

Melatonin Sensitize the Ovarian Cancer Cells to Cisplatin through suppression of PI3K/Akt pathway

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Abstract

Background: The present study elucidated the effect of melatonin on oxidative stress status, the expression of pro-apoptotic protein (caspase 3 and cleaved caspase 3), anti-apoptotic proteins (X-linked apoptosis inhibitor protein (XIAP) and Survivin), and the activity of PI3K/Akt signaling pathway in human ovarian cancer cell line.

Methods: Human ovarian cancer cells (OVCAR3) were treated with cisplatin, melatonin, cisplatin + melatonin, and siRNA Akt. Reactive oxygen species (ROS) levels were assessed using fluorimetric assay in the different groups. Moreover, protein expression of caspase-3, cleaved caspase 3, PI3K, Akt, phosphorylated (p)-Akt, XIAP, and Survivin were determined by Western blotting in all experimental groups.

Results: Our results showed that administration of melatonin significantly increased intracellular ROS generation, the cleavage of caspase 3 and phosphorylation of Akt. This effect was more prominent in the combination therapy with cisplatin versus cisplatin alone. Akt siRNA transfection had similar effects on ROS generation, the cleavage of caspase 3, and phosphorylation of Akt. Interestingly, the levels of XIAP, PI3K, and Survivin were not significantly changed by any of these treatments.

Conclusions: Taken together, this study suggests that combination therapy of cisplatin and melatonin increases apoptosis in the OVCAR-3 cells by inhibition of PI3K/Akt signaling pathway and exacerbation of oxidative stress.

Introduction

Ovarian cancer (OC) is the common cause of cancer mortality from gynecologic tumors in the worldwide [1, 2]. This cancer is often diagnosed in advanced stages when cure rates are low, which is possibly due to late presentation, heterogeneous nature, and lack of effective screening [2]. The main risk factors for OC are family history, early menarche, obesity, fertility treatments, diabetes, alcohol consumption, aging, and smoking [3–5]. Available standard treatments for diagnosed patients are surgery, as the initial choice, and chemotherapy, as the second step of treatment. In the advanced phase of OC, most of the patients are likely to develop chemotherapy resistance, which is the main cause of treatment failure [6]. Several molecular signaling pathways, including excessive accumulation of reactive oxygen species (ROS), inflammation, apoptosis cell death, and angiogenesis are implicated in the development of OC. The PI3K/Akt pathway is one of the deregulated molecular pathways in ovarian cancer [7]. Akt is a serine-threonine kinase which plays a key role in cellular survival, proliferation, cell growth, and drug resistance metabolism. Moreover, its biological activity depends on phosphorylation of Thr-308 and Ser-473 residues [8]. Accumulating evidence shows overexpression of Akt in OC and therefore, Akt ablation can be a successful approach in the treatment of advanced-stage OC [9]. Among different Akt isoforms, Akt1, Akt2, and Akt3, Akt1 is a chief isoform involve in ovarian cancer cell proliferation and protection against apoptosis [10]. Cisplatin is a DNA-damaging agent and the platinum analogs which has been

successfully used in the treatment of various types of cancer, including blood vessels, bone, muscle, soft tissue, and sarcoma cancer, and OC [11]. The main mechanisms underlying the antitumor activity of cisplatin are its ability to induce DNA damage and apoptosis cell death [12, 13]. However, cisplatin resistance happens in clinical practice that ultimately leads to treatment failure [14]. The mechanisms underlying chemoresistance still remain to be elucidated. Previous studies have shown that activation of Akt contributes to resistance to cisplatin in several cancers, namely OC, while inhibition of Akt sensitizes ovarian cancer cell to cisplatin, promotes the antitumor activity of cisplatin, and induces apoptosis [15–17]. Therefore, PI3K/Akt pathway inhibition can be considered as a useful therapeutic strategy either as monotherapy or in combination with chemotherapy drugs [18]. Melatonin (N-acetyl-5-methoxytryptamine) is an endogenous free-radical scavenger that synthesized and secreted by the pineal gland and other organs such as ovary, intestine, and testes [19]. Although melatonin prevents apoptosis in healthy cells, in many cancer types such as OC melatonin exerts antiproliferative, anti-migration, and pro-apoptotic properties [20–22]. Moreover, melatonin induces apoptosis via activation of caspases and downregulation of the phosphorylation of Akt in various cancers [21, 23]. However, evidence shows that the production of melatonin is decreased in women with OC compared to healthy women [24]. Since there is an urgent need for the development of safe and efficient novel drugs for the management of OC, the purpose of this study was to investigate the knockdown of Akt signaling pathway and the protective effects of melatonin and cisplatin therapies on the apoptosis and cell survival in an *in vitro* model of OC cells.

Material And Methods

Materials

Melatonin, cisplatin, and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] were obtained from Sigma Chemical Co. (St. Louis, MO). Human ovarian cancer cells, OVCAR3 cell line (NCBI code: C209), were purchased from the National Cell Bank of Iran (NCBI), Pasteur Institute. Cell culture reagents were obtained from appropriate commercially available suppliers.

Cell culture

Human ovarian cancer cells, OVCAR3 cell line, were cultured in RPMI 1640 (Sigma-Aldrich, USA) medium supplemented with 10% fetal bovine serum (FBS) (Gibco, USA), 100 U/ml penicillin and 100 µg/ml streptomycin (Gibco, USA) at 37°C in a 5% CO₂ humidified chamber.

Melatonin preparation

Melatonin was dissolved in dimethyl sulfoxide (DMSO, Merck, Germany) to prepare a 0.2 M (50 mg/ml) stock solution. Then, the stock solution was diluted with RPMI-1640 to prepare different concentrations of the working solution immediately before use.

Cell viability assay

The effects of cisplatin and melatonin therapies on the cell viability were evaluated by a colorimetric assay using (3-(4, 5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) (Sigma-Aldrich, USA). Briefly, OVCAR3 cells were seeded in 96-well plate and cultured in RPMI-1640 medium containing 10% FBS, then the cells were exposed to different concentrations of cisplatin or melatonin for 24, 48, and 72 h in the same medium supplemented with 2% FBS. Subsequently, 20 μ l of MTT solution (5 mg/ml) was added to each well and incubated for 4 h. All treatments were carried out in triplicate. The absorbance value was measured at a wavelength of 630 nm using a microplate reader (Biotek Instruments, USA) and the results were presented as a percentage of the viability of control cells. The drug concentration causing 50% cell growth inhibition (IC₅₀) values for each treatment were calculated from dose-response curve of percent growth inhibition against test concentrations. For evaluation of the synergizing function of melatonin and cisplatin, cells were co-treated with IC₅₀ value of 24 h of melatonin and various concentrations of cisplatin for 24 h and cell viability was determined.

siRNA transfection

For protein knockdown of Akt in OVCAR3 cells, small interfering RNA (Akt siRNA (h): sc-29195 siRNA) was used. Akt-3m-siRNA was used as the control siRNA for transfection: Briefly, in a six well tissue culture plate, 2×10^5 cells per well in 2 ml antibiotic-free normal growth medium were seeded, supplemented with FBS and incubated at 37° C in a CO₂ incubator until the cells reached to 60–80% confluence. A 0.8 ml mixture (1:1) of Solution A (siRNA duplex in siRNA Transfection Medium (sc-36868) and Solution B (siRNA Transfection Reagent (sc-29528) in siRNA Transfection Medium (sc-36868) was added onto the washed cells and incubated for 5–7 h at 37° C in a CO₂ incubator. Fluorescein Conjugated Control siRNA was incubated for a total 5–7 h at 37° C in a CO₂ incubator and assessed by fluorescent microscopy. Then, the transfection mixture was removed and replaced with 1x normal growth medium and incubated for an additional 18–24 h. The expression level of Akt protein was confirmed by Western blot analysis. Controls were transfected with non-specific siRNA under similar conditions.

Measurement of reactive oxygen species (ROS)

The intracellular ROS level was determined using a DCFH-DA (2',7'-dichlorodihydro fluorescein diacetate) assay kit. For this purpose, cells were seeded into a 96-well plate, washed with PBS twice, and then incubated with 100 μ M DCFH-DA in a fresh medium at 37°C for 30 min in the dark. Intracellular ROS production was detected by measuring the fluorescence intensity at 485 nm excitation and 530 nm emission wavelengths in a multiwell plate reader spectrofluorometer and photographed by fluorescence microscope (Olympus IX70, Tokyo, Japan). The measured fluorescence values were expressed as a percentage of the control.

Protein extraction and Western blot analysis

Cells were lysed in 100 μ l RIPA lysis buffer (25 mM HEPES, 1% Triton X-100, 2 mM EDTA, 0.1 M NaCl, 25 mM NaF, 1 mM Sodium Orthovanadate) containing protease inhibitor cocktail for 30 min on ice. Then, cell lysates were centrifuged at 12000 g for 20 min at 4°C and the supernatant was collected. Protein concentration in the supernatant was determined using the Bradford protein assay. The equal amount of

protein (~ 100 µg) loaded at 12% SDS- acrylamide gel followed by transferring onto a polyvinylidene difluoride (PVDF) membrane (Roche, UK). Non-specific binding reactions in the membranes were blocked by bovine serum albumin (BSA) 3% in Tris-buffered saline (pH 7.5) at room temperature for 1 h. Subsequently, the membranes were incubated overnight with diluted (1:500 concentrations) primary antibodies (Santa Cruz Biotechnology, U.S.A) against phospho-Akt (sc-52940), Akt (sc-5298), X-linked inhibitor of apoptosis protein (sc-55550), Survivin (sc-17779), caspase 3 (sc-136219), HIF1 (sc-13515), VEGF (sc-7269), GSK3β (sc-81462), p-GSK3β (sc-373800), p53 (sc-126), and β-actin (sc-47778) overnight at 4°C. Next, the membrane was incubated with appropriate horseradish peroxidase-conjugated (HRP) secondary antibody after washing three times with PBS at room temperature for 2 h. The antigen-antibody complexes were detected using an enhanced chemiluminescence (ECL) detection kit (Pierce, Rockford, IL). The density of each band was acquired by Image J software (version 1.62, National Institutes of Health, Bethesda, MD, USA) and normalized to β-actin ⁹.

Statistical analysis

Statistical analysis was performed using the GraphPad Prism 6 Scientific software (GraphPad Software, Inc., La Jolla, CA). The results were expressed as the mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) followed by Tukey post-hoc test was performed for multiple comparisons. A P value of < 0.05 was considered to indicate statistical significance.

Results

MTT assay

The IC₅₀ values of cisplatin or melatonin in single treatment after 24, 48, and 72 h, are shown in Fig. 1A and B, respectively. The IC₅₀ values of cisplatin alone were 12.8 µM at 24 h, 10 µM at 48 h, and 8.7 µM at 72 h. For melatonin alone, the IC₅₀ values were 4.9 mM at 24 h, 3 mM at 48 h, and 2.3 mM at 72 h. For evaluation of cell sensitivity of OVCAR3 to cisplatin in the presence of melatonin, we planned the MTT assay in the presence of constant values of melatonin (4.1 mM) with different concentrations of cisplatin on OVCAR3 cells (Fig. 1C). The results indicated that co-administration of melatonin with cisplatin significantly decreased IC₅₀ values of cisplatin to 4.1 µM, 3.8 µM, and 2.2 µM at 24 h, 48 h, and 72 h, respectively compared to the cisplatin alone ($p < 0.05$).

Intracellular ROS levels

As expected, cisplatin significantly elevated ROS production compared with the untreated control group ($p < 0.001$). Also, combined treatment with cisplatin and melatonin markedly increased ROS levels compared with cisplatin alone ($p < 0.001$). Moreover, the production of ROS was significantly decreased in the melatonin-treated Akt siRNA-treated groups compared to the cisplatin alone group ($p < 0.001$; Fig. 2).

Apoptosis markers

To demonstrate mechanisms underlying apoptotic induction, the expression of apoptosis-related proteins in the treated cells was investigated. The results of immunoblotting revealed that treatment with cisplatin had no effect on the expression of XIAP. Surprisingly, addition of melatonin to the OVCAR3 cells significantly increased protein levels of XIAP compared to the Cis group ($p < 0.05$, Fig. 3B). However, combination therapy of cisplatin and melatonin or Akt siRNA had no significant effects on protein expression of XIAP.

Moreover, the protein content of pro-caspase 3 was significantly decreased in single treatment with cisplatin ($p < 0.001$) or melatonin ($p < 0.05$) compared to the untreated control cells. However, combination therapy or Akt siRNA did not decrease pro-caspase 3 expression compared to the cisplatin group. Furthermore, cisplatin alone ($p < 0.001$) as well as combination therapy ($p < 0.001$) markedly increased cleaved caspase 3 levels compared to the untreated control cells. Nevertheless, cleaved caspase 3 level was significantly decreased in the melatonin alone, Akt siRNA, and control siRNA groups compared to the cisplatin-treated cells ($p < 0.001$, Fig. 3D).

Akt activity

As shown in Fig. 4, none of the treatments had significant effects on the expression of PI3K (Fig. 4B) and Survivin (Fig. 4E). Moreover, a significant increase in p-Akt levels was observed in the cisplatin-treated group versus untreated control cells ($p < 0.05$, Fig. 4C). Furthermore, melatonin alone ($p < 0.05$), combination therapy ($p < 0.05$), and Akt siRNA ($p < 0.01$) treatments significantly reduced p-Akt levels compared to the cisplatin-treated cells. As expected, Akt siRNA could significantly ($p < 0.001$) decrease Akt protein expression compared to the untreated control cells.

Angiogenesis and cancer proliferation

Expression of HIF1, VEGF, p-GSK3 β , GSK3 β and p53 as the main factors in angiogenesis process and cancer cell proliferation were evaluated (Fig. 5). The results of immunoblotting demonstrated a significant increase of HIF1 expression in cisplatin treatment compared to control ($p < 0.05$). In contrast, melatonin alone and Akt siRNA treatments significantly attenuated the expression of HIF1 ($p < 0.001$). HIF expression showed a marked drop in cisplatin + melatonin treatment group compared to cisplatin treatment ($p < 0.001$).

All treatment groups, except for control siRNA treatment, notably showed reduced VEGF protein expression as compared to untreated control group. Cisplatin treatment group had the lowest impact ($p < 0.05$) on VEGF expression, while combination treatment of cisplatin + melatonin had the highest impact ($p < 0.001$) with approximately 75% decline. Moreover, combination treatment showed a marked decrease in VEGF expression compared to treatment of cisplatin alone ($p < 0.001$).

As compared to control group, combination therapy of cisplatin + melatonin revealed the highest effect on diminishing p-GSK3 β by 50% ($p < 0.001$). Also, cisplatin, melatonin and Akt siRNA treatment groups showed a significant decrease ($p < 0.001$). It is noteworthy that combination therapy had a significant decrease on p-GSK3 β compared to cisplatin alone ($p < 0.05$).

The cisplatin alone, Akt siRNA and control siRNA treatment groups demonstrated minor decrease, while melatonin alone and cisplatin + melatonin combination therapy showed insignificant increase in GSK3 β expression compared to control group ($p > 0.05$). In this regard, there was no differences in single treatment of cisplatin compared to combination therapy ($p = 0.19$)

Among all treatment groups, combination therapy of cisplatin + melatonin significantly increased the expression of p53 almost by 80% ($p < 0.001$) compared with cisplatin alone treatment group. Also, melatonin-treated group showed a notable increase of p53 protein by 30% ($p < 0.01$) as compared to untreated control group. However, cisplatin alone ($p = 0.60$) and Akt siRNA ($p = 0.12$) treatment groups did not significantly alter the p53 expression.

Discussion

Apoptosis or programmed cell death is a critical physiological phenomenon playing an essential role in ovarian tissue homeostasis, the developmental process of organs, and deletion of potentially dangerous or defective cells [25]. However, any fault in the control of apoptosis could give rise to pathological conditions like various type of ovarian cancer [26]. Therefore, strategies targeting apoptosis-related molecules have a great value in the treatment of OC. Moreover, the failure of cells to undergo apoptosis may develop chemotherapy resistance, which is the main cause of treatment failure in OC [27, 28]. Inhibition of PI3K/Akt signaling and induction of oxidative stress have been known as two therapeutic strategies to sensitize the resistant tumoral cells to apoptosis [29]. In the current study, we demonstrated that melatonin sensitized the OVCAR3 cells to cisplatin, as a well-known chemotherapy agent, via induction of oxidative stress and inhibition of PI3K/Akt signaling. Overexpression and activation of PI3K/Akt signaling, as a critical cell process regulator of cell proliferation, has been demonstrated in OC [30], which can develop cisplatin resistance [31]. Hence, inhibition of PI3K/Akt signaling activation could be a therapeutic target for epithelial OC [29]. Many previous studies have also demonstrated that the PI3K/Akt signaling regulates ROS production and aberrant PI3K/Akt signaling contributes to the overproduction of ROS [32, 33]. On the other hand, excessive ROS levels can potentiate the activation of PI3K/Akt signaling mainly through inhibition of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) [33]. In this study, we used Akt siRNA to inhibit Akt protein expression. We observed that Akt knockdown was associated with decreased Akt phosphorylation, increased ROS generation, and apoptosis induction in the Akt siRNA group in comparison to the control siRNA. In line with our results, previous reports have also shown that inhibition of PI3K/Akt pathway is accompanied by excessive ROS production [33, 34]. It seems that Akt counteracts with ROS generation in oxidative stress pathway and consequently apoptosis in cancer cells. Several studies have been reported the beneficial effects of melatonin on the apoptosis of cancer cells by focusing on oxidative stress [35]. ROS play an important role in the activation of apoptosis pathway, so that elevation in ROS generation leads to an alteration in mitochondrial membrane potential (MMP) and defects in the respiratory chain, consequently initiates the apoptotic process [36, 37]. Our data demonstrated that co-treatment of OVCAR3 cells with melatonin sensitized the cells to cisplatin treatment via increased ROS production in this cell line. In line with our result, several studies have established that the antitumor activity of melatonin on cancer cells is

through ROS-dependent activation of apoptotic cell death [38, 39]. Likewise, we found that cisplatin as a clinically-proven drug to fight various cancer types caused an increase in ROS production, and this elevation in combination therapy with melatonin was much more prominent than cisplatin alone. Recently, many researchers reported that cisplatin-induced cytotoxicity is strongly associated with increased ROS generation [40]. Moreover, we observed a significant decrease in the p-Akt levels in the melatonin, combination therapy, and Akt siRNA groups versus cisplatin-treated cells. It seems that the simultaneous reduction of Akt phosphorylation and ROS production are rationales to the sensitization of OVCAR3 cells to cisplatin. Moreover, activated Akt has the ability to phosphorylate one of its targets, GSK3 β [41]. Multiple consequences may occur in presence of activated (non-phosphorylated) or inactivated (phosphorylated) form of GSK3 β . NF- κ B activity is positively regulated by GSK3 β , thus, aberrated Akt pathway may lead to high level of GSK3 β , increased NF- κ B function and finally OC proliferation [42]. As expected, we observed decreased level of p-GSK3 β in Akt siRNA treatment group as well as combination therapy group. Notably, our results demonstrated that neither cisplatin nor melatonin treatments could alter the expression level of GSK3 β . In concordant with other reports, we demonstrated the OVCAR3 cells sensitized to cisplatin and melatonin by regulating the activity of GSK3 β [43]. Akt signaling pathway may be activated by integrin ligation which triggers integrin-linked kinase activation and subsequently leads to elevation of Hypoxia-Induced Factor (HIF)-1 and Vascular Endothelial Growth Factor (VEGF) expression [44]. HIF1 is a crucial factor in angiogenesis which expressed in response to low oxygen concentration and contributes to survival and proliferation of cancer cells [45]. Following the phosphorylation of Akt, HIF1 enters the nucleus, acts as a transcription factor and triggers the transcription of VEGF gene. This signaling pathway leads to angiogenesis and tumor proliferation [46]. On the other hand, p53 can negatively modulates this pathway through expression induction of MDM2 which subsequently triggers the degradation of HIF1 by the proteasome pathway [47]. Zhihong et.al have hypothesized that downregulating HIF-1 would be an efficient strategy for overcoming cisplatin resistance of human OC cells [48]. They demonstrated that cisplatin has the ability to downregulates HIF1 expression, thus inhibits the proliferation of cancer cells by induction of ROS production [48]. Accumulating studies revealed the potential of cisplatin to abolish the expression of HIF1 and VEGF in OC [49, 50]. Furthermore, increasing evidence indicated that melatonin potentiates to affects the angiogenesis by targeting HIF-1 under hypoxic conditions [51, 52]. These findings were in line with our results, in which we showed the combination therapy would significantly downregulates both HIF1 and VEGF expression level compared to untreated control group and even cisplatin alone and melatonin alone treatment. Furthermore, align with our results, p53 expression has been demonstrated to be associated with cisplatin sensitivity in OC [53]. We showed that combination treatment of cisplatin and melatonin had a positive significant association with p53 expression level as compared to untreated control and cisplatin treated groups. In concordance to our results, several studies revealed that p53 facilitates the apoptosis by cisplatin treatment [54–56].

In line with our finding, melatonin has been reported to reduce phosphorylation of Akt in different cancer cells such as hepatoma cells [57], lung cancer [58], and breast cancer [59, 60]. Activation of caspase 3, as an irreversible step of apoptosis, plays an important role in the apoptosis process and any defect in its

function or downregulation of its expression may lead to development of carcinogenesis [61]. Moreover, it has been shown that the expression of caspase-3 is decreased or undetectable in OC cells, which is one of the causes of resistance to chemotherapy agents [62]. However, its expression is increased in response to anti-cancer therapy leading to apoptosis in cancer cells [63]. As shown in Henkels et al. study [62], we also found that cisplatin increased the cleavage of caspase 3 in OVCAR3 cells and combination therapy with melatonin also elevated caspase 3 activation, indicating that melatonin sensitized OVCAR3 cell to cytotoxic effect of cisplatin and promoted cell death. According to the results of MTT (Fig. 1C), 24 h treatment with cisplatin along with melatonin lowered the IC₅₀ of cisplatin more than 50% in compare to cisplatin alone (4.1 μ M vs. 12.8 μ M). XIAP, a direct inhibitor of caspase 3 and 7, and Survivin proteins are inhibitors of apoptosis which can promote cell cycle progression [64]. Evidence shows that cancer cells have elevated expression levels of apoptosis inhibitor proteins promoting cell survival and tumor growth and consequently chemoresistance [65]. Therefore, therapies targeting inhibitors of apoptosis protein in cancer may improve the sensitivity of cancer cells to chemotherapies and hence potentiate cell death. In this study, we observed a slight reduction, but not significantly, in XIAP protein expression in the cisplatin and combined therapy with melatonin. Surprisingly, melatonin-treated cells displayed elevated expression of XIAP compared to the treatment with cisplatin alone. Elevated expression of XIAP along with elevated caspase 3 activation is an inconsistent point in our results which should be further investigated to be clarified. Asselin et al. have reported that cisplatin decreases XIAP content at protein levels which in turn induces the cleavage of pro-caspase 9, pro-caspase 3, as well as Akt and therefore apoptosis in ovarian cancer cells [66]. Conversely, overexpression of XIAP prevents cisplatin-induced Akt cleavage and increases p-Akt content, which in turns protects OC cells by inhibiting apoptosis [65].

Survivin is another apoptosis inhibitor protein that controls cell proliferation and suppresses apoptosis-induced cell death [67]. Accumulating studies have shown that cancer cells have a higher expression level of Survivin [68, 69]. Overexpression of Survivin in normal and tumor cells has prevented cell death that promotes by apoptotic stimuli, such as caspases and anti-cancer drugs. Importantly, down-regulation of Survivin is accompanied by overexpression of caspase 3 which could have therapeutic benefits in OC cells [70]. The blockade of Survivin expression has also been reported to improve the antitumor activity of chemotherapeutic drugs in OC [71]. In this study, unexpectedly, melatonin had no effect on the protein expression of Survivin. Moreover, only a reduction in Survivin expression was observed in the Akt siRNA group, indicating the critical role of Akt in cell proliferation. In contrast to our results, Fan et al. have demonstrated that melatonin reverses apoptosis resistance in human hepatocellular carcinoma by inhibition of Survivin and XIAP [69]. However, many previous studies have confirmed pro-apoptotic, and oncostatic properties of melatonin [70]. Pro-apoptotic effect of melatonin in cancer cells is mainly through modulation of the release of pro-apoptotic (BAX, caspase 3, and cytochrome c) and anti-apoptotic (Bcl-2) proteins, as well as oxidative stress [72–74]. Yun et al. also found that melatonin attenuated phosphorylation of Akt and induced caspase 3 activity and hence increased apoptosis in lung cancer cells [58]. These discrepancies highlight the need to more elucidating investigations in future.

Conclusion

In conclusion, the result of the present study showed that melatonin increased cisplatin efficiency in induction of apoptosis in OVCAR3 cells. This effect may be partially mediated by ROS production and PI3K/Akt signaling down-regulation.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SBS and MB collected and analyzed the data as well as performed the statistical analysis, drafted the manuscript, and conceived the main idea of the study. IA and FS participated in the acquisition of data and in the design and intellectual conception of the study. All authors approved the final version of the manuscript.

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Figures

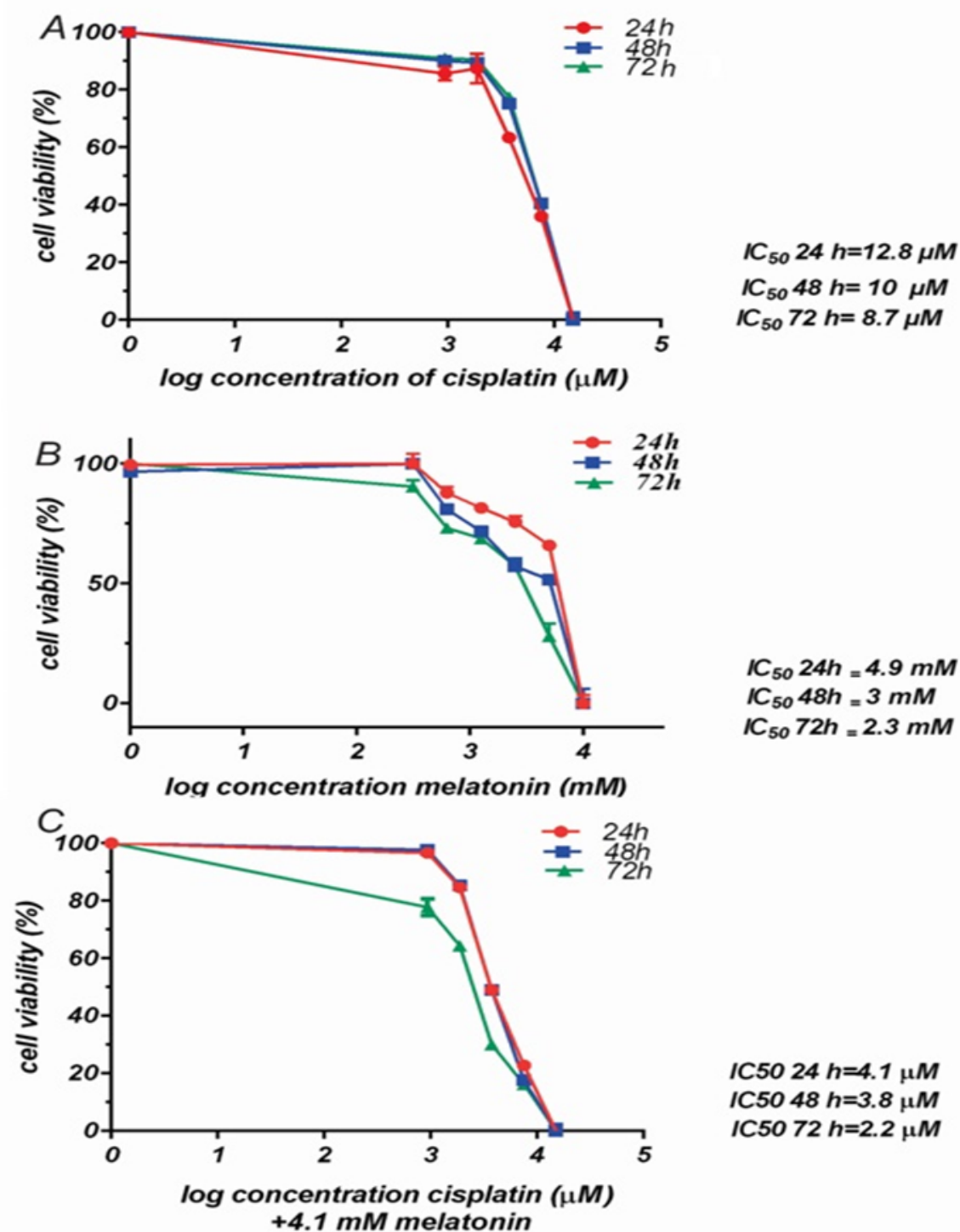


Figure 1

Effect of cisplatin and melatonin on cell viability of ovarian cancer cells alone or in combination. OVCAR3 cells were treated with different concentrations of A) cisplatin, B) melatonin, and C) cisplatin+ melatonin in combination for 24, 48 and 72 h and analyzed by MTT assay. Data were obtained from triplicate tests and expressed as mean \pm SEM.

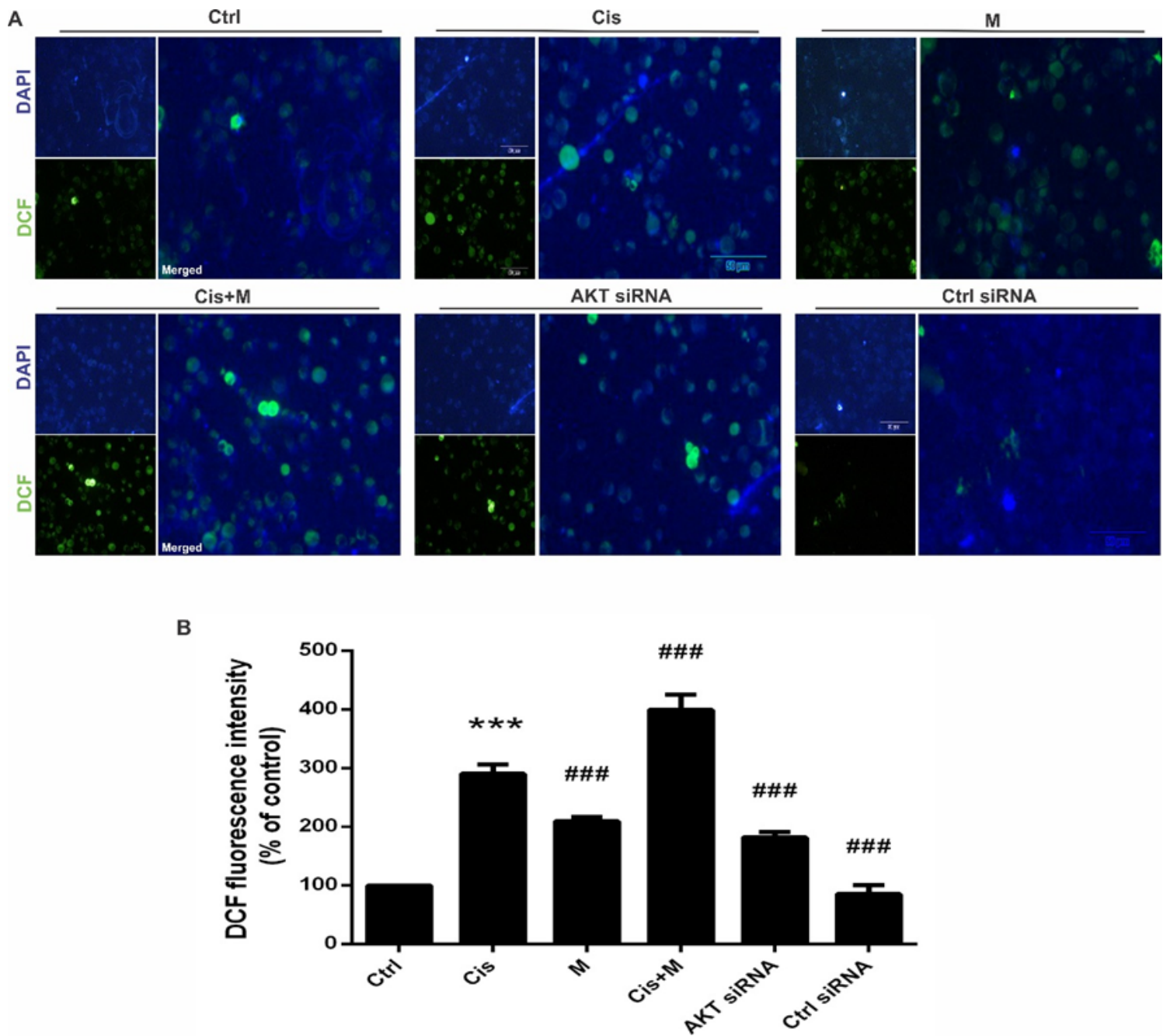


Figure 2

Effect of different interventions on reactive oxygen species (ROS) production in OVCAR3 cells. Data are presented as means \pm SEM: *** $p < 0.001$ vs. Ctrl group; ### $p < 0.001$ vs. Cis group. (Ctrl: control; Cis: cisplatin, M: melatonin, Cis+M: Cisplatin + melatonin).

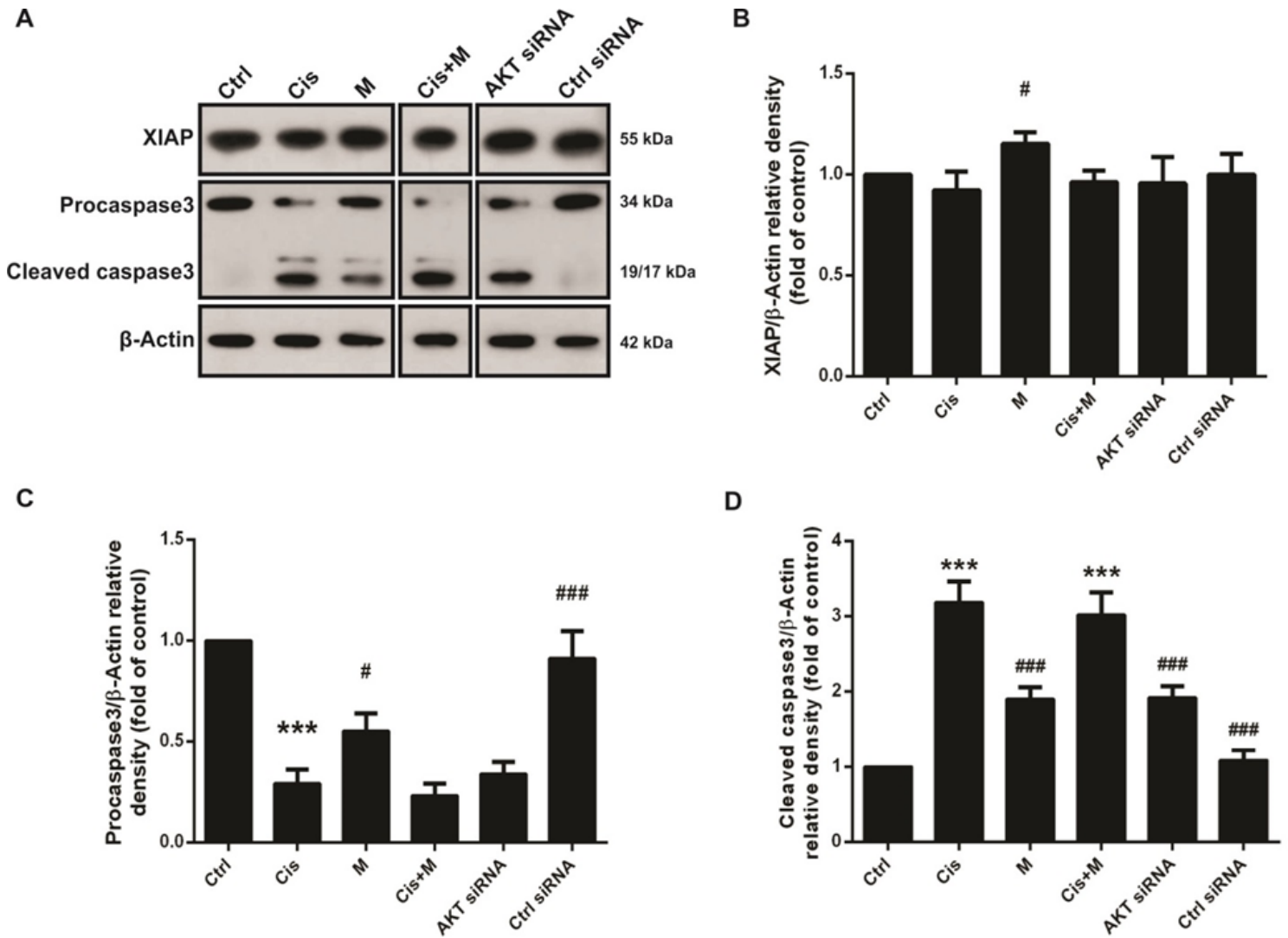


Figure 3

The effect of different treatments on the protein expressions of XIAP, caspase 3, and cleavage of caspase 3 in the OVCAR3 cells. A) Immunoblotting images of XIAP, pro-caspase 3, cleaved caspase 3, and β -actin proteins detected by Western blot. Quantification of band densities of B) XIAP, C) pro-caspase 3, and D) cleaved caspase 3 in different experimental groups. Data are presented as means \pm SEM (triplicate): *** p <0.001 vs. Ctrl group; # p <0.05, ### p <0.001 vs. Cis group. (Ctrl: control; Cis: cisplatin, M: melatonin, Cis+M: Cisplatin + melatonin).

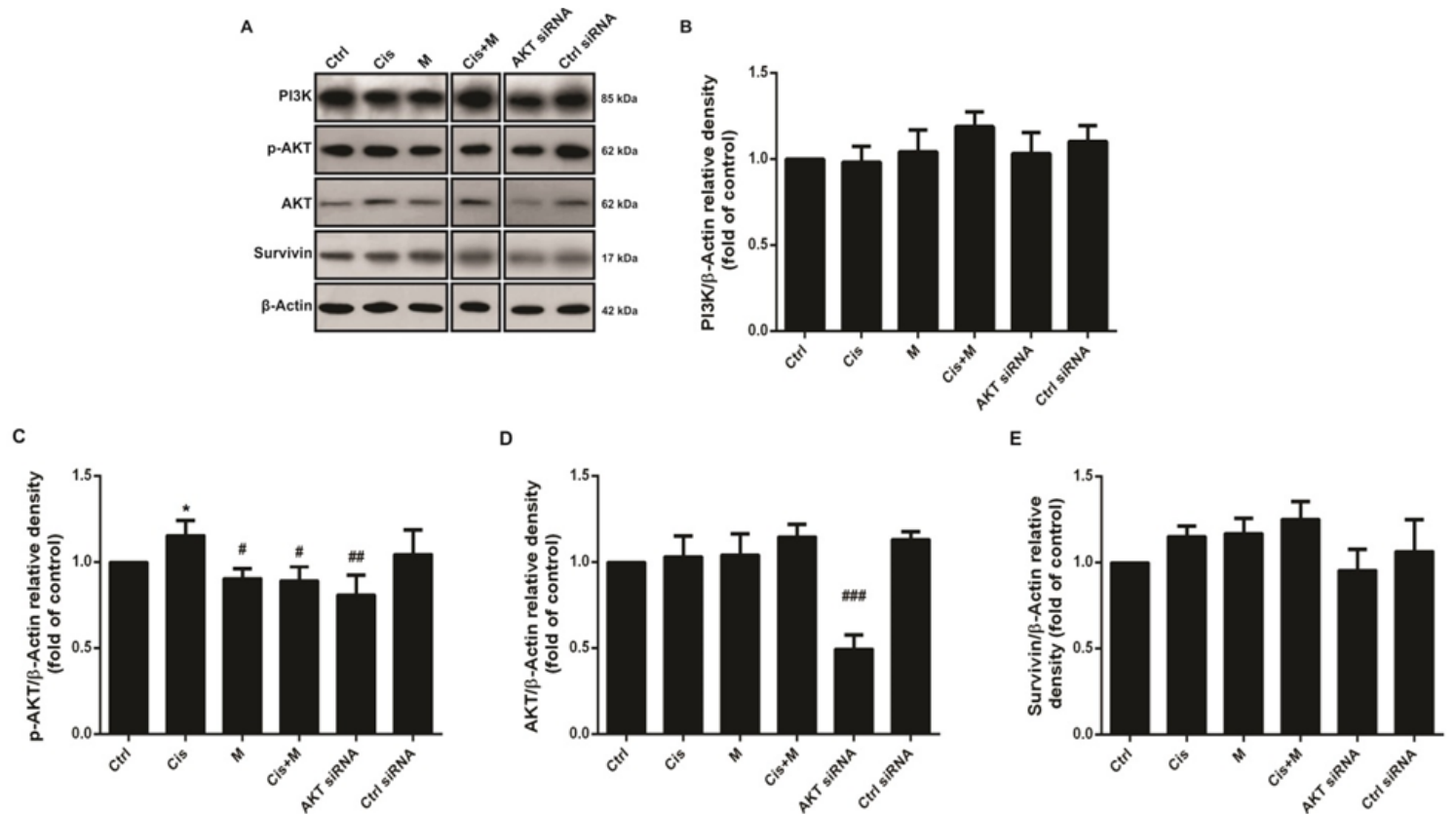


Figure 4

The effect of different treatments on the protein expressions of PI3K, Akt, and Survivin, as well as p-Akt levels in the OVCAR3 cells. A) Immunoblotting images of PI3K, p-AKT, Akt, Survivin, and β -actin proteins detected by Western blot. Quantification of band densities of B) PI3K, C) p-Akt, D) Akt, and E) Survivin in different groups. Data are presented as means \pm SEM (n=3): * p<0.05 vs. Ctrl group. # p<0.05, ## p<0.01, and ### p<0.001 vs. Cis group. (Ctrl: control; Cis: cisplatin, M: melatonin, Cis+M: Cisplatin + melatonin)

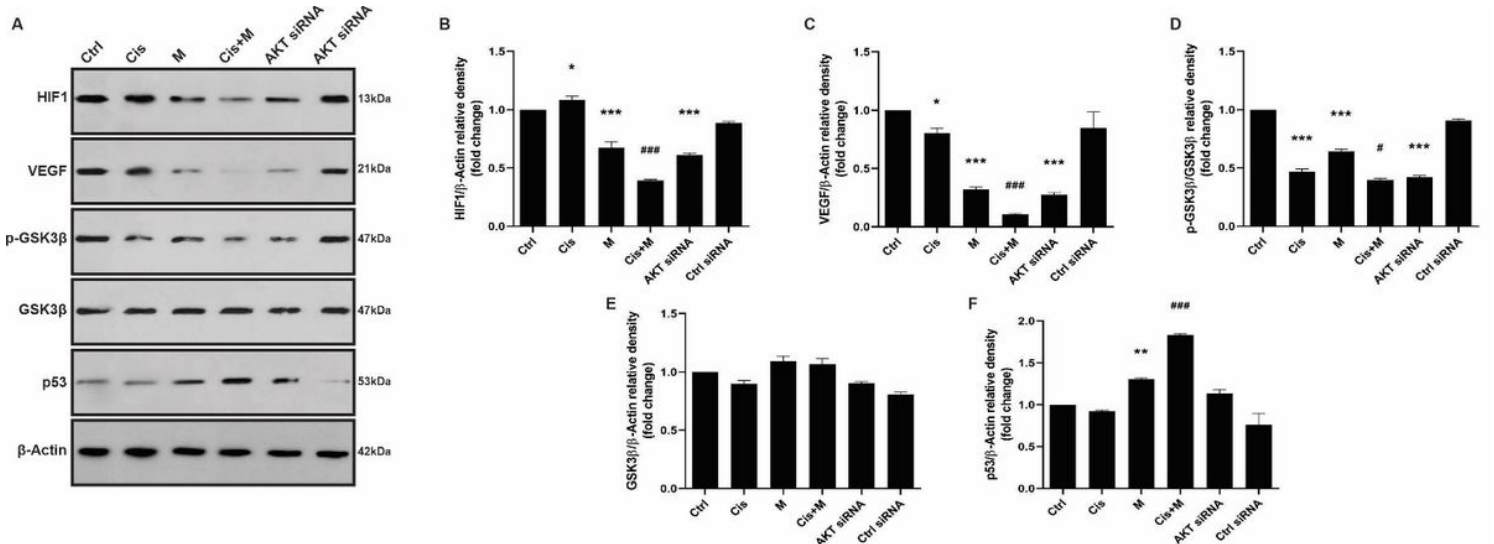


Figure 5

Evaluating the effect of different treatments on the protein expressions of HIF1, VEGF, p-GSK3 β , GSK3 β and p53 in the OVCAR3 cells. A) Immunoblotting images of the proteins detected by western blot after various treatments on OVCAR3 cell line. Quantification of band densities of HIF1 (B), VEGF (C), p-GSK3 β (D), GSK3 β (E) and p53 (F) in different experimental groups. Data are presented as means \pm SEM (triplicate): ***p<0.001 vs. Ctrl group; # p<0.05, ### p<0.001 vs. Cis group. (Ctrl: control; Cis: cisplatin, M: melatonin, Cis+M: cisplatin + melatonin).