

Melatonin Increases the Sensitivity of Osteosarcoma Cells to Chemotherapy Drug Cisplatin

Authors

Forough Hosseini¹, Dariush Shanehbandi², Jafar Soleimanpour³, Bahman Yousefi¹, Forough Alemi¹

Affiliations

- 1 Department of Biochemistry and Clinical Laboratories, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
- 2 Molecular Medicine Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
- 3 Department of Orthopedics Surgery, Shohada Teaching Hospital, Tabriz University of Medical Sciences, Tabriz, Iran

Forough Alemi

Tabriz University of Medical Sciences Clinical Biochemistry and Laboratory Medicine

29 Bahman Blvd

Tabriz

5166616471 East Azerbaijan

Iran (the Islamic Republic of)

forough.alemi@gmail.com

Key words

Bone cancer, cisplatin, combination therapy, P53, BCL2, non-coding RNA

received 15.01.2022

accepted 12.04.2022

published online 30.05.2022

Bibliography

Drug Res 2022; 72: 312–318

DOI 10.1055/a-1830-8716

ISSN 2194-9379

© 2022. Thieme. All rights reserved.

Georg Thieme Verlag, Rüdigerstraße 14,
70469 Stuttgart, Germany

Corresponding

Bahman Yousefi

Tabriz University of Medical Sciences Clinical Biochemistry

Azadi Street

Toba Station

Motahari Building, 606

Tabriz

5166/15731

Iran (the Islamic Republic of)

Tel.: +989143456196

yousefib@tbzmed.ac.ir

ABSTRACT

Chemotherapy, which is one of the common treatments for osteosarcoma (OS), has many side effects and in some cases has low effectiveness due to chemoresistance, hence it is vital to study new therapies for OS. In this regard, we combined melatonin with cisplatin and evaluate their effect on MG63 OS cells. Since melatonin has anti-cancer properties, we hypothesized that its combination with cisplatin could increase the effectiveness of cisplatin. Firstly, MTT assay was used to evaluate the cell viability and cytotoxicity of cisplatin on MG63 cells and the results showed that melatonin in combination with cisplatin increases the sensitivity of MG63 cells to cisplatin. In addition, qRT-PCR results showed that the expressions of miR-181 and P53, CYLD, CBX7 and BCL2 genes change in MG63 cells after treatment with the combination of cisplatin and melatonin, so that the expression of P53, CYLD and CBX7 increased and the expression of BCL2 and miR-181b decreases significantly. Furthermore, analysis of Annexin V/FITC assay data revealed that the rate of apoptosis in MG63 OS cell line remarkably promoted after treated with cisplatin and melatonin combination. As a result, our findings show that melatonin in combination with cisplatin increases the effectiveness of cisplatin in osteosarcoma cells and this study provides a new therapeutic approach for OS.

Introduction

Osteosarcoma (OS) is a primary bone malignancy that affects long bones and mostly occurs in young people [1]. OS usually metastasizes to the lungs and it is a sign of poor prognosis in OS patients [2]. In 1970, the prevalent treatment of OS was amputation which was not effective on patients' survival rate, while in 1980, neoad-

juvant chemotherapy and surgery to some extent increased patient survival, but the survival rate was still low in patients with metastasis [3]. Since chemotherapy and surgery have side effects and are invasive treatment methods, efforts are being made to discover new strategies in OS treatment.

Cisplatin (cis-diamminedichloroplatinum II, DDP) is a platinum-based chemotherapy drug which is used against a variety of cancers such as ovarian cancer, lung cancer, and OS. Cisplatin binds to DNA through N7 nucleophilic sites and inhibits the production of mRNA and protein, which ultimately prevents tumor cells proliferation and initiates apoptosis [4, 5]. Although cisplatin is widely used in chemotherapy, its side effects and resistance of OS cells to cisplatin are two of the most important issues that have led researchers to seek new therapies [6]. One of the novel strategies based on cisplatin is combinational therapy in which a low dose of cisplatin combines with other agents to improve its efficacy on tumor cells [6, 7].

Melatonin (N-acetyl-5-methoxytryptamine, MLT) is a physiological hormone that regulates the sleep-wake cycle in the human body. Furthermore, studies have proven that MLT plays an important role in cancer. MLT can be a pro-apoptotic and anti-metastatic agent in cancers including OS. Thus, it reduces tumor development and improves immune system activity. MLT can be used as a combination agent along with chemotherapy drugs to reduce their side effects and increase the efficacy of chemotherapy drugs in OS [8–10].

microRNAs (miRNAs) are small and non-coding RNAs (ncRNAs) which regulate gene expression through binding to 3'-UTR of mRNA [11]. miRNAs play an important role in cancers and usually act as suppressor tumors or oncogenes. Accordingly, they can be used as diagnostic or therapeutic, or prognostic biomarkers in cancers [12, 13]. miR-181 family includes four miRNAs: miR-181a, miR-181b, miR-181c, miR-181d. miR-181b upregulates in pancreatic and bladder cancer and OS while it downregulates in gastric and prostate cancer [11]. Upregulation of miR-181b increases OS proliferation and metastasis [14]. In some types of cancer miR-181b target some specific genes, for instance, it is shown that miR-181b targeted CBX7, and reciprocally CBX7 negatively regulated miR-181b in human breast adenocarcinoma cells [15]. Furthermore, CYLD is another potential target of miR-181b in breast cancer and thyroid papillary cancer. miR-181b contributes to cell growth in breast cancer and thyroid cancer by suppressing the expression of CYLD [16, 17].

BCL2 is an anti-apoptotic protein that prevents the release of cytochrome c from mitochondria, thereby inhibiting caspase and apoptosis activation. Hence, BCL2 increases tumorigenesis. High expression of BCL2 in OS is associated with poor prognosis and low survival [18–20].

P53 is a tumor suppressor gene that has several roles in cells such as growth arrest, apoptosis, and DNA stability. The expression of P53 is decreased in OS which is correlated with poor prognosis in patients and chemotherapy resistance. Low expression of P53 reduces apoptosis rate in OS cells. On the other hand, upregulation of P53 leads to chemosensitivity in OS [21, 22].

Materials and Methods

All experimental procedures were applied in accordance with the approval from the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.VCR.REC.1398.265).

Materials

The material used for cell culture included RPMI-1640 medium, fetal bovine serum (FBS), and trypsin solution. Chemical reagents

included MLT (Cayman, USA), cisplatin (Cayman, USA). RT-PCR reagents included RNA extraction solution (Cinnagen), SYBR green master mix, and reverse transcriptase kit which were purchased from Bioneer company.

Cell Culture

For cell culture, the Human OS cell line MG63 was provided from the Pasteur Institute of Iran (Tehran, Iran). Saos2 was cultured in RPMI-1640 medium containing 10% FBS and incubated at 37°C, 5% CO₂, and 95% humidity. The culture medium is sterilized by 0.22 μm microbiological filters and stored at 4°C. The cells were passaged with wash solution (PBS) and 1 ml of trypsin.

Cell viability and proliferation assay

Cell proliferation was determined by MTT assay. MG63 cell line was seeded into 96-well plates (5 × 10³ cells per well). The plate was divided into 4 groups: 1. Control without any treatment, 2. MLT, 3. cisplatin, 4. combination of MLT and cisplatin (50/50). The plate was incubated at 37°C with CO₂ and 95% humidity for 24–72 hours. After this period, the media were removed and washed with Phosphate Buffered Saline (PBS), and then MTT solution was added to each well then incubated for 4 hours. After 4 hours, 200 μL of DMSO was added to each well. The absorption was measured by an enzyme-linked immunosorbent assay (ELISA) reader at 570 nm.

Evaluation of cell apoptosis

10⁶ of MG63 cells per well were seeded in 6 wells and treated with MLT, cisplatin, and a combination of them. The cells were detached with trypsin and centrifuged. Then, the cells were washed with 1 mL of PBS and 100 μL of binding buffer to remove trypsin. The MG63 cells were dissolved in FITC-Annexin V and incubated for 10 min and then 400 μL of binding buffer was added to cells. Finally, apoptosis was analyzed by flow-cytometer.

Quantitative RT-PCR

Total RNA was extracted from cells using Trizol reagent and its concentration was determined by NanoDrop. RNA was treated with DNase and reverse transcribed with RT kit (Bioneer) for cDNA synthesis. Then, cDNA was quantified on real-time PCR detection system using SYBR Green PCR master mix. The expression levels of genes were measured based on the comparative C_t method (2^{-ΔC_t}). The reverse and forward primer sequences are listed in ► **Table 1** (► **Table 1**).

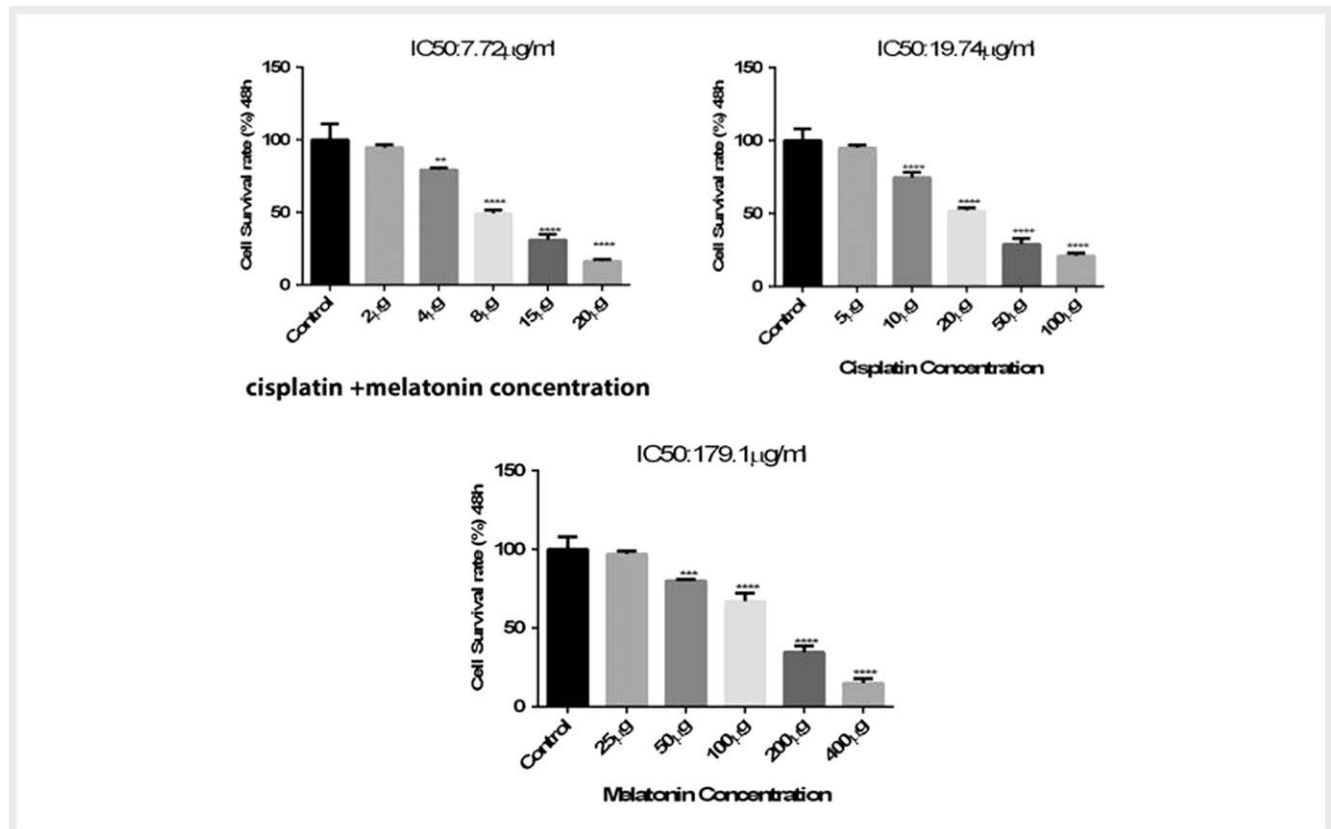
Results

The effect of melatonin on cytotoxicity of cisplatin in MG63 osteosarcoma cells

MTT assay has been used to evaluate the cytotoxic effect of melatonin, cisplatin, and their combination in different doses on MG63 OS cells. IC₅₀ of each of the above drugs was measured. The IC₅₀s obtained for cisplatin and melatonin and their combinations are 19.74 μg/ml, 179.1 μg/ml, and 7.72 μg/ml. After 48 h, the survival rate of MG63 OS cells was significantly reduced (► **Fig. 1**). IC₅₀ of the combination of MLT and cisplatin diminished and this means that the sensitivity of MG63 OS cells to cisplatin has been improved

► **Table 1** PCR Primers Sequences.

Gene	Forward	Reverse	Annealing temperature
CBX7	CATGGAGCTGTCAGCCATC	CTGTACTTTGGGGCCATC	59.0 °C
BCL2	CCTCCAGGTAGGCCGTTTT	GGCCTCTGTTCCCTCCCTC	57.5 °C
P53	GCGTGTGGAGTATTGGATG	GTACAGTCAGAGCCAACCTC	61.0 °C
CYLD	CCTTTATGTCAAGAGGTGGTG	GAGTAATGATTGGAAAGAAG	59.0 °C
miR-181b	CGTGATTGACAAGCTG	GAACATGTCTGCGTATCTC	58.5 °C

► **Fig. 1** cytotoxicity of cisplatin, MLT, and cisplatin-MLT combination on MG63 OS cells; the combination of cisplatin and MLT inhibited cell viability in low concentration.

(► **Fig. 1**). These results prove that MLT enhances the cytotoxic effect of cisplatin on OS cells.

Cisplatin-MLT combination downregulates expression of BCL2 and miR-181b

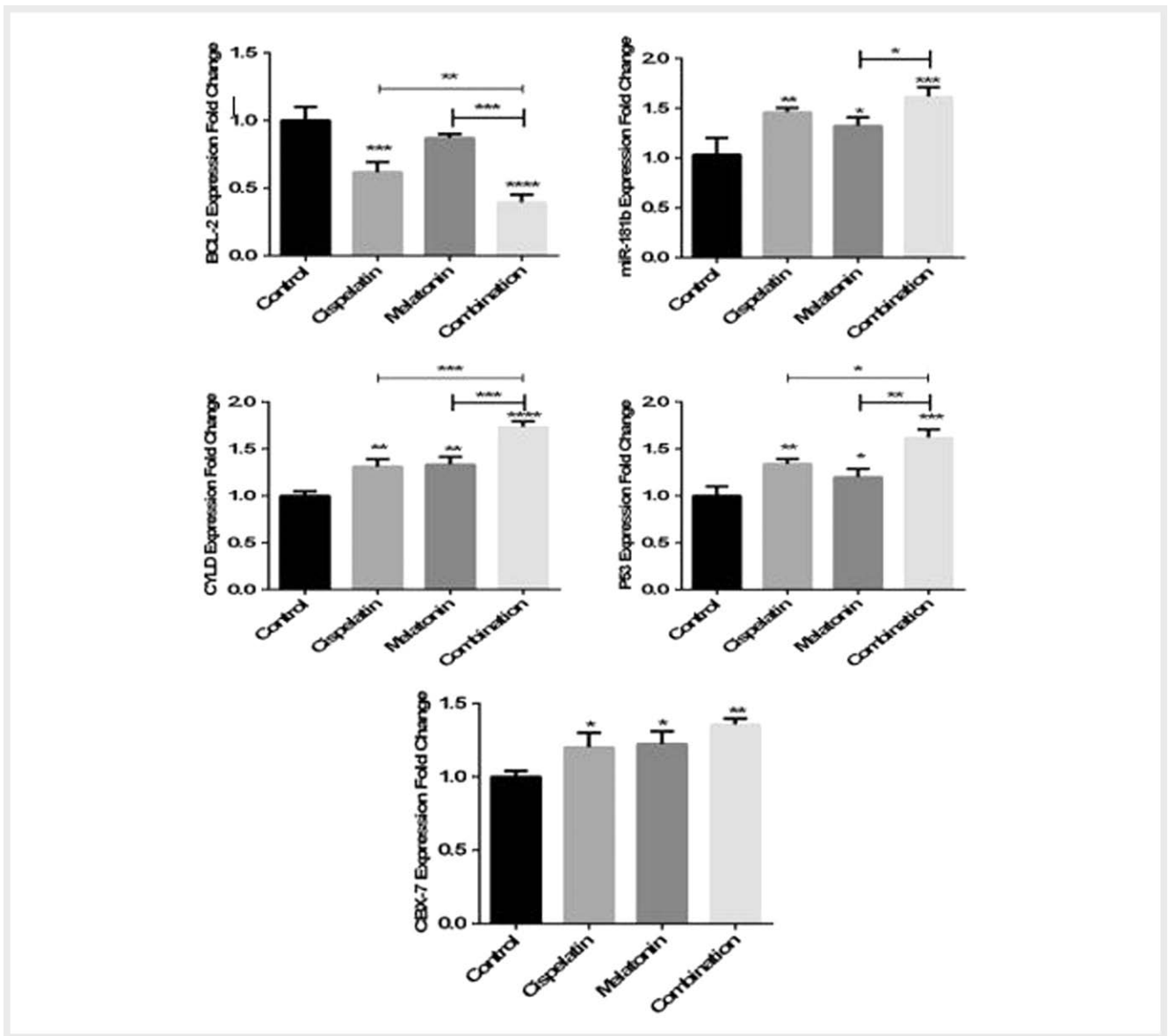
BCL2 and miR-181b play an oncogenic role and their expression increases in OS. Cisplatin notably reduces BCL2 expression while MLT slightly downregulates it. The combination of cisplatin and MLT significantly downregulates BCL2 expression (► **Fig. 2**). Cisplatin-MLT combination was expected to decrease the expression of miRNA-181b in the MG63 OS cell, however the data show that the combination of cisplatin-MLT increased miR-181b expression unexpectedly (► **Fig. 2**).

Cisplatin-MLT combination upregulates expression of CYLD, CBX-7, and p53

CYLD, CBX-7, and p53 act as tumor suppressors, and their expression reduce in OS. PCR results revealed that cisplatin and melatonin each promote the expressions of CYLD and CBX-7 slightly (► **Fig. 2**). Increased expression of p53 by cisplatin was slightly greater than the increased expression of p53 by MLT. Our results demonstrated that the cisplatin-MLT combination remarkably enhances the expression of these genes (► **Fig. 2**).

The effect of cisplatin-MLT on apoptosis

Flow cytometry results demonstrated that the rate of apoptosis in MG63 OS cells was increased meaningfully after treatment with cisplatin, MLT, and cisplatin-MLT compared to untreated cells. In ► **Fig. 3**, the cells are alive in the Q1-LL region and Annexin V⁻ and PI⁻, in the Q1-LR region, cells enter the early apoptosis phase, in which Annexin



► **Fig. 2** qRT-PCR results after 48 h treatment with cisplatin, MLT and cisplatin-MLT in MG63 cells. A) The expression level of oncogenic genes such as BCL2 and miR-181b decreases; B) the expression of tumor suppressor genes including CYLD, p53, CBX-7 increases.

V⁺ and PI⁻ and in the Q1-UR region, cells are in the late stage of apoptosis and the cells die, where Annexin V⁺ and PI⁺. As shown in the ► **Fig. 3**, apoptosis increased after the cells were treated with a combination of MLT and cisplatin (► **Fig. 3**).

Statistical Analysis

All experimental data were analyzed using Student t-test and SPSS software.

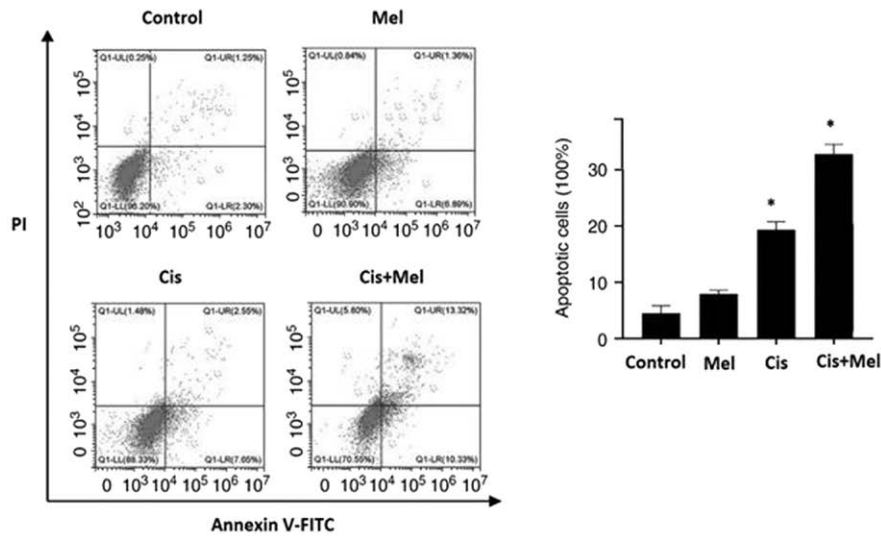
Discussion

Melatonin has an anti-cancer role in several cancers through its anti-oxidant, pro-apoptotic, anti-inflammatory and anti-angiogenic properties [9, 23]. In this study, we investigated that MLT can alleviate the resistance of MG-63 OS to cisplatin and in other words,

MLT can increase chemosensitivity in OS. More studies need to confirm our results.

In recent years, common treatments for OS, including neoadjuvant chemotherapy and surgery, do not have acceptable outcomes in OS patients with metastasis [24, 25], it is vital to study new treatments strategies for osteosarcoma such as combination therapy and targeted therapy.

MLT plays different roles in cancers such as restoring drug sensitivity, inducing apoptosis, and inhibiting cell growth and metastasis, therefore it is a hormone as well as a cell protector and can be utilized as a natural agent in OS treatment [26, 27]. It is identified that melatonin inhibits the proliferation and invasion of osteosarcoma cells in a dose-dependent manner. It also inhibits VEGFA and angiogenesis by targeting miR-424-5p [26, 28].



► **Fig. 3** Apoptosis rate in MG63 OS cells treated with cisplatin, MLT, and cisplatin-MLT; apoptosis rate of MG63 cells increases after treatment with cisplatin-MLT and more cells enter the early apoptosis and death phase.

The combination of MLT with chemotherapy drugs increases their effectiveness and reduces the resistance of cancer cells to them. The combination of MLT with chemotherapy drugs, such as cisplatin or methotrexate, increases the survival rate of normal cells while decreasing the viability of cancer cells [29]. In this regard, Mir et al. proposed that MLT in combination with doxorubicin (DOX) decreases the survival of MG63 and Saos2 OS cells and also promotes the therapeutic effect of DOX on OS cells through downregulating the expression of X-linked Inhibitor of Apoptosis (XIAP) and Survivin [30].

miRNAs are associated with tumorigenesis and their expression is altered in cancers [12, 31]. They play a role in drug sensitivity and metastasis by regulating signaling pathways or regulating their target genes involved in cancers [32]. The expression of miR-181b is dysregulated in various cancers and it is one of the important miRNAs in cancers progression [31, 33]. It is identified that low expression of miR-181b is associated with cisplatin resistance in small cell lung cancer cells and its upregulation inhibits this resistance by downregulation of Bcl2 [31]. Furthermore, the expression of miR-181b upregulates in OS, and its high expression increases proliferation and metastasis while reducing the apoptosis rate in OS cells. According to previous studies, miR-181b regulates apoptosis and invasion in MG63 and U2OS cells by targeting p53 [13].

Bcl2 is an antiapoptotic protein that is altered in cancers. Bcl2 inhibits apoptosis in cancer cells by removing the pro-apoptotic and inducing anti-apoptotic genes [34]. Bcl2 is an ideal agent for targeting therapies and combination therapies in cancers [35]. Chemotherapy drugs including cisplatin induce DNA damage which release cytochrome C. Release of cytochrome C activates caspase-9 and consequently activates caspase-3 [36, 37]. Since Bcl2 is the substrate of caspase-3, caspase-3 cleaves Bcl2 at 34th amino acid and deactivates it. The product of this cleavage acts as a pro-apoptotic protein [36]. Therefore, cisplatin downregulates Bcl2 expres-

sion indirectly through regulating caspase-3. MLT promotes apoptosis by activating MAPK pathway. In this mechanism, MLT induces phosphorylation of JNK, ERK and p38, consequently increases caspase 3 cleavage and Bax expression and reduces the expression of Bcl2 [38, 39]. In addition, in mitochondrial-dependent pathway, increased Bax translocation to mitochondria by MLT, enhances mitochondrial membrane permeability and caspase activation, and eventually decreases Bcl2 expression [39]. MLT in combination with cisplatin induce apoptosis through downregulating Bcl2 in HepG2 hepatocellular carcinoma cells [40]. According to Xiyue Zhang et al. study, miR-181b-5p targeted Bcl2 by binding to its 3'-UTR and downregulated its expression. Moreover, the combination of miR-181b-5p and temozolomide also reduces the expression of Bcl2 in glioma cells [41].

p53 is a tumor suppressor gene that induces apoptosis by inducing cell cycle arrest and also determines the cytotoxicity of chemotherapeutic drugs in cancer and its upregulation promotes the sensitivity of OS cells to chemotherapeutic agents [42, 43]. As mentioned earlier, cisplatin interferes with N7 purine bases in DNA and forms inter-strand and intra-strand crosslinks which can inhibit the cell cycle and activate p53 and apoptosis [44, 45]. MLT reduces antioxidant defense in cancer cells and produces ROS which can increase p53 protein expression and apoptosis [46]. Moreover, according to another study, MLT promotes P53 to phosphorylation at serine 15, thereby inhibits cell proliferation and DNA damage in transformed cells [47]. Bennukul et al. demonstrated that melatonin along with cisplatin increase apoptosis and decreases oxidative stress by targeting and regulating p53 in hepatocellular carcinoma cells [40].

CBX7 mostly acts as a tumor suppressor whose low expression is associated with poor prognosis in various cancers and is involved in cancer progression by regulating EMT and drug resistance related genes. In this regard, Rong Li et al. identified that CBX7 down-

regulates in cervical cancer, and high expression of CBX7 increases apoptosis and reduces proliferation in cervical cancer [48].

CYLD is a deubiquitination enzyme and plays tumor-suppressive role in malignancies whose expression is downregulated in several cancers [16, 49]. In addition, CYLD is a negative regulator of the NF-KB signaling pathway and studies have shown that CYLD promotes the sensitivity of cells to chemotherapy agents by inhibiting the activity of the NF-KB pathway in gastric cancer [50].

Conclusion

In this study, we identified that MLT improve the cytotoxic effect of cisplatin, which is one of the common therapeutic agents in cancers, on OS cancer cells. Our findings show that the combination of MLT and cisplatin promotes the apoptosis rate and the expression of tumor suppressive genes including CBX7, CYLD and p53, while this combination reduces survival of cancer cells and suppresses the expression of oncogenic genes such as Bcl2 and miR-181b in MG63 OS cell line. Taken together, these results suggest that MLT lead to chemo-sensitizing OS cells to cisplatin and can be considered as an enhancer agent of the therapeutic effect of chemotherapy drugs on cancers.

Acknowledgements

The authors would like to thank Shohada Clinical Research Development Unit, Shohada Hospital, Tabriz University of Medical Science, Tabriz, Iran.

Funding

The research grant was provided by Clinical Research Development Unit, Shohada Hospital, Tabriz University of Medical Sciences (grant no: 64876).

Conflict of Interest

The authors declare that they have no conflict of interest with the contents of this article.

References

- [1] Czarnecka AM, Synoradzki K, Firliej W, Bartnik E, Sobczuk P, Fiedorowicz M et al. Molecular biology of osteosarcoma. *Cancers*. 2020; 12: 2130
- [2] Harrison DJ, Geller DS, Gill JD, Lewis VO, Gorlick R. Current and future therapeutic approaches for osteosarcoma. *Expert review of anticancer therapy* 2018; 18: 39–50
- [3] Zhao X, Wu Q, Gong X, Liu J, Ma Y. Osteosarcoma: a review of current and future therapeutic approaches. *BioMedical Engineering OnLine* 2021; 20: 1–14.
- [4] Ghosh S. Cisplatin: The first metal based anticancer drug. *Bioorganic chemistry* 2019; 88: 102925
- [5] Kim M, Jung J-Y, Choi S, Lee H, Morales LD, Koh J-T et al. GFRA1 promotes cisplatin-induced chemoresistance in osteosarcoma by inducing autophagy. *Autophagy*. 2017; 13: 149–68.
- [6] Wang Y, Deng X, Yu C, Zhao G, Zhou J, Zhang G et al. Synergistic inhibitory effects of capsaicin combined with cisplatin on human osteosarcoma in culture and in xenografts. *Journal of Experimental & Clinical Cancer Research* 2018; 37: 1–17
- [7] Dasari S, Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action. *European journal of pharmacology* 2014; 740: 364–378
- [8] Lu K-H, Lin R-C, Yang J-S, Yang W-E, Reiter RJ, Yang S-F. Molecular and cellular mechanisms of melatonin in osteosarcoma. *Cells*. 2019; 8: 1618
- [9] Fathizadeh H, Mirzaei H, Asemi Z. Melatonin: an anti-tumor agent for osteosarcoma. *Cancer cell international* 2019; 19: 1–8
- [10] Reiter RJ, Rosales-Corral SA, Tan D-X, Acuna-Castroviejo D, Qin L, Yang S-F et al. Melatonin, a full service anti-cancer agent: inhibition of initiation, progression and metastasis. *International journal of molecular sciences* 2017; 18: 843
- [11] Liu J, Shi W, Wu C, Ju J, Jiang J. miR-181b as a key regulator of the oncogenic process and its clinical implications in cancer. *Biomedical reports* 2014; 2: 7–11
- [12] Wang M, Xie R, Si H, Shen B. Integrated bioinformatics analysis of miRNA expression in osteosarcoma. *Artificial cells, nanomedicine, and biotechnology* 2017; 45: 936–943
- [13] Wan J, Long F, Zhang C, Liu Y. miR-181b-p53 negative feedback axis regulates osteosarcoma cell proliferation and invasion. *International journal of molecular medicine* 2020; 45: 1803–1813
- [14] Xu M, Li J-M. MicroRNA-181b promotes osteosarcoma cell proliferation, invasion and migration in vitro via targeting RASSF8. *International Journal Of Clinical And Experimental Pathology* 2016; 9: 6145–6153
- [15] Mansueto G, Forzati F, Ferraro A, Pallante P, Bianco M, Esposito F et al. Identification of a new pathway for tumor progression: MicroRNA-181b up-regulation and CBX7 down-regulation by HMGA1 protein. *Genes & cancer* 2010; 1: 210–224
- [16] Andalib A, Rashed S, Dehbashi M, Hajati J, Noorbakhsh F, Ganjalikhani-Hakemi M. The upregulation of hsa-mir-181b-1 and downregulation of its target CYLD in the late-stage of tumor progression of breast cancer. *Indian Journal of Clinical Biochemistry* 2020; 35: 312–321
- [17] Li D, Jian W, Wei C, Song H, Gu Y, Luo Y et al. Down-regulation of miR-181b promotes apoptosis by targeting CYLD in thyroid papillary cancer. *International journal of clinical and experimental pathology* 2014; 7: 7672
- [18] Chen J, Zhou J, Chen X, Yang B, Wang D, Yang P et al. miRNA-449a is downregulated in osteosarcoma and promotes cell apoptosis by targeting BCL2. *Tumor Biology* 2015; 36: 8221–8229
- [19] Nedelcu T, Kubista B, Koller A, Sulzbacher I, Mosberger I, Arrich F et al. Livin and Bcl-2 expression in high-grade osteosarcoma. *Journal of cancer research and clinical oncology* 2008; 134: 237–244
- [20] Zhao Y, Zhang C-I, Zeng B-f, Gao T-T, Oda Y. Enhanced chemosensitivity of drug-resistant osteosarcoma cells by lentivirus-mediated Bcl-2 silencing. *Biochemical and biophysical research communications* 2009; 390: 642–647
- [21] Ye S, Shen J, Choy E, Yang C, Mankin H, Hornicek F et al. p53 overexpression increases chemosensitivity in multidrug-resistant osteosarcoma cell lines. *Cancer chemotherapy and pharmacology* 2016; 77: 349–356
- [22] Yao D, Cai G-H, Chen J, Ling R, Wu S-X, Li Y-P. Prognostic value of p53 alterations in human osteosarcoma: a meta analysis. *International journal of clinical and experimental pathology* 2014; 7: 6725
- [23] Cutando A, Lopez-Valverde A, Arias-Santiago S, De Vicente J, De Diego RG. Role of melatonin in cancer treatment. *Anticancer research* 2012; 32: 2747–2753

- [24] Walters DK, Steinmann P, Langsam B, Schmutz S, Born W, Fuchs B. Identification of potential chemoresistance genes in osteosarcoma. *Anticancer research* 2008; 28: 673–679
- [25] Lilienthal I, Herold N. Targeting molecular mechanisms underlying treatment efficacy and resistance in osteosarcoma: a review of current and future strategies. *International Journal of Molecular Sciences* 2020; 21: 6885
- [26] Li Y, Zou J, Li B, Du J. Anticancer effects of melatonin via regulating lncRNA JPX-Wnt/ β -catenin signalling pathway in human osteosarcoma cells. *Journal of Cellular and Molecular Medicine* 2021; 25: 9543–56.
- [27] Li Y, Li S, Zhou Y, Meng X, Zhang J-J, Xu D-P et al. Melatonin for the prevention and treatment of cancer. *Oncotarget*. 2017; 8: 39896
- [28] Vimalraj S, Saravanan S, Raghunandhakumar S, Anuradha D. Melatonin regulates tumor angiogenesis via miR-424-5p/VEGFA signaling pathway in osteosarcoma. *Life Sciences* 2020; 256: 118011
- [29] Najafi M, Salehi E, Farhood B, Nashtaei MS, Hashemi Goradel N, Khanlarkhani N et al. Adjuvant chemotherapy with melatonin for targeting human cancers: A review. *Journal of cellular physiology* 2019; 234: 2356–72.
- [30] Mir SM, Yousefi B, Marjani A, Rahimi M, Quejeq D. The sensitization of melatonin in osteosarcoma cells by suppression of anti-apoptotic proteins. *Pharmaceutical Sciences* 2020; 26: 159–64.
- [31] Liu H-N, Qie P, Yang G, Song Y-B. miR-181b inhibits chemoresistance in cisplatin-resistant H446 small cell lung cancer cells by targeting Bcl-2. *Archives of medical science: AMS* 2018; 14: 745
- [32] Wang X, Chen X, Meng Q, Jing H, Lu H, Yang Y et al. MiR-181b regulates cisplatin chemosensitivity and metastasis by targeting TGF β R1/Smad signaling pathway in NSCLC. *Scientific reports* 2015; 5: 1–15
- [33] Zhao L-D, Zheng W-W, Wang G-X, Kang X-C, Qin L, Ji J-J et al. Epigenetic silencing of miR-181b contributes to tumorigenicity in colorectal cancer by targeting RASSF1A. *International journal of oncology* 2016; 48: 1977–1984
- [34] Li W-h, Wu H-j, Li Y-x, Pan H-g, Meng T, Wang X. MicroRNA-143 promotes apoptosis of osteosarcoma cells by caspase-3 activation via targeting Bcl-2. *Biomedicine & pharmacotherapy* 2016; 80: 8–15
- [35] Radha G, Raghavan SC. BCL2: A promising cancer therapeutic target. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*. 2017; 1868: 309–314
- [36] Biswas SK, Huang J, Persaud S, Basu A. Down-regulation of Bcl-2 is associated with cisplatin resistance in human small cell lung cancer H69 cells. *Molecular cancer therapeutics* 2004; 3: 327–334
- [37] Kutuk O, Arisan ED, Tezil T, Shoshan MC, Basaga H. Cisplatin overcomes Bcl-2-mediated resistance to apoptosis via preferential engagement of Bak: critical role of Noxa-mediated lipid peroxidation. *Carcinogenesis*. 2009; 30: 1517–1527
- [38] Li W, Wu J, Li Z, Zhou Z, Zheng C, Lin L et al. Melatonin induces cell apoptosis in Mia PaCa-2 cells via the suppression of nuclear factor- κ B and activation of ERK and JNK: A novel therapeutic implication for pancreatic cancer. *Oncology reports* 2016; 36: 2861–2867
- [39] Li W, Fan M, Chen Y, Zhao Q, Song C, Yan Y et al. Melatonin induces cell apoptosis in AGS cells through the activation of JNK and P38 MAPK and the suppression of nuclear factor-kappa B: A novel therapeutic implication for gastric cancer. *Cellular Physiology and Biochemistry* 2015; 37: 2323–2338
- [40] Bennukul K, Numkliang S, Leardkamolkarn V. Melatonin attenuates cisplatin-induced HepG2 cell death via the regulation of mTOR and ERCC1 expressions. *World journal of hepatology* 2014; 6: 230
- [41] Zhang X, Yu J, Zhao C, Ren H, Yuan Z, Zhang B et al. MiR-181b-5p modulates chemosensitivity of glioma cells to temozolomide by targeting Bcl-2. *Biomedicine & Pharmacotherapy* 2019; 109: 2192–202.
- [42] Sun Y, Xia P, Zhang H, Liu B, Shi Y. P53 is required for Doxorubicin-induced apoptosis via the TGF-beta signaling pathway in osteosarcoma-derived cells. *American journal of cancer research* 2016; 6: 114
- [43] Chen X, Lv C, Zhu X, Lin W, Wang L, Huang Z et al. MicroRNA-504 modulates osteosarcoma cell chemoresistance to cisplatin by targeting p53. *Oncology letters* 2019; 17: 1664–1674
- [44] Han J-Y, Chung Y-J, Park SW, Kim JS, Rhyu M-G, Kim H-K et al. The relationship between cisplatin-induced apoptosis and p53, bcl-2 and bax expression in human lung cancer cells. *The Korean journal of internal medicine* 1999; 14: 42
- [45] Casares C, Ramírez-Camacho R, Trinidad A, Roldán A, Jorge E, García-Berrocal JR. Reactive oxygen species in apoptosis induced by cisplatin: review of physiopathological mechanisms in animal models. *European Archives of Oto-Rhino-Laryngology* 2012; 269: 2455–2459
- [46] Gholami M, Saki G, Hemadi M, Khodadadi A. Effect of melatonin on the expression of apoptotic genes in vitrified-thawed spermatogonia stem cells type A of 6-day-old mice. *Iranian journal of basic medical sciences* 2013; 16: 906
- [47] Santoro R, Marani M, Blandino G, Muti P, Strano S. Melatonin triggers p53Ser phosphorylation and prevents DNA damage accumulation. *Oncogene*. 2012; 31: 2931–2942
- [48] Li R, Yan Q, Tian P, Wang Y, Wang J, Tao N et al. CBX7 inhibits cell growth and motility and induces apoptosis in cervical cancer cells. *Molecular Therapy-Oncolytics* 2019; 15: 108–16.
- [49] Hayashi M, Jono H, Shinriki S, Nakamura T, Guo J, Sueta A et al. Clinical significance of CYLD downregulation in breast cancer. *Breast cancer research and treatment* 2014; 143: 447–457
- [50] Zhu M, Zhou X, Du Y, Huang Z, Zhu J, Xu J et al. miR-20a induces cisplatin resistance of a human gastric cancer cell line via targeting CYLD. *Molecular medicine reports* 2016; 14: 1742–50.