 11320074, Dulbecco's Modified Eagle Medium/ Nutrient Mixture F-12) as solvent. The cell suspension with lyophilization additive was divided into $15X10^7$ cells per vial. The vials were lyophilized at a half-capped state. Then, 1.5 million MSC cells were placed into each vial.

Inoculation of lyophilized MSCs cells into A549 cells

A549 cells were cultured for 24 hours and then treated with lyophilized MSC cells. Serum-free DMEM was used as a solvent to prepare the dilutions of lyophilized MSC cells. In addition, considering that the JAK/STAT pathway is a fast pathway due to STAT proteins that pass directly to the nucleus with the transcription factor, a 24-hour incubation period was preferred for exposure.

Cell Viability Assessment

In the study, exposure doses and times for MSC-L cells were determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. The working principle of MTT assay is based on measuring the color change, which results from the production of purple formazan dye by the proliferating cells using tetrazolium, a yellow compound, through increasing dehydrogenase activity, as absorbance with a microplate reader or spectrophotometer. Analyses to determine cell viability were repeated three times.

Gene Expression Analyses

After exposure to MSC-L cells at concentrations and times determined by the MTT assay, the A549 cells were washed three times with HBSS and the cells were detached using Trypsin-EDTA, resulting in total RNA isolation. Expression levels of SOCS2, Bcl-xL, c-MYC, p21, p53 genes that are regulated by the JAK/STAT 3 cascade, which plays an important role in cell proliferation, were determined. Gene expression analyses were performed in triplicate.

RNA Isolation and cDNA Synthesis

The A549 cells seeded at $5X10^4$ cells/ml in 6-well cell culture dishes for RNA isolation were washed three times with PBS when the bottom of the plate was covered by 70%. Then, the MSC-L cells suspended in serum-free DMEM were added to the A549 cells at concentrations of $5X10^5$ cells/well and $20X10^5$ cells/well. After 24 hours, the supernatant of A549 cells was discarded and the cells were washed three times with PBS. The washed A549 cells were harvested by detaching from the base of the flask using Trypsin-EDTA (1X) (0.25%). RNA isolation was performed using the commercial kit Qiagen; RNeasy Mini Kit, (Catalog number: 74104), and cDNA synthesis using Thermo; HighCapacity cDNA Reverse Transcription Kit, (Catalog number: 4368814).

Primers Used in the Study

The specificity of the gene sequences used in the study was tested (Table 2).

Table 2. Forward and Reverse Coding of Gene Sequences Used in the Study

Genes	Primers	Reference
STAT3	Forward CAGCAGCTTGACACACGGTA Reverse AAACACCAAAGTGGCATGTGA	Yu et al. (18)
SOCS2	Forward TTAAAAGAGGCACCAGAAGGAAC Reverse AGTCGATCAGATGAACCACACT	Zhu et al. (19)
c-MYC	Forward GTCAAGAGGCGAACACACAAC Reverse TTGGACGGACAGGATGTATGC	Cao et al. (20)
p21	Forward TGTCCGTCAGAACCCATGC Reverse AAAGTCGAAGTTCCATCGCTC	Meng et al. (21)
p53	Forward CAGCACATGACGGAGGTTGT Reverse TCATCCAAATACTCCACACGC	Yang et al. (22)
Bcl-xL	Forward GAGCTGGTGGTTGACTTTCTC Reverse TCCATCTCCGATTCAGTCCCT	Zhao et al. (23)

Real-time PCR

Gene expression analyses were performed in the QIAGEN-Rotor-Gene Q Real-time PCR instrument, using the commercial kit Thermo; Applied Biosystems Power SYBR Green PCR Master Mix (Catalog number: 4367659). The primer sequences of the analyzed genes are presented in Table 3. The primer of the GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase) gene, which was used as the housekeeping gene, was purchased commercially from Qiagen. Melting curve analysis was performed to check for non-specific products and the presence of primer-dimer. Experiments were performed in triplicate and each sample was studied in duplicate. The experiments results were expressed as fold-change.

Table 3. Incubation of Tubes according to the Real-Time PCR Protocol

Temperature	Time	Number of Cycles
95°C	10 min	1
95°C	15 sec	40
60°C	60 sec	

The Real-Time PCR Protocol

1. To each sample tube, 12.5 µL of Power SYBR Green PCR Master Mix, 100 nM of primer, 6 µL of RNase-free water, and 100 ng of cDNA were added, and the tubes were capped.

2. The tubes were placed in the device and incubated under the conditions specified in Table 3.

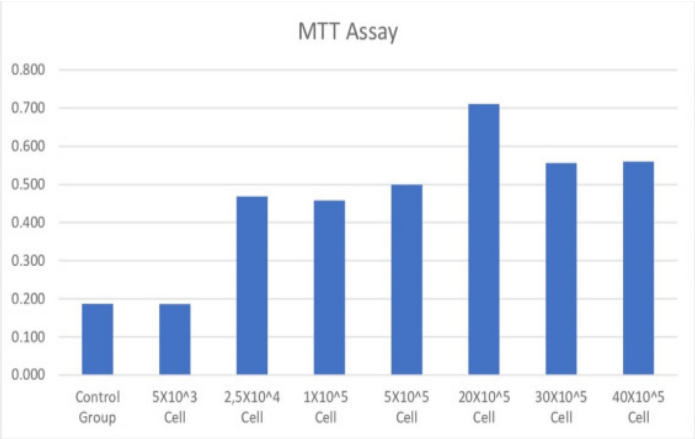

Figure 1. Changes in Proliferation Balance of A549 Cell Line with Exposures to Increasing Numbers of MSC-L Cells

Legal and Ethical Considerations

Since the study was not an experimental animal or a clinical study and it was a laboratory study ethical approval for cell use was obtained from Istanbul Acibadem University. After obtaining the necessary legal permissions, MSC-L was obtained from the Tissue Typing Laboratories, Istanbul Acıbadem University (the umbilical cord was used as the source to derive MSCs).

Statistical Analysis

The data on cell viability was assessed using the GraphPad Prism 5.01 (GraphPad Software Inc., USA) software. Statistical analyses of the study results were conducted using the Statistical Package for Social Sciences (SPSS) for IBM 25 package program. Descriptive statistical methods (number, percentage, minimum and maximum, mean and standard deviation) were used to assess numerical data. The Mann-Whitney U test was used to determine the difference between the means. Statistical analysis of the Real-Time PCR data was conducted using RT2 Profiler PCR Array Data Analysis version 3.5 software. Experiments were repeated three times. Results were presented as fold-change, assessed at 95% confidence interval and a significance level of $p<0.05$.

Results

The MTT Assay

The MTT assays revealed that cell proliferation continued to increase until exposure to lyophilized containing $20X10^5$ cells, while exposure to a higher amount of cells inhibited cell proliferation and caused cytotoxicity. For exposure, $20X10^5$ at which statistically significant change began and a lower digit of this level were preferred. Therefore, it was decided to examine the gene expression regulations resulting from exposure to $5X10^5$ and $20X10^5$ MSC-L in the gene expression analyses (Table 4).

Table 4. Statistical data on the MTT Assay

N		Meant ± SD	Min–Max	U, p
Control	6	0.19±0.02	0.16–0.22	U=0.790 p=0.810
$5x10^3$	6	0.19±0.02	0.17–0.22	
Control	6	0.19±0.02	0.16–0.22	U=0.210 p=0.016*
$2.5x10^4$	6	0.47±0.2	0.21–0.61	
Control	6	0.19±0.02	0.16–0.22	U=1.231 p=0.004**
$1x10^5$	6	0.46±0.22	0.22–0.73	
Control	6	0.19±0.02	0.16–0.22	U=1.123 p=0.004**
$5x10^5$	6	0.5±0.23	0.23–0.76	
Control	6	0.19±0.02	0.16–0.22	U=0.513 p=0.004**
$20x10^5$	6	0.71±0.11	0.62–0.87	
Control	6	0.19±0.02	0.16–0.22	U=2.214 p=0.004**
$30x10^5$	6	0.56±0.05	0.49–0.61	
Control	6	0.19±0.02	0.16–0.22	U=0.312 P=0.004**
40x105	6	0.56±0.16	0.39–0.75	

U; Mann-Whitney U Test, *p<0.05, **p<0.01.

In the study conducted by adjusting the cell concentration to $5x10^3$ cells/well, the MTT assay results did not show a statistically significant difference when compared to the control group ($p>0.05$). When compared control and exposure groups for all other cell concentrations, the MTT assay results were statistically higher for all exposure groups ($p<0.05$).

Table 5 shows the comparison of fold-change values in gene expressions between the control group and the A549 cells exposed to $5X10^5$ MSC-L cells/well.

Table 5. Comparison of fold-change values in gene expressions between the control group and the A549 cells exposed to $5X10^5$ MSC-L cells/well

p53	XX ± SD	Min–Max	U, p
Control	1±0	1–1	U=3.00 p=0.487
$5X10^5$ MSC-L	1.02±0.12	0.91–1.14	
c-MYC	**XX±SD**	**Min–Max**	**U,p**
Control	1±0	1–1	U=4.500 p=0.999
$5X10^5$ MSC-L	0.96±0.08	0.87–1.01	
SOCS2	**XX±SD**	**Min–Max**	**U,p**
Control	1±0	1–1	U=3.00 p=0.487
$5X10^5$ MSC-L	0.91±0.12	0.8–1.04	
p21	**XX±SD**	**Min–Max**	**U,p**
Control	1±0	1–1	U=3.00 p=0.487
$5X10^5$ MSC-L	1.27±0.38	0.97–1.7	
Bcl-xL	**XX±SD**	**Min–Max**	**U,p**
Control	1±0	1–1	U=0.001 p=0.037*
$5X10^5$ MSC-L	1.2±0.12	1.06–1.28	

U; Mann-Whitney U Test, **p < 0.01, *p < 0.05

The p53, c-MYC, SOCS2, and p21 values did not differ statistically between the groups (p>0.05). The Bcl-xL value was higher in the 5X10^5 MSC-L group than in the control group, which was statistically significant (p=0.037).

Table 6 shows the comparison of fold-change values in gene expressions between the control group and the A549 cells exposed to 20X10^5 MSC-L cells/well.

There was no statistically significantly different change in the expression levels of p53, c-MYC, and SOCS2 compared to the control group (p>0.05). The p21 gene expression level was statistically significantly higher in the 20X10^5 MSC-L experimental group than in the control group (p=0.037). The Bcl-xL value was higher in the 20X10^5 MSC-L experimental group than in the control group, which was statistically significant (p=0.034).

Table 6. Comparison of fold-change values in gene expressions between the control group and the A549 cells exposed to 20X10^5 MSC-L cells/well

p53	**XX ± SD**	**Min–Max (Median)**	**U, p**
Control	1±0	1–1	U=3.00 p=0.487
20X10^5 MSC-L	1.01±0.09	0.94–1.11	
c-MYC	***XX±SD***	**Min–Max**	**U,p**
Control	1±0	1–1	U=3.00 p=0.487
20X10^5 MSC-L	1.07±0.17	0.96–1.27	
SOCS2	**XX±SD**	**Min–Max**	**U,p**
Control	1±0	1–1	U=3.00 p=0.487
20X10^5 MSC-L	1±0.18	0.87–1.21	
p21	**XX±SD**	**Min–Max**	**U,p**
Control	1±0	1–1	U=0.001 p=0.037*
20X10^5 MSC-L	1.14±0.14	1.02–1.29	
Bcl-xL	**XX±SD**	**Min–Max**	**U,p**
Control	1±0	1–1(1)	U=0.001 p=0.034*
20X10^5 MSC-L	1.22±0.15	114–1.4	

U; Mann-Whitney U Test, **p < 0.01, *p < 0.05

Discussion

STAT3 is a member of the STAT group that is known to be effective in cell proliferation and tumor formation. Following the binding of cytokines to the cell surface receptor, STAT3 proteins form a dimeric structure, transmitting extracellular signals directly to the cell nucleus via a signal transduction pathway with an extremely rapid mechanism. The signal transduction pathway is critical for the response of JAK-STAT to extracellular factors, conversion of the signaling pathway response, and the regulation of cell growth/apoptosis [24]. Cytokines bind to dimerized receptors of growth factors and phosphorylated JAK through STAT activation-inducing ligands. A previous study has indicated that STAT3 is associated with unregulated cell proliferation and malignant transformation [25]. SOCS2 proteins directly bind to JAK proteins that are involved in the initiation of the JAK-STAT3 pathway and act as a feedback inhibitor of the STAT3 pathway. The expression levels of SOCS2 protein are directly controlled by STAT3 [26, 27]. Therefore, JAK-STAT3, which induces SOCS2 expression, regulates its expression levels through SOCS2 proteins [26]. In addition, STAT3 activators and signal converters were found to induce Bcl-xL expression [28].

The results of the MTT assay conducted in our study revealed that cell proliferation continued to increase until exposure to lyophilized containing 20X10^5 cells, while exposure to a higher amount of cells inhibited cell proliferation. For exposure, 20X10^5 at which statistically significant change began and a lower digit of this level were preferred. Therefore, it was decided to examine the gene expression regulations caused by 5X10^5 and 20X10^5 MSC-L in the gene expression analyses. Our study observed no STAT3 value change during A549 cell proliferation in the control and both experimental groups. Based on the data from our study, it is possible to say that the JAK-STAT3-Bcl-xL pathway is not responsible for the proliferation of A549 cells exposed to MSC-L. A similar study found the JAK/STAT5-Bcl-xL pathway to be responsible for anti-apoptotic activities, and to regulate the anti-apoptotic activities of granulocyte-macrophage colony-stimulating factor (GM-CSF) and the expression of genes activating the Bcl-2 family proteins [28]. The same study determined that the JAK-STAT3-Bcl-xL pathway was not responsible for antiapoptotic processes. The absence of any change in the gene expression of STAT3 may indicate that the MAPK/Erk pathway, which is one of the important regulators of the STAT3 protein, is not actively involved in proliferation.

In our study, the Bcl-xL value was statistically significantly higher in the 5X10^5 and 20X10^5 MSC-L experimental groups than in the control group (p<0.05). Even though the expression level of the anti-apoptotic Bcl-xL protein is regulated by the JAK-STAT3 pathway, the increase or decrease in the expression level of the Bcl-xL protein alone cannot be evidence for the activity of the JAK-STAT3 pathway. To detect the activation of the JAK-STAT3 pathway, it is required to determine the STAT3/pSTAT3 ratio by a method such as Western Blot. This is the subject of another study that can be conducted in the future. p53 plays an essential role in arresting cell proliferation at the G1/S phase in cells with DNA damage [29]. p53 arrests the cell cycle until the DNA damage is repaired in the cell and controls the transition of the cell to apoptosis. After DNA damage, the p53 protein levels increase significantly within minutes in cells with DNA damage. p21, in turn, negatively regulates the cell cycle through CDK2 together with p53. p21 is one of the main targets of p53 after DNA

damage, and an increased intracellular p53 concentration elevates P21 expression levels [3,4]. It is stated in the literature that the proto-oncogene c-MYC is the master regulator of cell proliferation and transformation [29]. In our study, the statistically significant increased level of p21 expression in the $20x10^5$ experimental group compared to the control group can be associated with the survival of the cells and interpreted as contributing to cell proliferation [30]. Our study did not establish any statistically significant difference in p53, c-MYC, SOCS2 gene expression levels between the control and experimental groups ($p>0.05$). In addition, the p21 level was statistically significantly higher in the $20X10^5$ MSC-L experimental group than in the control group ($p=0.037$).

There are studies in the literature showing that MSCs lead to a high level of increase in A549 cell proliferation [31,32]; however, such studies did not provide any data on the signaling pathway. Besides, some studies have demonstrated that tumors can integrate MSCs into the microenvironment, where they can affect tumor cell survival, angiogenesis, and metastasis [33,34]. Acceleration of cell proliferation, increase in cell migration, and suppression of apoptosis accelerate wound healing [35]. In addition, MSCs with strong potential and differentiation ability improve the regeneration capacity of the tissue [36,37]. It has been emphasized in the literature that MSCs increase the neovascularization of ischemic tissues [38,39]. The present study established that the change in the expression levels of p21 and Bcl-xL genes that are believed to be upregulated by the JAK-STAT3 pathway, which is believed to be involved in cell proliferation, was associated with MSC-L exposure. This, in turn, revealed an important effect of MSC-L on the cell cycle.

Our study is the first to examine the effect of umbilical cord-derived MSC-L on A549 cell proliferation. In this study, which examined the effect of MSC-L on the lung adenocarcinoma cell line, there were remarkable changes in some parameters of proliferation in the experimental groups due to the effect of MSC-L application. The results from this study suggest that MSC-L can accelerate wound healing. However, we estimate that the improving effect was not realized through the JAK-STAT3 pathway but under the control of other signaling pathways contributing to cell proliferation. Further preclinical and clinical studies are required to demonstrate the wound healing-accelerating effect of MSC-L. The limitations of the study included the lack of testing on primary cell lines, the absence of performing analyses such as Western blot, and the non-optimal number of similar studies.

Conclusion

Changes in some parameters that were identified in our study examining the effects of MSC-L on A549 cell proliferation via the JAK-STAT3 signaling pathway are important findings. No exposure was applied to A549 cells in the control group and gene expression analyses of STAT3, p53, c-MYC, SOCS2, P21, and Bcl-xL proteins were performed during cell proliferation. Analyses showed that the expression level of the Bcl-xL gene increased significantly with exposure to both 5X105 and 20X105 cells, while p21 increased significantly only with exposure to 20X105 cells. There was no change in the expression levels of other genes. This gives clues that the JAK-STAT3 pathway is not directly responsible for the proliferation of A549 cells exposed to MSC-L exposure.

It is known that the p53 and p21 levels increase when the cell DNA is damaged. In our study, the insignificant difference in p53 elevation between the control and experimental groups is a promising development regarding the lack of DNA damage in the produced cells. However, the p21 level is statistically significantly higher in the $20X10^5$ MSC-L experimental group than in the control group suggests that this topic should be explored in more depth.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
All authors declare no financial support.

Ethical approval
Informed consent was obtained from all individuals participating in the study and study was approved by Acıbadem University

References

1. Bose S, Banerjee S, Mondal A, et al. Targeting the JAK/STAT signaling pathway using phytocompounds for cancer prevention and therapy. Cells..2020;9:1451.
2. Machado FS, Johndrow JE, Esper L et al. Anti-inflammatory actions of lipoxin A4 and aspirin-triggered lipoxin are SOCS-2 dependent. Nat. Med. 2006;12:330–4.
3. Celepli S, Bigat İ, Celepli P, Karagin PH. Apoptoz ve Apoptotik Yolların Gözden Geçirilmesi. Güncel Gastroenteroloji Dergisi. 2020;24:103-11
4. Schmidt AK, Pudelko K, Boekenkamp JE, et al. The p53/p73-p21 CIP1 tumor suppressor axis guards against chromosomal instability by restraining CDK1 in human cancer cells. Oncogene. 2021;40:436-51.
5. Lam AT, Reuveny S, Oh SKW. Human mesenchymal stem cell therapy for cartilage repair: review on isolation, expansion, and constructs. Stem Cell Res. 2020;44:101738.
6. Mushahary D, Spittler A, Kasper C, et al. Isolation, cultivation, and characterization of human mesenchymal stem cells. Cytometry Part A. 2018;93:19-31.
7. Jessop ZM, Manivannan S, Zhang Y, et al. Tissue specific stem/progenitor cells for cartilage tissue engineering: A systematic review of the literature. Applied Physics Reviews. 2019;6:031301.
8. Gönen ZB, Şahin NS. Mesenchymal Stem Cell Production for Clinical Usage. Akd Med J. 2019;5:169-75.
9. Schmitt A, van Griensven M, Imhoff AB, Buchmann S. Application of stem cells in orthopedics. Stem Cells Int. 2012;1-12.
10. Liu L, Michowski W, Kolodziejczyk A, Sicinski P. The cell cycle in stem cell proliferation, pluripotency and differentiation. Nature cell biology, 2019;21:1060-7.
11. Bromberg JF, Wrzeszczynska MH, Devgan G, et al. Stat3 as an oncogene. Cell. 1999,98;295-303.
12. Catlett-Falcone R, Landowski TH, Oshiro MM, et al. Constitutive activation of Stat3 signaling confers resistance to apoptosis in human U266 myeloma cells. Immunity. 1999;10:105-15.
13. Bowman T, Broome MA, Sinibaldi D, et al. Stat3-mediated Myc expression is required for Src transformation ve PDGF-induced mitogenesis. Proc Natl Acad Sci USA. 2001;98:7319-24.
14. Kiuchi N, Nakajima K, Ichiba M, et al. STAT3 is required for the gp130-mediated full activation of the c-myc gene. J Experimental Med. 1999;189:63-73.
15. Zhang F, Li C, Halfter H, Liu J. Delineating an oncostatin M-activated STAT3 signaling pathway that coordinates the expression of genes involved in cell cycle regulation and extracellular matrix deposition of MCF-7 cells. Oncogene. 2003;22:894-905.
16. Sinibaldi D, Wharton W, Turkson J,et al. Induction of p21 WAF1/CIP1 and cyclin D1 expression by the Src oncoprotein in mouse fibroblasts: role of activated STAT3 signaling. Oncogene. 2000;19:5419-27.

17. Croker BA, Kiu H, Nicholson SE. SOCS regulation of the JAK/STAT signalling pathway. Semin Cell Dev Biol. 2008;19:414-22.

18. Yu P, Xiao L, Lin L,et al. STAT3-mediated TLR2/4 pathway upregulation in an IFN-gamma-induced Chlamydia trachomatis persistent infection model. Pathog Dis. 2016;74:1-12.

19. Chen SS, Wu WZ, Zhang YP, Huang WJ. Gene polymorphisms of SOCS1 and SOCS2 and acute lymphoblastic leukemia. Eur Rev Med Pharmacol Sci. 2020;24:5564-72.

20. Cao XP, Cao Y, Zhao H, et al. HMGA1 promoting gastric cancer oncogenic and glycolytic phenotypes by regulating c-myc expression. Biochem Biophys Res Commun. 2019;516:457-65.

21. Meng W, Wang B, Mao W, et al. Enhanced efficacy of histone deacetylase inhibitor combined with bromodomain inhibitor in glioblastoma. J Exp Clin Cancer Res. 2018;37:1-11.

22. Yang X, Ye H, He M, et al. LncRNA PDIA3P interacts with c-Myc to regulate cell proliferation via induction of pentose phosphate pathway in multiple myeloma. Biochem Biophys Res Commun. 2018;498:207-13.

23. Zhao S, Guo J, Zhao Y, et al. Chidamide, a novel histone deacetylase inhibitor, inhibits the viability of MDS and AML cells by suppressing JAK2/STAT3 signaling. Am J Transl Res. 2016;8:3169-78.

24. Pencik J, Pham HTT, Schmoellerl J, et al. JAK-STAT signaling in cancer: From cytokines to non-coding genome. Cytokine. 2016;87:26-36.

25. Gao B, Shen X, Kunos G, et al. Constitutive activation of JAK–STAT3 signaling by BRCA1 in human prostate cancer cells. FEBS letters. 2001;488:179-84.

26. Croker BA, Kiu H, Nicholson SE. SOCS regulation of the JAK/STAT signalling pathway. Semin Cell Dev Biol. 2008;19:414–22.

27. Ortiz-Munoz G, Lopez-Parra V, Lopez-Franco O, et al. Suppressors of cytokine signaling abrogate diabetic nephropathy. J Am Soc Nephrol. 2010;21:763–72.

28. Choi JK, Kim KH, Park H, et al. Granulocyte macrophage-colony stimulating factor shows anti-apoptotic activity in neural progenitor cells via JAK/STAT5-Bcl-2 pathway. Apoptosis. 2011;16:127-34.

29. Liu X, Yu H, Cai H, Wang Y. Expression of CD24, p21, p53, and c-myc in alpha-fetoprotein-producing gastric cancer: Correlation with clinicopathologic characteristics and survival. J Surg Oncol. 2014;109:859-64.

30. Seo YH, Joo YE, Choi SK, et al. Prognostic significance of p21 and p53 expression in gastric cancer. Korean J Int Med. 2003;18:98.

31. Zhang YM, Zhang ZM, Guan QL, et al. Co-culture with lung cancer A549 cells promotes the proliferation and migration of mesenchymal stem cells derived from bone marrow. Exp Ther Med. 2017;14:2983–91.

32. Zhang YM, Liu YQ, Liu D, et al. The effects of astragalus polysaccharide on bone marrow-derived mesenchymal stem cell proliferation and morphology induced by A549 lung cancer cells. Medical Science Monitor: Int Med J Experimental Clin Res. 2019;25:4110.

33. Karnoub AE, Dash AB, Vo AP, et al. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature. 2007;449:557–63.

34. Beate H, Douaa D, Salita E, et al. Role of mesenchymal stem cell-derived fibbrinolytic factor in tissue regeneration and cancer progression. Cell Mol Life Sci. 2015;72:4759–70.

35. Ma T, Fu B, Yang X, et al. Adipose mesenchymal stem cell-derived exosomes promote cell proliferation, migration, and inhibit cell apoptosis via Wnt/β-catenin signaling in cutaneous wound healing. J Cell Biochem. 2019;120:10847-54.

36. Assis-Ribas T, Forni MF, et al. Extracellular matrix dynamics during mesenchymal stem cells differentiation. Dev Biol. 2018;437:63-74.

37. Kariminekoo S, Movassaghpour A, Rahimzadeh A, et al. Implications of mesenchymal stem cells in regenerative medicine. Artif Cells Nanomed Biotechnol. 2016;44:749-57.

38. Ko SH, Nauta AC, Morrison SD, et al. PHD-2 suppression in mesenchymal stromal cells enhances wound healing. Plast Reconstr Surg. 2018;141:55-67.

39. Motegi S, Ishikawa O. Mesenchymal stem cells: the roles and functions in cutaneous wound healing and tumor growth. J Dermatol Sci. 2017;86:83-9.

40. Benekli M, Xia Z, Donohue KA, et al. Constitutive activity of signal transducer and activator of transcription 3 protein in acute myeloid leukemia blasts is associated with short disease-free survival. Blood. 2002;99:252-7.