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Relationship between the Release of Interleukin 12 and the Expression of PCNA, Cyclin A2, and CDK2 in Lung Cancer Cells

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ABSTRACT

Objective: Mesenchymal stem cell (MSC)-based IL-12 release therapy may be less toxic than direct administration of IL-2. We investigated the release of interleukin (IL)-12 in MSCs and A549 cocultures and identified the time points at which IL-12 is secreted. We also examined changes in the expression of proliferative genes, such as proliferating cell nuclear antigen (PCNA), cyclin A2, and cyclin-dependent kinase 2 (CDK2).

Material and Methods: This study was conducted by culturing A549 cells, MSCs, and MSC-A549 cells at 3 different time points. The release of IL-12p70 from cells in the collected culture media was determined by Enzyme-Linked ImmunoSorbent Assay. The expression of the *PCNA*, cyclin *A2*, and *CDK2* genes involved in the cell cycle was determined quantitatively by reverse transcriptase polymerase chain reaction.

Results: The expression of PCNA was the lowest, and the expression of the cyclin *A2* and *CDK2* genes was the highest in cocultured A549 cells, whereas the expression of the IL-12 protein was the highest at the 12th h of coculture. The expression levels of the cyclin *A2* and *CDK2* genes decreased in cocultured A549 cells at the 24th h in the presence of released IL-12. However, in A549 cells cultured alone, when the IL-12 level was the highest at the 24th h, the cyclin A2 and CDK2 expression levels were high compared to those in the control group.

Conclusion: IL-12 protein affects cell cycle regulatory genes, slows down cell proliferation, and may affect the prognosis of patients with this disease.

Keywords: Lung neoplasms; mesenchymal stem cells; interleukin-12; cell cycle proteins

INTRODUCTION

Lung cancer is the leading cause of cancer-related death worldwide. Although lung neoplasms are a heterogeneous group of more than 50 histomorphological subtypes, they are generally classified as non-small cell lung carcinoma (NSCLC) or small cell lung carcinoma (SCLC) because limited treatment options do not require considerable morphological subclassification. While NSCLCs account for 80-85% of all lung cancers, their most common histological subtype is adenocarcinoma, with a rate of about 40%.[1,2]

Stem cells are undifferentiated and can regenerate, differentiate, form clones derived from a single cell, and ensure the continuity and regeneration of tissues.[3,4] They are responsible for the regeneration of cells that are damaged in tissues due to physiological or pathological processes, the production of soluble factors necessary for cell survival and reproduction, and the regulation of the immune response.[5,6] Among adult stem cells, the most commonly used cell types in stem cell therapy are mesenchymal stem cells (MSCs) and hematopoietic stem cells.[3]

MSCs originate from the stroma and can be obtained from many tissues in the organism. Compared to bone marrow-derived stem cells, adipose-derived MSCs have greater isolation efficiency and proliferation capacity.[7] MSCs can

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migrate to ischemic areas and primary or metastatic tumor sites, and this migration makes them a tool for targeted therapy.[8] The use of genetically modified MSCs in targeted treatments ensures that anticancer agents are continuously distributed.

Cytokines act as cell signal proteins in intercellular communication. They bind to their receptors on the target cell after they are released. The activation resulting from this binding initiates intracellular signaling, which changes cell functions.[9] Cytokines are not constantly released; rather, they are usually produced and released in response to stimuli. They are involved in adaptive inflammatory host defense, cell growth, differentiation, and death, angiogenesis, maintenance of homeostasis, and regulation of immune responses.[10] Among these cytokines, interleukin 12 (IL-12) suppresses the growth of cancer cells by stimulating immune system cells via the production of antiangiogenic factors in cancerous tissue.[11] IL-12 plays an important role in the production of interferon gamma, the differentiation of helper T-cells, the proliferation of activated T and natural killer cells, and the stimulation of anticancer responses.[12,13] Cytokine-based immunotherapy can effectively treat many malignancies. As IL-12 causes tumor cell death, it can be considered a strong candidate for immunotherapy-based interventions. However, systemic administration of IL-12 is highly toxic; therefore, alternative methods, such as IL-12 delivery or release from cells, are needed.[14] MSC-mediated IL-12 release may be less toxic than direct administration of IL-2.

Many studies have reported that the abnormal activity of cell cycle proteins, such as cyclin-dependent kinase 2 (CDK2) and cyclin A2, which play important roles in the progression of the cell cycle, is closely related to cancer.[15-17] Abnormal activity of CDK2 causes proliferation in prostate cancer and NSCLC, as well as the transformation of mammary epithelial cells into cancer cells.[18] The expression of cyclin A2 is associated with cellular proliferation and is an indicator of poor prognosis.[19] Cyclin A2 deficiency causes cells to display characteristics similar to those of cancer stem cells.[20] Additionally, studies have shown shortened survival in patients with NSCLC positive for cyclin A.[21] Proliferating cell nuclear antigen (PCNA) acts as a bridge between CDK2 and its substrates by binding with the cyclin A-CDK2 complex during the cell cycle.[22] A high level of expression of PCNA in NSCLC is associated with a poor prognosis, and patients with PCNA-positive carcinoma have a shorter survival time than patients with PCNA-negative carcinoma.[23,24] Studies on the effects of IL-12 on cyclin A2, CDK2, and PCNA are rare.

In this study, MSCs and A549 lung cancer cells were cultured alone and together in vitro to analyze spontaneous IL-12 release from cells into the medium and monitor changes in the expression of cell cycle genes, including PCNA, cyclin A2, and CDK2, in cultured cells under these conditions. The data related to the effects of IL-12 on cyclin A2, CDK2, and PCNA are limited.

MATERIAL AND METHODS

Cell Culture

Adipose-derived MSCs (obtained from Acıbadem University) were added to DMEM F12 (Dulbecco's modified Eagle's medium/nutrient mixture F-12; Gibco, Carlsbad, CA, US) supplemented with 1% penicillin/streptomycin (Gibco, Carlsbad, CA, US) and 25% fetal bovine serum (FBS; Gibco, Carlsbad, CA, US). A549 cancer cells (obtained from Yeditepe University) were cultured in DMEM F12 medium containing 1% penicillin/streptomycin and 10% FBS. The cells were grown in a CO_2 incubator with 95% O_2 and 5% CO_2 at 37 °C overnight. The next day, the cells were seeded in a serum-free medium. Cell culture experiments were performed by establishing 3 independent cell groups: A549 cell culture alone, MSC culture alone, and MSC-A549 coculture. The Transwell system was used to assess the paracrine effects of the combination of MSCs and A549 cells. This system consisted of 2 chambers separated by a semipermeable membrane with a pore size of 0.4 µm (FALCON, Tewksbury, MA, USA). A549 cells (3×10^5 cells/well in six-well plates) were cultured in the upper chamber, and MSCs (3×10^5 cells/well in six-well plates) were cultured in the lower chamber of the Transwell inserts.

Preparation of the Conditioned Medium

After 2, 12, and 24 h, the medium was aspirated from the Transwell insert systems and centrifuged at 1000×g for 10 min. The supernatant obtained was filtered through 0.2 µm pores (Millipore Corporation SCILOGEX, Bedford, MA, USA) and stored at -80 °C for Enzyme-Linked ImmunoSorbent Assay (ELISA).

Culture Supernatant Analysis by ELISA

After 2, 12, and 24 h of A549 alone, MSC alone, and A549+MSC coculture, the supernatants were collected and ELISA was performed with these media to measure the level of the cytokine IL-12p70 secreted by A549 cells and MSCs. These measurements were made using an ELISA kit (Picokine, Valley Ave Pleasanton, CA, USA) following the manufacturer's protocols, and the absorbance of each well at 450 nm was measured using a microplate reader. The IL-12 protein concentration was calculated based on standard curves.

Detection of Change in Gene Expression Levels with QRT-PCR Application

For each experimental design, 3×10^5 cells were plated in six-well plates. The following day, the medium was aspirated, the cells were washed with phosphate saline buffer (PBS, Gibco, Carlsbad, CA, US), and FBS-free medium was added to the wells. The medium was aspirated, the cells were washed with phosphate buffer, and 0.05% trypsin-EDTA (Gibco, Carlsbad, CA, US) was added to the A549 cancer cells. Next, 0.25% trypsin-EDTA was added to the MSCs, which were incubated for 2, 12, or 24 h in a serum-free medium for RNA isolation. RNA was isolated using a NucleoSpin (Macherey Nagel, Bethlehem, PA, USA) RNA isolation kit following the manufacturer's protocol. The concentration of RNA was measured using a NanoDrop system (Multiskan Go Microplate Spectrophotometer, Thermo Fisher Scientific, Ratastie, P.O., Finland).

The cDNA synthesis kit (ProtoScript, BioLabs, New England) was used to synthesize cDNA from RNA, following the manufacturer's protocol. The amount of RNA used for cDNA synthesis was fixed to 1 µg. Then, cDNA was synthesized using a Bio-Rad T100 Thermal Cycler (Dubai, PO, United Arab Emirates).

The expression levels of the target genes were quantitatively determined by the delta delta Ct ($2^{-\Delta\Delta Ct}$) method, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) used as a reference gene. Quantitative gene expression RT-PCR was performed using a Bio-Rad Real-Time System (Bio-Rad, Dubai, PO, United Arab Emirates). The NCBI, Primary Blast, and Primer3 programs were used for primer design and were purchased from the Sentromer Company. The primer list is provided in Table 1.

All experiments were repeated twice biologically and twice technically.

Statistical Analysis

All data were analyzed using GraphPad Prism Statistics version 9. The mRNA expression levels of the genes and the mean and standard deviation of the ELISA results were determined. Two-way analysis of variance was performed to determine differences in the data between the 2 groups. All differences were considered to be statistically significant at $p<0.01$.

RESULTS

Assessment of the IL-12P70 Level by ELISA

The media obtained from the cocultures were used to measure cytokine levels by ELISA. The presence and amount of IL-12p70 in the media collected from the 3 different cell culture groups were analyzed after they were cultured for 2, 12, and 24 h. The data obtained in the second hour were used as a control for each cell culture group. The results obtained at the 12^{th} h and 24^{th} h were compared to those of the control group.

As shown in Figure 1, the measured levels of IL-12p70 (Table 2) in the culture media following 12 h and 24 h of incubation were significantly different from those of the control time point for each culture group. The highest IL-12p70 protein levels in the coculture medium were obtained after 12 h ($p<0.0001$). However, when the cells were evaluated separately without coculturing, the highest protein content was detected in the culture media of A549 cells and MSCs after 24 h of incubation, and the difference was statistically significant ($p<0.0001$).

Determination of mRNA Levels of Genes by qRT-PCR

At the end of 12 h and 24 h of culture, the MSCs and A549 cells were cultured alone, and the cocultured MSCs and A549 cells were harvested. Then, RNA was isolated, and the expression levels of the PCNA, cyclin A2, and CDK2 genes were determined. MSCs were used as a healthy control group

TABLE 1: Primers for qRT- PCR.

Primers	Sequences
Proliferatin cell nuclear Antige11 (PCNA)	Fonvard-S'-CCAGAGCTCTTCCCTTACGC-3' Reverse- 5'-TCTAGCTGGTTTCGGCTTCA -3'
Cydin A2	Fonvard- S'-AAGACTGGCATCCAAGAAGTTT-3' Revers&- 5'-TGGTTTTACTCTCATCTTGCCAC-3'
Cycline dependent kinase 2 (CDK2)	Fomard- S'-GGATGCCTCTGCTCTCACTG-3' Revers&- 5'-GAGGACCCGATGAGAATGGC -3'
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Fonvard- S'-CGAGATCCCTCCAAAATCAA-3 ' Reverse- S'-TTCACACCCATGACG AACAT-3'

qRT-PCR: Real-time polymerase chain reaction.

to determine target gene expression levels between each cell group, and expression at 2 h of incubation was used as a control for time-dependent comparisons of target gene expression in each cell group separately.

As shown in Figure 2, after 12 h of incubation, when the IL-12p70 level was the highest, the expression of PCNA, a cell cycle marker, decreased 0.683-fold in A549 cells cultured alone and 0.134-fold in cocultured A549 cells compared to the control cells ($p<0.0001$). In contrast, after 24 h of incubation, when the level of the IL-12 protein started decreasing, the maximum level of PCNA was detected in cocultured A549 cells, with a 1.196-fold increase ($p=0.0008$). At the end of 12 h, the level of expression of the PCNA gene in cocultured A549 cells was determined to be 0.225-fold lower than that in A549 cells cultured alone, and the difference was significant ($p=0.0047$). When IL-12 protein levels in the medium were high after 24 h of incubation, the protein expression in cocultured A549 cells was 1.785 times greater than that in A549 cells alone, and the difference was significant ($p=0.0093$). The expression levels of PCNA are shown in Table 3.

As shown in Figure 3, the level of cyclin A2, another cell cycle promoter, increased 12.88-fold in cocultured A549 cells after 12 h but 7.46-fold after 24 h of incubation ($p<0.0001$). After 12 and 24 h of incubation, A549 cells cultured alone presented a 2.86-fold and 13.34-fold increase in mRNA expression levels, respectively, compared to those at the control time point ($p<0.0001$). At the end of 12 h, the expression level of the

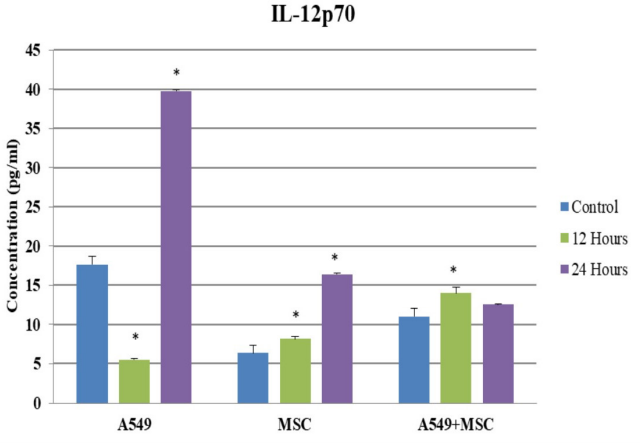

FIGURE 1: IL-12p70 results obtained by ELISA. *IL-12p70 levels are significantly different compared to those in the control group ($p<0.01$).

IL: Interleukin; MSC: Mesenchymal stem cell

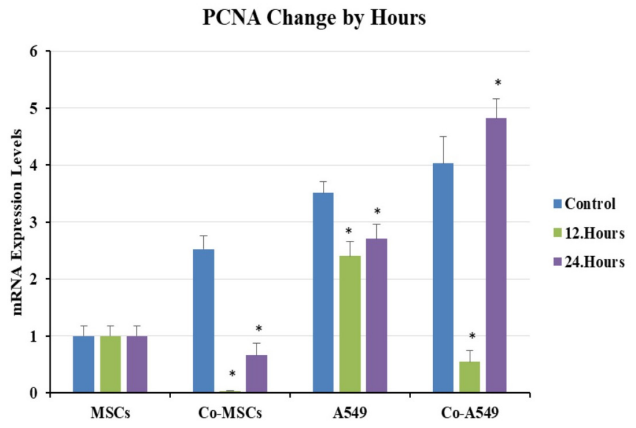

FIGURE 2: PCNA mRNA expression results obtained by qRT-PCR. Changes in the expression levels of the mRNAs in MSCs alone, A549 cells alone, and cocultured cell groups over time ($p<0.01$).

MSC: Mesenchymal stem cell; qRT-PCR: Real-time polymerase chain reaction; PCNA: Proliferating cell nuclear antigen

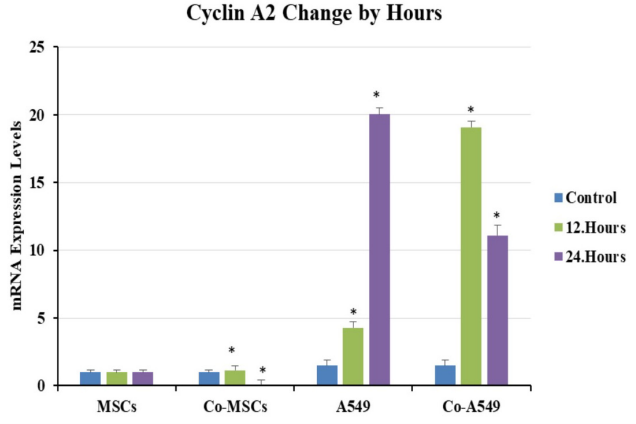

FIGURE 3: Cyclin A2 mRNA expression results obtained by qRT-PCR. Changes in the expression levels of the mRNAs in MSCs alone, A549 cells alone, and cocultured cell groups over time ($p<0.01$).

MSC: Mesenchymal stem cell; qRT-PCR: Real-time polymerase chain reaction

TABLE 2: IL-12p70 protein levels obtained by ELISA at the selected time points.

Groups	IL-12p70 levels after 2 b Mean ± SD	IL-12p70 levels after 12 b Mean ± SD	IL-12p70 levels after 24 b Mean ± SD
AS49 cells	17.65±3.42 pg/mL	5.525±1.18 pg/mL	39.775±1.71 pg/mL
MSC	6.40±0.26 pg/mL	8.150±3.16 pg/mL	16.40±2.10 pg/mL
A549+MSC	11.025±3.03 pg/mL	14.025±7.77 pg/mL	12.525±0.92 pg/mL

IL: Interleukin; ELISA: Enzyme-linked ImmunoSorbent assay; MSC: Mesenchymal stem cell, SD: Standard deviation.

cyclin A2 gene in cocultured A549 cells was upregulated 4.44-fold compared to that in A549 cells cultured alone (p=0.0002). At the end of 24 h, the expression level in cocultured A549 cells was downregulated 0.55-fold compared to that in A549 cells cultured alone (p=0.0001). The expression levels of cyclin A2 are shown in Table 3.

As shown in Figure 4, the mRNA levels of the *CDK2* gene in cocultured A549 cells increased 1.06-fold (p=0.5273) after 12 h of incubation and decreased 0.69-fold after 24 h of incubation (p=0.0010). A549 cells cultured alone increased by 1.25-fold (p=0.0774) and 1.71-fold after 12 and 24 h of culture, respectively (p<0.0001). At the end of 12 h, the CDK2 expression level of the cocultured A549 cells was upregulated 1.46-fold compared to that of the A549 cells cultured alone (p=0.0085), and the results were statistically significant. At the end of 24 h, the expression level of the cocultured A549 cells was downregulated 0.69-fold compared to that of the A549 cells cultured alone, and the results were not statistically significant (p=0.1541). The expression levels of CDK2 are shown in Table 3.

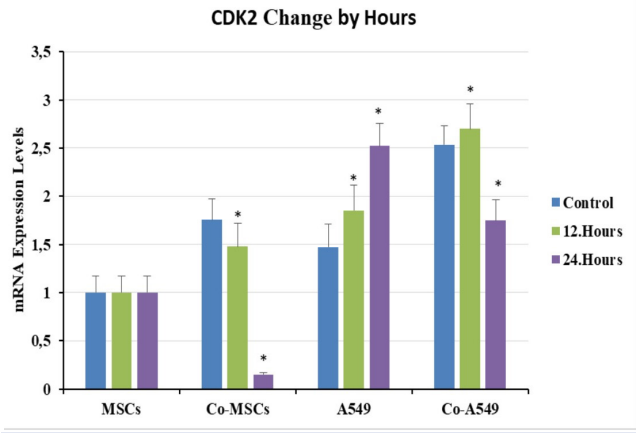

FIGURE 4: CDK2 mRNA expression results obtained by qRT-PCR. Changes in the expression levels of the mRNAs in the MSCs alone, A549 cell alone, and cocultured cell groups over time (p<0.01).

MSC: Mesenchymal stem cell; qRT-PCR: Real-time polymerase chain reaction; CDK2: Cyclin-dependent kinase 2

DISCUSSION

In this study, the IL-12p70 levels significantly changed over time when the cells were cocultured, and the effects of these changes on gene expression were significant. The results revealed that IL-12p70 levels varied over time and that their effects on cellular processes were significant. IL-12p70 content reached its maximum level during 12 h of incubation, and the decrease in the expression of PCNA, a cell cycle marker, in A549 cells during this period supported the suppressive effect of IL-12 on proliferation. In contrast, the decrease in IL-12p70 levels at the end of 24 h, followed by an increase in the expression of PCNA, suggested that the effect of IL-12 may change over time and that this change may be related to the dynamics of cellular responses. These findings indicated that IL-12 may regulate the cell cycle through direct or indirect effects as an immune modulator. The results of gene expression analyses also revealed the effect of IL-12p70 levels on the expression of cell cycle regulatory genes such as cyclin A2 and CDK2. The significant increase in cyclin A2 levels in the coculture medium at the end of 12 h suggested that IL-12 can stimulate the expression of cell cycle-related genes. However, the decrease in cyclin A2 expression observed at 24 h of incubation suggested that the effect of IL-12 on the cell cycle varies with time. Similarly, the increase in CDK2 expression peaked at 12 h and decreased at 24 h, suggesting

TABLE 3: mRNA expression levels and fold change in expressions of the cultured cells at the selected time points.

	Control	12 hours			24 hours		
	$\bar{X}$ ± SD	$\bar{X}$ ± SD	Fold change		$\bar{X}$ ± SD	Fold change	
MSCs_PCNA	1±0.17	1±0.17	1		1±0.17	1	
Co-MSCs_PCNA	2.52±0.23	0.03±0.01	↓0.01		0.66±0.22	↓0.26	
A549_PCNA	3.51±0.2	2.4±0.26	↓0.68	↓0.255	27.±0.26	↓0.88	↑1.785
Co-A549_PCNA	4.03±0.46	0.54±0.20	↓0.13		4.82±0.34	↓1.196	
MSCS_Cyclin A2	1±0.17	1±0.17	1		1±0.17	1	
Co-MSCS_Cyclin A2	1±0.17	1.15±0.30	↑1.15		0.34±0.34	↓0.34	
A549_Cyclin A2	1.5±0.43	4.29±0.43	↑2.86	↑4.44	20.01±0.46	↑13.34	↓0.55
Co-A549_Cyclin A2	1.49±0.43	19.08±0.47	↑12.88		11.09±0.74	↑7.46	
MSCs_CDK2	1±0.17	1±0.17	1		1±0.17	1	
Co-MSCs_CDK2	1.76±0.22	1.48±0.24	↓0.84		0.15±0.02	↓0.08	
A549_CDK2	1.47±0.23	1.85±0.26	↑1.25	↑1.46	2.52±0.23	↑1.71	↓0.69
Co-A549_CDK2	2.53±0.20	2.7±0.26	↑1.06		1.75±0.22	↓0.69	

↓ : Fold decrease in expression, ↑: Fold increase in expression; SD: Standard deviation; Co: Bicultural.

that IL-12 can regulate not only proliferation but also different stages of the cell cycle.

Stem cells are undifferentiated cells that can renew themselves and differentiate into different types of cells.[25] Stem cells obtained from adipose tissue are the focus of attention in cell therapy, as they can be isolated from adipose tissue via a less invasive procedure than other sources of stem cells.[26,27] In cancer treatment, stem cells increase vascularization, suppress immune reactions, and ultimately promote the growth and invasion of the mass. On the other hand, they are used as vectors in targeted therapy and exhibit antitumor effects. Studies have shown that human MSCs decrease the growth of cancer when they are administered to Kaposi's sarcoma model mice. The communication between MSCs and cancer cells is facilitated by the cytokines secreted from them, and the presence of cytokines in the environment reduces the reliability of stem cell treatments.[28-31] The main finding of this study is that stem cell-produced IL-12 affects cell cycle regulatory genes, and further studies may reveal its effects on cell proliferation and the prognosis of this disease.

Cytokines are signal proteins involved in communication between cells. After being released, they are activated by binding to their receptors on the target cell, triggering signal transduction in the cell.[10] IL-12 is a powerful anticancer cytokine that suppresses the growth of cancerous tissue by suppressing angiogenetic factors such as Vascular endothelial growth factor, stimulating apoptosis, and consequently increasing the activation of p53.[11,32]

IL-12 consists of the subunits p40 and p35. The p40 subunit is present in the cell as monomers and homodimers under normal conditions, and in the presence of p35, it creates p70 through a reduction in both forms. Some studies have shown that the half-life of p35 (one of the subunits) is 2 h, and in the presence of p40, this period may extend up to 4 h. The half-life of the p40 subunit is more than 4 h, and it is not affected by the presence of p35. However, for IL-12p70 to be released, it must be activated by combining with its receptors on the target cell. Therefore, optimal production occurs due to the balanced combination of the two subunits of the IL-12 protein and its receptors.[33,34] The half-life of IL-12 produced via recombinant DNA technology is 12 h. The desire to extend this period has contributed to the use and development of other methods, such as viral vectors, exosomes, and gene therapy, in cell treatments.[35,36]

The ELISA results revealed that the maximum level of IL-12 was released at the 24th h in the A549 and MSC groups cultured alone. However, at the end of the 12th h in A549 cells, IL-12 release was minimal compared to that of the control. This occurred probably because of an imbalance in the production of IL-12 subunits or receptors. On the other hand, maximum cellular secretion of endogenously produced IL-12 protein in the medium was first detected at 12 h in the MSC-A549 coculture. This occurred probably because the interaction of 2 different cell lines in the environment increased the expression of IL-12 receptors and subunits, and therefore, the release of IL-12.

The PCNA protein is responsible for DNA synthesis, cell cycle control, and DNA damage repair by wrapping chromatin. An increase in the expression of cell cycle genes is an indicator of cell proliferation, and this is considered to be an indicator of poor prognosis for diseases with high cell proliferation, such as cancer. Hu et al.[37] examined the expression levels of PCNA and E-cadherin in gastric cancer patients and reported that E-cadherin may play a protective role in the prognosis of patients when both markers are positive. However, tumor proliferation and metastasis increase in PCNA-positive E-cadherin-negative patients.[38] In this study, the PCNA mRNA expression level was minimal in cocultured A549 cells at the 12th h, when the maximum amount of IL-12 was released in the medium. This finding may provide evidence that IL-12-mediated suppression of the cell cycle produces an anticancer response due to a decrease in PCNA levels.

The expression levels of cyclins and CDKs, which are involved in the regulation of the cell cycle, increase in proliferative diseases such as cancer. Gopinathan et al.[17] reported that CDK2 and cyclin A2 knockout in mice reduced tumorigenesis. Dobashi et al.[15] studied lung carcinomas and reported that excess CDK2/cyclin A2 expression in malignant areas was associated with a poor prognosis. Unlike PCNA, the increase in cyclin A2 and CDK2 expression at the 12th h in cocultured A549 lung cancer cells indicated that these cell cycle markers may not be affected by the release of IL-12. However, the decrease in cyclin A2 and CDK2 expression after 24 h of incubation in cocultured A549 cells suggested that IL-12 levels may affect these cell cycle markers after affecting PCNA. Other studies have reported that PCNA is activated from the middle of the G1 phase to the end of the S phase of the cell cycle, whereas cyclin A2 and CDK2 are activated from the S phase to the middle of the G2 phase.[22,39] These cell cycle phases, in which PCNA, cyclin A2, and CDK2 are functional, might explain why PCNA is downregulated earlier than cyclin A2 and CDK2. An increase in PCNA levels following 24 h of coculture might be important for DNA repair in the G2 phase of the cell cycle when DNA replication ceases. Although A549 cells had the highest IL-12 levels at the 24th h compared to all experimental groups, cyclin A2 and CDK2 were not downregulated in A549 cells cultured alone. IL-12 secreted by A549 cells that are cultured alone starts autocrine proliferative signaling, which is widely observed in cancer cells.[40] These findings

suggest the importance of culturing lung cancer cells with MSCs in a coculture since these markers are downregulated in cocultured A549 cells.

CONCLUSION

Systemic administration of IL-12 in patients is toxic, and there are no data related to the spontaneous release of IL-12 in lung cancer cell culture studies. Therefore, we determined the level of IL-12 released from cocultured and cultured MSCs and A549 cells. In this study, novel data showed that the expression of the cell cycle markers PCNA, cyclin A2, and CDK2 decreased in cocultured A549 cells when IL-12 levels were high but not in A549 cell cultures alone; these findings highlighted the importance of coculturing lung cancer A549 cells with MSCs. These results provided insights into the use of MSC-mediated IL-12-related anticancer therapies as alternative methods for administering IL-12.

Ethics

Ethics Committee Approval: Not necessary.

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.B.I.Y., S.T., Concept: M.B.I.Y., S.T., Design: M.B.I.Y., S.T., Data Collection or Processing: M.B.I.Y., S.T., Analysis or Interpretation: M.B.I.Y., S.T., Literature Search: M.B.I.Y., S.T., Writing: M.B.I.Y., S.T.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The experiments were conducted at Haliç University.

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