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Evaluation of IKK-β, NF-kβ, p53, and Ki-67 Protein and Gene Expression in Neuroblastoma Cells Treated with Cisplatin

Sisplatin ile Tedavi Edilen Nöroblastoma Hücrelerinde IKK-β, NF-kβ, p53, Ki-67 Protein ve Gen Ekspresyonunun Değerlendirilmesi

Elif Kağa[1], Zafer Söylemez[2], Sadık Kağa[3]

[1]*Afyonkarahisar Health Sciences University, Şuhut Health Services Vocational School, Clinic of Medical Services and Techniques, Afyonkarahisar, Türkiye*

[2]*Afyonkarahisar Health Sciences University Faculty of Medicine, Department of Medical Biology, Afyonkarahisar, Türkiye*

[3]*Afyon Kocatepe University Faculty of Engineering, Department of Biomedical Engineering, Afyonkarahisar, Türkiye*

ABSTRACT

Background: Neuroblastomas are extracranial solid tumors caused by the differentiation and uncontrolled proliferation of immature sympathetic nervous system cells. This study investigated the effects of the anticarcinogenic cisplatin (Cis) on IKK-β, NF-κβ, p53, Ki-67 protein, and gene expression in neuroblastoma cells.

Materials and Methods: SH-SY5Y cells were treated for 48 hours with various doses of Cis (1, 3 and 10 μM). *IKBK1, MKI67, TP53,* and *NFKB1* gene expression analyses were completed by transcription-quantitative polymerase chain reaction (RT-qPCR). The levels of IKK-β, NF-κβ, p53, and Ki-67 proteins were detected by Western blotting.

Results: IKBKB gene expression was significantly increased at 1 and 3 μM and reduced significantly 10 μM compared to the control. *NFKB1* gene expression significantly increased at 1 μM Cis, whereas it decreased when Cis concentration increased. *TP53* gene expression significantly increased at 1 and 3 μM. MKI67 gene expression decreased with an increase in Cis concentration. In the low concentration (1 μM) of Cis group, while there was no significant change in IKK-β and p53 protein expressions, NF-κβ expression significantly increased. However, in the groups administered with high-dose Cis (3 μM, 10 μM), where Cis showed a significant antiproliferative effect, IKK-β expressions decreased significantly, while NF-κβ and p53 expression increased. The expression of the proliferation marker Ki-67 was also reduced in this group.

Conclusion: These results highlight the antiproliferative activity of Cis and its related NF-κβ/IKK-β, p53, and Ki-67 proteins and the marked changes in gene expression in neuroblastoma cells.

Keywords: SH-SY5Y, neuroblastoma, NF-kβ, p53, IKK-β, Ki-67

ÖZ

Amaç: Nöroblastomlar, olgunlaşmamış sempatik sinir sistemi hücrelerinin farklılaşması ve kontrolsüz çoğalmasının neden olduğu ekstrakraniyal katı tümörlerdir. Çalışmada nöroblastom hücrelerinde antikanserojen sisplatinin IKK-β, NF-κβ, p53, Ki-67 protein ve gen ekspresyonu üzerine etkilerinin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: SH-SY5Y hücrelerine, 48 saat boyunca çeşitli dozlarda sisplatin (1, 3 ve 10 μM) uygulanmıştır. *IKBK1, MKI67, TP53, NFKB1* gen ekspresyon analizi transcription-quantitative polymerase chain reaction (RT-qPCR) ile gerçekleştirildi. IKK-β, NF-κβ, p53, Ki-67 protein düzeyleri Western blot yöntemiyle tespit edildi.

Bulgular: IKBKB gen ekspresyonu, kontrole kıyasla 1 ve 3 μM'de önemli ölçüde arttı ve 10 μM'de önemli ölçüde azaldı. *NFKB1* gen ekspresyonu 1 μM sisplatinde önemli ölçüde artarken, sisplatin konsantrasyonu arttığında azaldı. *TP53* gen ekspresyonu 1 ve 3 μM'de önemli ölçüde arttı. MKI67 gen ekspresyonu sisplatin konsantrasyonunun artmasıyla azaldı. Düşük konsantrasyonlu (1 μM) sisplatin grubunda IKK-β ve p53 protein ekspresyonlarında anlamlı bir değişiklik olmazken, NF-κβ ekspresyonunda anlamlı artış görüldü. Ancak sisplatinin anlamlı antiproliferatif etki gösterdiği yüksek doz sisplatin (3 μM, 10 μM) uygulanan gruplarda IKK-β ekspresyonları anlamlı derecede azalırken NF-κβ ve p53 ekspresyonları arttı. Bu grupta prolifrasyon belirteci Ki-67'nin ifadesi de azalmıştır.

Sonuç: Bu sonuçlar, sisplatinin ve bununla ilişkili NF-κβ/IKK-β, p53 ve Ki-67 proteinlerinin antiproliferatif aktivitesini ve nöroblastoma hücrelerinde gen ekspresyonlarının belirgin şekilde değiştiğini vurgulamaktadır.

Anahtar Kelimeler: SH-SY5Y, nöroblastoma, NF-kβ, p53, IKK-β, Ki-67

Address for Correspondence: Elif Kağa, Afyonkarahisar Health Sciences University, Şuhut Health Services Vocational School, Clinic of Medical Services and Techniques, Afyonkarahisar, Türkiye

Phone: +90 532 584 57 67 E-mail: elif.kaga@afsu.edu.tr **ORCID ID:** orcid.org/0000-0002-2279-6105

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Introduction

Neuroblastoma is a type of cancer that develops from the uncontrolled proliferation of precursor nerve cells. Metastasis is one of the most common causes of mortality in patients with neuroblastoma (1). Surgery, chemotherapy, and radiotherapy are the current treatment methods for neuroblastoma. Although these current treatments are effective in patients with neuroblastoma, the prognosis of these patients is still poor (2). Cisplatin (Cis) is a cytotoxic drug that inhibits cancer cell proliferation by damaging DNA and is generally accepted as an effective drug used in chemotherapy for neuroblastoma (3). Cis plays a role in inflammation by activating proinflammatory cytokines and nuclear factor kappa-B kinase subunit beta (IKK-β) /nuclear factor-κβ (NF-κβ) or p53 pathways (4).

The IKK complex is activated in the immunoresponse, cell survival, and cancer. The IKK complex comprises catalytic subunits (IKK-α, IKK-β kinases) and a regulatory subunit (IKK-γ). IKK-β mediates phosphorylation of IκB, an important step in multiple signaling pathways leading to NF-κβ activation (5). Inflammation is a process that is often associated with the development and progression of cancer through the activation of multiple signaling pathways, including the NF-κβ pathways (6). It has been shown that over expression of NF-κβ promotes cancer cell proliferation, survival, and metastasis (7). In addition, it was reported that NF-kβ expression increased significantly in mice kidney tissue after Cis administration compared with the control group (8).

The protein complex known as NF-κβ is responsible for regulating the expression of genes that play important roles in immune response and cell survival. *NFKB1* encodes the p105 protein, which is a precursor of the NF-κβ transcription factor that gives rise to the p50 subunit of NF-κβ upon proteolytic processing. NF-κβ p65, on the other hand, is a constitutively expressed subunit of NF-κβ that directly participates in gene regulation upon nuclear translocation, often forming heterodimers with other NF-κβ subunits. Both subunits play an important role in the NF-κβ signaling pathway and are involved in various cellular processes, including inflammation, immunity, cell proliferation, and apoptosis (8,9).

The tumor suppressor p53 protein is a multifunctional protein that regulates a multitude of cellular processes. The p53 protein is a nuclear transcription factor that activates numerous target genes related to multiple cellular events and regulates cell cycle progression and DNA repair (10). P53 is an important transcription regulator in Cis nephrotoxicity. It has been shown that Cis treatment induces p53 phosphorylation and protein accumulation in p53 knockout C57BL/6 mice (11). Increased p53 and NF-κβ expression was observed in neuroblastoma cells treated with the anti-carcinogenic Doxorubicin. Loss of NF-κβ activity inhibited p53-induced apoptosis. These results show that NF-κβ plays a critical role in p53-mediated cell death (5).

We analyzed the protein and gene expression profiles of IKKβ, NF-κβ, p53, and Ki-67 to investigate the mechanism of action of Cis in Neuroblastoma. We report the functional importance and interconnection of IKK-β, NF-κβ, and p53 in Cis-induced neuroblastoma cell death. Comprehending the intricate molecular processes that underlie the development of neuroblastoma is of utmost importance for devising tailored treatment strategies and enhancing the prognosis of patients. Comprehending the interactions and regulatory mechanisms of these genes may offer insights into the etiology of neuroblastoma and facilitate the identification of potential targets for therapeutic intervention.

Materials and Methods

Because a commercially purchased cell line was used in the study, ethical approval is not required in terms of the method.

Reagents

Cell Counting Kit 8 (CCK-8) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The neuroblastoma cell line (SH-SY5Y-CRL-2266) was obtained the American Type Culture Collection [(ATCC), USA]. Dulbecco's modified Eagle's medium (DMEM), heat-inactivated fetal bovine serum (FBS) and Antibodies against IKKβ, NF-κβ-p65, p53, Ki-67, β-Actin were purchased from Invitrogen (Thermo Fisher Scientific Inc. USA).

Cell Culture

Neuroblastoma cells were cultured in DMEM containing 10% FBS and 1% penicillin-streptomycin at 37 °C in a 5% CO_2 environment.

Proliferation Assay

Neuroblastoma cells ($3x10^3$ cells/well) were seeded into 96-well plates. After 24 hours, cells were treated with increased Cis concentrations (1, 3,10 μM) for 48 hours at 37 °C. 10 μl of CCK-8 solution was added to the wells, and the cells were further incubated for 4 hours at 37 °C. The absorbance was then determined at 450 nm using a plate (Thermo Scientific Mutilskan FC, Thermo Fischer Scientific, USA). Cell viability was measured compared with the optical density value of the control group. Proliferation analysis was performed in four replicates.

Western Blotting

After treatment, cells were washed twice with phosphate-buffered saline and lysed with radioimmunoprecipitation assay buffer supplemented with protease inhibitors (Santa Cruz Biotechnology, Inc. Europe). The protein concentration of samples was determined using a Bicinchoninic acid protein assay kit (Thermo, Rockford, IL, USA). 30 µg of protein was subjected to SDS-PAGE on 12% gel. The protein was then transferred onto a nitrocellulose membrane (Bio-Rad, Philadelphia, PA, USA). Nitrocellulose membranes were blocked with 5% non-fat dried milk in TBS with 0.1% Tween for 1 hour. After blocking, the membrane was incubated with primary antibodies at 4 °C overnight and then washed three times in Tris-buffered saline with 0.1% Tween® buffer. Mebrane was incubated in horseradish peroxidase-conjugated secondary antibody (Thermo Fisher Scientific Inc. USA) for 1 hour. Specific protein bands were detected using an enhanced chemiluminescence Western Blotting Substrate (Thermo Fisher Scientific Inc. USA). Bands were quantified using ImageJ Software.

RNA Extraction and Genetic Analyses

Total RNA was extracted from SH-SY5Y cells using PureZole (#7326890, Biorad, USA) for genetic analysis. The purity and quantity of the RNA samples were determined using Nanodrop ND-1000 spectrophotometer V3.7. Following this, all RNA samples were converted into cDNA (1708890, Biorad, USA). The transcription-quantitative polymerase chain reaction (RT-qPCR) method with the Step-One-Plus Thermocycler (Applied Biosystems) was employed to analyze the expression of *IKBKB*, *NFKB1*, *MKI67*, and *TP53* mRNA. The amplifications were performed using cDNA, site-specific primers (Oligomer Biotechnology, Ankara), SYBR Green (1725270, Biorad, USA), and nuclease-free water. To ensure internal control, *GAPDH* was used. The primer sequences of *IKBKB*, *NFKB1*, *MKI67*, and *TP53* were designed on the basis of the FASTA format from NCBI (Table 1) (12).

The subsequent RT-qPCR protocol was employed: for *IKBKB*, *NFKB1*, and *TP53*, 95 °C for a duration of 30 s, 40 cycles consisting of 5 s at 95 °C and 30 s at 60 °C. Additionally, for MKI67, 30 s at 95 °C, 40 cycles consisting of 5 s at 95 °C and 30 s at 58 °C. For confirmation of singular product amplification, a melting curve analysis was performed at the conclusion of the PCR. Each run was executed in triplicate.

Table 1. Primer sequences

Gene	Forward primer 5'→3'	Reverse primer 5'→3'
TP53	CCGGGGACACTTTGCGTT	AGTCTGGCCAATCCAGGGAAG
MKI67	GTGGTTCGACAAGTGGCCTT	AGTTGGGTCTCCCCCTGTAA
NFKB	TGTGCTTCGAGTGACTGACC	TCACCCCACATCACTGAACG
IKBK	TCTCACGTCTGACGGACTCT	CCTGGCATTCCTTAGTGGCA
GAPDH	CATTGCCCTCAACGACCACTTT	GGTGGTCCAGGGGTCTTACTCC

Statistical Analysis

Data were analyzed using the ordinary One-Way analysis of variance test with GraphPad Prism 8.0 software (GraphPad Software, Inc., San Diego, CA). Statistical significance was considered *p<0.05, **p<0.01 compared with the control group. Genetic analysis was performed using REST 2009 V2.0.13 (13) where p<0.05 is considered to be statistically significant.

Results

Genetic Analysis

The mRNA levels of *IKBKB*, *NFKB1*, *TP53*, and *MKI67* genes expressed in SH-SY5Y neuroblastoma cancer cell lines exposed to 1, 3, 10 µM Cis for 48 hours were compared with control cells. The mRNA levels of *IKBKB* were increased in 1 µM and 3 µM Cis and decreased 10 µM Cis compared with the control (2.27*; 5.61*; 0.47*, fold change, respectively, *p<0.001). The mRNA levels of *NFKB1* were increased in 1 µM and 3 µM Cis and decreased 10 µM Cis compared with the control (1.34*; 1.05; 0.81*, fold change, respectively, *p<0.001). The mRNA levels of *TP53* were increased in 1 µM and 3 µM Cis and decreased 10 µM Cis compared with the control (2.86*; 2.36*; 0.40, fold change, respectively, *p<0.001). The mRNA levels of *MKI67* were decreased in 1 µM and 3 µM Cis compared with the control (0.76; 0.68 fold change, respectively, no data obtained at 10 µM amplification plot) (Figure 1).

Cis-induced Antiproliferative Effect on Neuroblastoma Cells

To investigate the antiproliferative effect of Cis, we tested a human neuroblastoma cell line using the CCK-8 kit. According to our results, Cis inhibited cell proliferation in a dose-dependent manner. In the 3 µM Cis-treated group, 19% proliferation inhibition was determined, and in the 10 µM Cis-treated group, 77% proliferation inhibition was determined compared with the untreated control. 1 µM of Cis did not affect the proliferation of the cells compared with the untreated control (Figure 2).

Determining IKKβ, NF-κβ, p53, and Ki-67 Expression Profiles

We examined total protein levels of IKKβ, NF-κβ, p53, and Ki-67 in neuroblastoma cells treated with 1 µM, 3 µM and 10 µM Cis. NF-κβ signaling has an essential role in the regulation of inflammation, cell survival, and cell proliferation. NF-kβ signaling is also active in neuroblastoma and has various functions in cancer progression (14). As shown in Figure 2, the NF-κβ protein expression significantly increased after

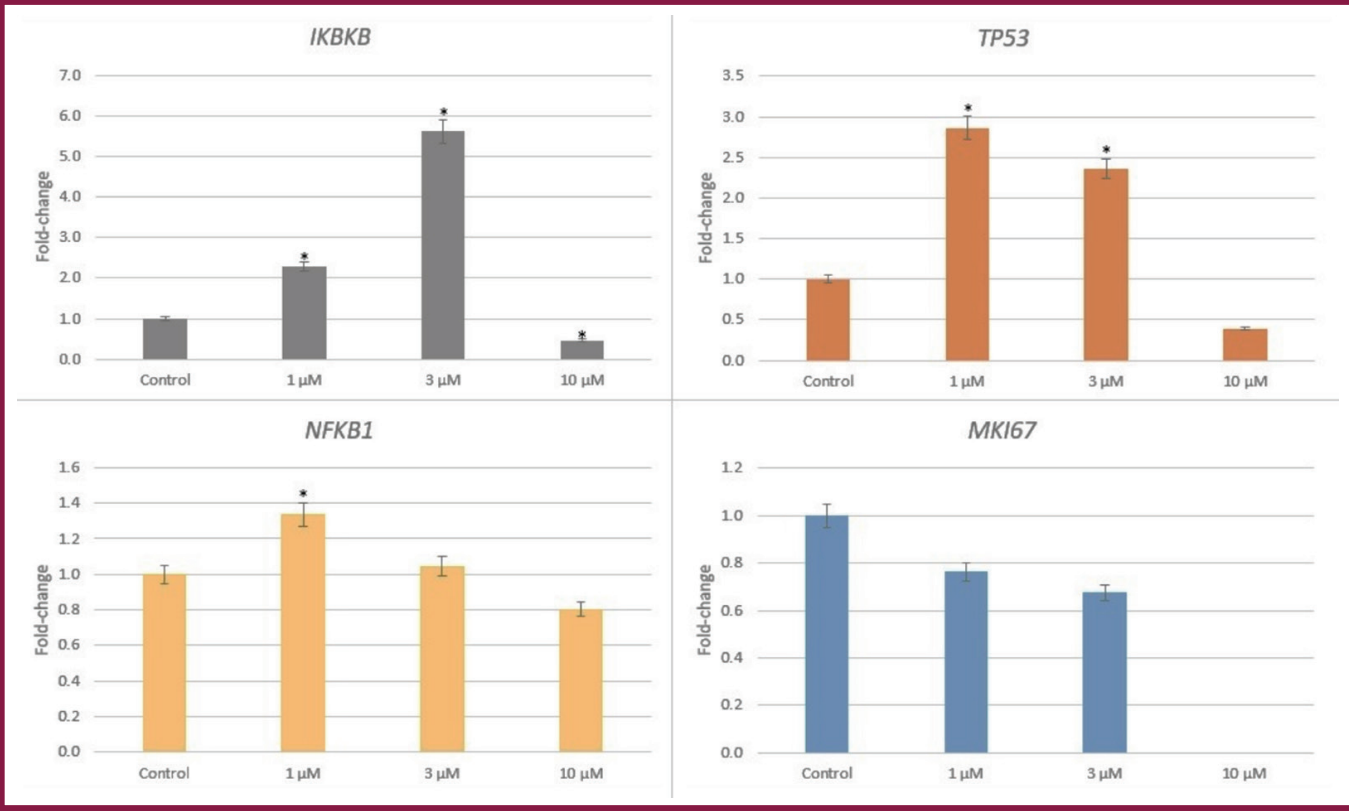

Figure 1. The results of real-time PCR. Relative mRNA expression of *IKBKB*, *NFKB1*, *TP53*, and *MKI67* genes in SH-SY5Y neuroblastoma cells exposed to Cis are given as fold change compared with the control. *Represents the significance of p<0.001. *GAPDH* was the reference gene for normalization

PCR: Polymerase chain reaction, Cis: Cisplatin, GAPDH: Glyceraldehyde-3-phosphate dehydrogenase

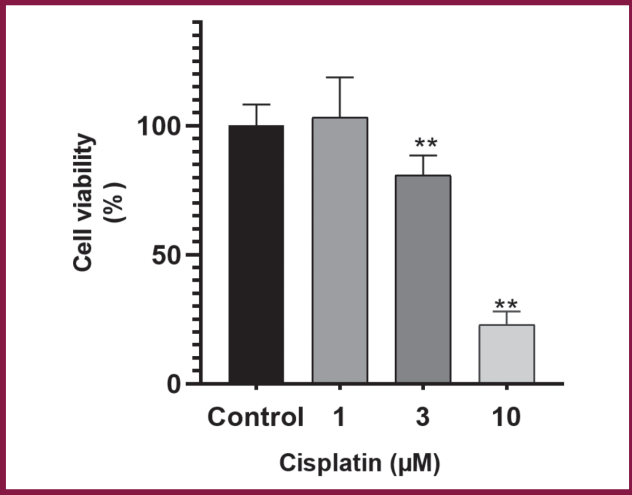

Figure 2. Cytotoxicity analysis of Cis (1, 3, and 10 µM) in SH-SY5Y neuroblastoma cells for 48 hours. Results are presented as mean ± SD of triplicate experiments. **p<0.01 compared with control

Cis: Cisplatin, SD: Standard deviation

exposure to Cis (1, 3 and 10 µM) compared to untreated control in neuroblastoma cells.

The IKK complex is an important regulator for NF-κβ Activation in cancer progression. The kinase IKK activates NF-κβ signaling molecules by phosphorylating the inhibitor I-κβ. As shown in Figure 3, the IKKβ protein markedly increased in the 1 µM and 3 µM Cis treated groups but decreased in 10 µM Cis group compared with the control. Ki-67 is an important prognostic marker in various tumors, including neuroblastoma (15). As shown in Figure 3, Ki-67 expression significantly decreased in 10 µM Cis group compared with the control.

P53 protein expression was analyzed in 64 human esophageal squamous cell carcinoma tumor tissues, and it was shown that p53 expression was higher than that in non-neoplastic tissues (16). P53 protein acts as an activator for apoptosis induced by DNA damage, such as Cis (17). Similarly, p53 protein expression levels increased in the 3 µM and 10 µM Cis treated groups compared with the control group (Figure 3).

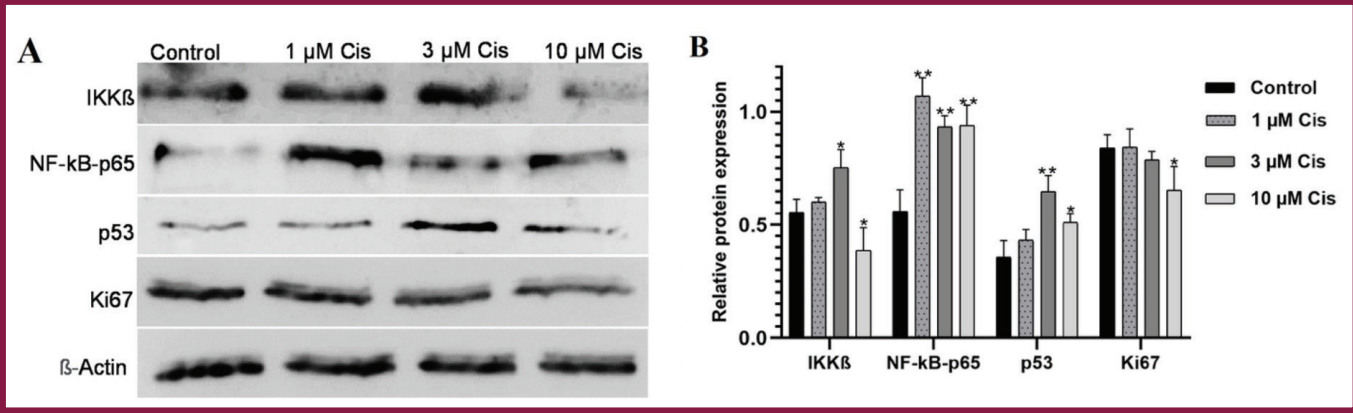

Figure 3. A) Western blot results of protein expression of IKKβ, NF-κβ, p53, Ki-67, β-actin. Cells were treated with increased Cis concentrations (1, 3, and 10 μM). **B)** Densitometric analysis of each band was performed using ImageJ. The relative density ratio of each band was normalized to β-actin as the internal control. Results are presented as mean ± SD. *p<0.05, **p<0.01 (n=3)

Cis: Cisplatin, SD: Standard deviation

According to the results, protein expression changes were correlated with Cis doses. The expression of IKKβ, NF-κβ, p53, and Ki-67 proteins was increased in the low-dose Cis (1, 3 μM) treated group. However, IKKβ expression significantly decreased in 10 μM Cis treated group. Similarly, Ki-67 expression was decreased in the high-dose Cis (10 μM) treated group.

These results highlight the antiproliferative activity of Cis and its related IKK-β/NF-κβ, p53 and Ki-67 protein expression changes in neuroblastoma cells.

Discussion

Neuroblastoma, an oncological condition originating from nascent nerve cells distributed across various regions of the human anatomy, predominantly affects infants and children. Elucidating the interconnection between genetic elements within the ambit of neuroblastoma can offer valuable knowledge regarding the molecular processes underpinning its formation and to identify potential targets for therapeutic interventions. Treatment of neuroblastoma usually includes surgery, chemotherapy, and radiotherapy. However, poor prognosis in the metastatic stage limits treatments (1).

The genes *NFKB1*, *TP53*, *IKBKB*, and *MKI67* are involved in different cellular processes and pathways, but they can interact indirectly through various signaling networks. Although these genes may not directly interact at the gene or protein level, their functions intersect within broader cellular signaling networks involved in inflammation, apoptosis, cell cycle regulation, and tumorigenesis. In the context of neuroblastoma or other cancers, dysregulation of these pathways can contribute to tumor development and progression. Within the realm of neuroblastoma, the interplay between these genes can influence tumor development, progression, and response to therapy.

Dysregulation of NF-κβ signaling, often through aberrant activation or overexpression of *NFKB1*, has been implicated in various cancers (18,19), including neuroblastoma (1,20). NF-κβ signaling is known to be dysregulated in neuroblastoma, and increased expression of *NFKB1* mRNA and NF-κβ-65 protein may indicate activation of the NF-κβ pathway. Zhi et al. (1) suggested that targeting NF-κβ signaling or its downstream target *CXCR4* may be a potential therapeutic strategy for inhibiting neuroblastoma metastasis and improving patient outcomes. The suppression of neuroblastoma cell migration and invasion is postulated to result from the inhibition of NF-κβ activity (21). In the present study, treatment resulted in a significant increase in *NFKB1* mRNA at 1 μM Cis compared with the control, whereas it decreased when the Cis concentration was increased. Beside, NF-κβ-65 protein level was increased, especially at 1 μM Cis concentration in cultured SH-SY5Y cells. Both subunits (*NKFB1* p105 and NF-κβ-65) play an important role in the NF-κβ signaling pathway.

IKBK1, is inhibitor for IKK-β, encodes a protein referred to as IKK-β. This protein functions as a subunit within the IKK complex and is responsible for initiating the NF-κβ signaling pathway. It is plausible that *IKBK1* contributes to the promotion of neuroblastoma cell growth and survival using activating NF-κβ. The IKK-β functions as an inhibitor within the Iκ-β kinase (IKK) complex, which in turn stimulates the activation of the transcription factor NF-κβ (22). Decreased *IKBKB* expression and *IKBKB* protein activity increase

anticancer treatment effectiveness (23). Inactive NF-κβ/I-κβ complex is activated by phosphorylation at specific serine residues of Iκ-β proteins. Phosphorylated I-κβ is degraded by the proteasomal system, resulting in NF-κβ activation and translocation to the nucleus (24). It has been shown that the migration and invasion properties and IKK-β/NF-κB activity of Cis-resistant HNSCC cells have increased compared with the control groups (25). Cis treatment results in a cardiac inflammatory response accompanied by an increase in tumor necrosis factor-alpha and interleukin-6 levels. These results showed that Cis treatment caused a significant upregulation of cardiac NF-κβ, STAT-3, and p-STAT-3, and Iκβ levels were significantly reduced (26). In our study, the content of IKK-β protein significantly increased at 3 μM and decreased significantly at 10 μM; similarly, *IKBKB* gene expression significantly increased at 1 and 3 μM and decreased significantly at 10 μM. This indicates that Cis treatment modulates the transcriptional regulation of *IKBKB* in a dose-dependent manner. Overall, these results suggest that Cis treatment modulates the expression of *IKBKB* and *NFKB1* in neuroblastoma cells, with complex effects on both protein and mRNA levels. In addition, the response of NF-κβ protein levels to Cis treatment may involve regulatory mechanisms distinct from *NFKB1* mRNA expression. Further studies are needed to elucidate the precise molecular mechanisms underlying these observed effects and their implications for neuroblastoma biology and treatment.

p53, a widely recognized tumor suppressor protein, plays a critical role in upholding genomic stability and averting cancer development. It governs cell cycle progression, apoptosis, DNA repair, and senescence. Genetic analyses indicate that the p53 protein plays a pivotal role in safeguarding cells from both genome instability and malignant transformation. It is noteworthy that the majority of neuroblastomas exhibit wild-type p53 with unimpaired transcriptional function, thereby suggesting that the mutated *TP53* gene, which encodes p53, is not commonly implicated in this particular type of cancer. In fact, the mutation rate of *TP53* in neuroblastomas does not surpass a mere 1-2% (27). The p53 tumor suppressor plays a pivotal role in triggering cell death or halting cell division in response to DNA harm and stress within the cell (28,29). Mutations or aberrant regulation of p53 are frequently observed in diverse malignancies, including neuroblastoma. The absence of functional p53 can instigate unbounded cell proliferation, survival, and resistance to therapeutic interventions in neuroblastoma. Moreover, research has suggested that the signaling pathway of p53 is operational in neuroblastoma (30,31,32). p53 protein expression was analyzed in 64 human esophageal squamous cell carcinoma tumor tissues and it was shown that the p53 expression level was higher than that in non-neoplastic tissues (16). p53 protein acts as an activator for apoptosis induced by DNA damage, such as Cis (17). Similarly, p53 protein expression levels increased in the 3 μM and 10 μM Cis treated groups compared with the control group. Similarly, *TP53* mRNA levels were increased by Cis treatment. The increase in mRNA and protein levels may indicate the anti-proliferative effect of Cis. In contrast, *TP53* is a tumor suppressor gene mostly mutated in neuroblastoma. The increase in p53 protein levels and *TP53* gene expression at 3 and 10 μM Cis concentrations may indicate the activation of p53-mediated apoptotic pathways in response to Cis-induced DNA damage.

The tumor suppressor p53 is involved in cell signaling, apoptosis, and DNA repair. It can interact with NF-κβ signaling by modulating the activity of NF-κβ target genes and influencing cell fate decisions. Cis treatment in neuroblastoma can influence p53 expression and activation. The functional status of p53 plays a significant role in determining cellular responses to Cis-induced DNA damage, apoptosis, and overall treatment outcomes. Further research into the molecular mechanisms underlying the interaction between Cis and p53 signaling pathways is needed to develop more effective treatment strategies for neuroblastoma.

MKI67 is a protein-coding gene. *MKI67*, also known as the marker of proliferation Ki-67, is responsible for the synthesis of the Ki-67 protein. This protein serves as a cellular indicator of cell proliferation. For decades, the Ki-67 protein has served as a prominent marker for measuring the proliferation of human tumor cells. Recent studies have shed light on the diverse molecular functions of this sizable protein. Ki-67 is involved in various activities in both interphase and mitotic cells, with its cellular localization undergoing significant alterations throughout the cell cycle (33). Ki-67 expression is observed during the active phases of the cell cycle, namely G1, S, G2, and M phases, but is absent in resting G0 cells. Its widespread application in cancer diagnostics and prognosis stems from its role as a marker of cell proliferation. Notably, an elevated level of Ki-67 expression often correlates with heightened tumor cell proliferation and increased aggressiveness (34,35). Ki-67 is a marker of cell proliferation, and its increased expression at lower Cis concentrations (1 μM) followed by a decrease at higher concentrations suggests a potential cytotoxic effect of Cis on neuroblastoma cells. Similarly, the changes in *MKI67* gene expression parallel the alterations in Ki-67 protein levels, reflecting changes in cell proliferation dynamics in response to Cis treatment. The genes *NFKB1*, *TP53*, and *IKBKB* are all associated with the NF-κβ signaling pathway. In the context of neuroblastoma, the interaction

between these genes can influence tumor development, progression, and response to therapy.

We analyzed the predictive value of *IKBK1*, *MKI67*, *TP53*, *NFKB1*, protein, and gene expression for the efficacy of Cis in neuroblastoma cell lines. We reported the functional importance and interconnection of *IKBK1*, *NFKB1*, *TP53*, *and MKI67* in Cis-induced neuroblastoma cell death. The results show that increasing concentrations of Cis was induced NF-κβ, p53 activation, and cell death in neuroblastoma cells. IKK-β/NF-κβ/p53 signaling can be suggested as an alternative therapeutic target for increasing the effectiveness of Cis treatment in neuroblastoma.

Conclusion

In brief, the aforementioned genes fulfill significant functions in the molecular pathways that underlie the development and progression of neuroblastoma. Disruption of these genes may contribute to irregular cell proliferation, survival, and other characteristic features of cancer. Comprehending the interactions and regulatory mechanisms of these genes may offer insights into the etiology of neuroblastoma and facilitate the identification of potential targets for therapeutic intervention.

Overall, our results provide insights into the molecular mechanisms underlying the response of neuroblastoma cells to Cis treatment, highlighting the relationship between NF-κβ signaling, p53-mediated apoptosis, and cell proliferation pathways. These results highlight the antiproliferative activity of Cis and related NF-κβ/IKβ/p53 and Ki-67 proteins and gene expression changes in neuroblastoma cells.

Ethics

Ethics Committee Approval: In the study, ethical approval is not required in terms of the method.

Informed Consent: Ethical approval is not required for our study so we do not need consent.

Authorship Contributions

Surgical and Medical Practices: E.K., Z.S., Concept: E.K., S.K., Design: E.K., S.K., Data Collection or Processing: E.K., S.K., Analysis or Interpretation: E.K., Z.S., Literature Search: E.K., Z.S., S.K., Writing: E.K., Z.S., S.K.

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

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