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ORIGINAL PAPER

Melatonin synergistically enhances docetaxel induced endoplasmic reticulum stress to promote apoptosis by suppressing NF-κB activation in cervical cancer

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Abstract

Cervical cancer is the fourth most common malignancy in women globally. Although chemotherapy significantly improves the survival of cervical cancer patients, the development of drug resistance is inevitable. In the present study, our study showed that melatonin suppressed the proliferation, cell survival, colony formation, and the ability of adhering to fibronectin in cervical cancer cells. Our data suggested that docetaxel insensitivity was caused by NF-κB pathway activation, and followed by reducing endoplasmic reticulum stress and apoptosis. We showed that melatonin functioned as an oncostatic agent via inhibition of NF-κB signaling in cervical cancer cells. Interestingly, melatonin not only reduced the basal and inducible NF-κB pathway activation, but also prevented docetaxel induced NF-κB pathway activation by stabilizing IκBα protein. Importantly, inhibition of NF-κB pathway activation by melatonin abrogated the protective effect of NF-κB activation on docetaxel provoked endoplasmic reticulum stress, and further enhanced endoplasmic reticulum stress and apoptosis to produce synergistic oncostatic effects in cervical cancer cells. In summary, we revealed that melatonin was a novel agent to enhance docetaxel sensitivity by abolishing NF-κB activation and aggravating endoplasmic reticulum stress. Our results might provide a rationale for the clinical application of melatonin to overcome docetaxel resistance in cervical cancer patients.

Keywords Melatonin · Cervical cancer · Docetaxel · Endoplasmic reticulum stress · NF-κB · Apoptosis

Abbreviations

ER	Endoplasmic reticulum
UPR	Unfolded protein response
FBS	Fetal bovine serum
CI	Combination index
TNF	Tumor necrosis factor
HPV	Human papillomavirus

Introduction

Cervical cancer is a prevalent female reproductive system disease [1]. Cervical cancer is currently treated with surgery, chemotherapy, and radiation. Drug resistance may arise in tumor cells as a result of constant exposure to different chemotherapeutic medications [2]. Such resilience lowers tumor cell susceptibility to chemotherapy, resulting in therapeutic failure. Thus, elucidating the mechanism of

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drug resistance and developing strategies to enhance drug sensitivity are urgently needed.

NF-κB, a family of pleiotropic transcription factors, is aberrantly activated in a wide range of human cancers, including cervical cancer, in which NF-κB promotes the survival and malignancy properties by upregulating multiple genes, which involved in the regulation of immune and inflammatory responses, cell proliferation, apoptosis and metastasis [3]. Compelling evidence has established the crucial role of NF-κB signaling in cervical cancer pathogenesis [3]. The upregulation of NF-κB target genes leads to NF-κB signaling pathway addiction and sensitivity to apoptosis upon inhibition of NF-κB pathway [4]. Recent studies show that docetaxel might trigger an unexpected NF-κB activation [5, 6]. These studies imply that blocking docetaxel induced constitutive NF-κB activation could be an effective strategy to enhance docetaxel sensitivity and overcome drug resistance.

The endoplasmic reticulum is a major site of protein synthesis, transport and protein folding [7]. endoplasmic reticulum homeostasis is essential for the correct cellular function [7]. When the accumulation of unfolded and/or misfolded protein exceeds the rate of protein refolding and degradation, endoplasmic reticulum stress occurs followed by the activation of three well-known pathways, including IRE1, ATF6 and PERK [7]. The unfolded protein response (UPR) is activated to resolve the stress and restore endoplasmic reticulum homeostasis [8]. Chronically elevated levels of endoplasmic reticulum stress have been implicated in a number of diseases, including cancers [9]. Available evidences indicate that endoplasmic reticulum stress and NF-κB interact at multiple levels. The activation of NF-κB has been reported to be a consequence of endoplasmic reticulum stress [10–12]. Moreover, activation of NF-κB represses endoplasmic reticulum stress and apoptotic genes expression to increase cell survival during endoplasmic reticulum stress [13]. Thus, modulating NF-κB activation and endoplasmic reticulum stress level might function as an effective strategy to enhance docetaxel sensitivity.

Melatonin (N-acetyl-5-methoxytryptamine) is an indoleamine compound mainly synthesized and secreted by the pineal gland to regulate the circadian dark/light rhythm of the human body [14]. Melatonin has diverse

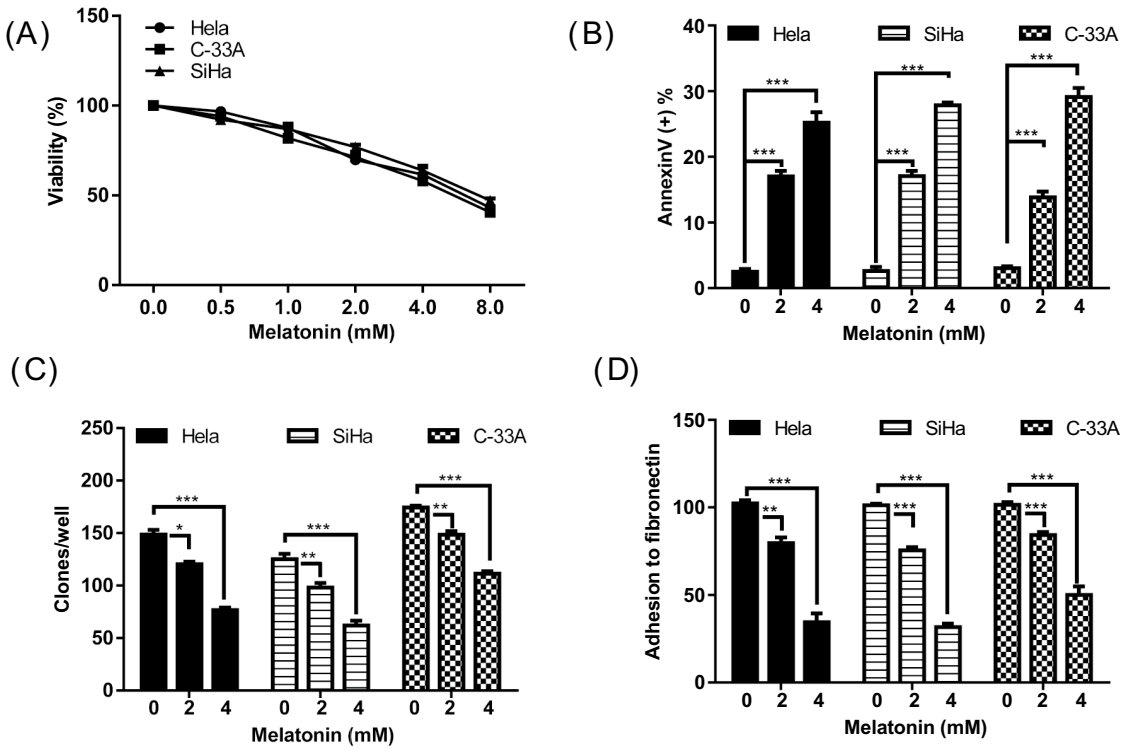


Fig. 1 Melatonin inhibits malignant properties of cervical cancer cells. **A** Hela, C-33A, and SiHa cells were treated with indicated concentration of melatonin for 48 h. Cell viability was evaluated by MTT assay. **B** Hela, C-33A, and SiHa cells (2×10^6) were treated with indicated concentration of melatonin for 48 h. Cells were subjected for apoptosis analysis. **C** Hela, C-33A, and SiHa cells (1×10^3) were cultured in soft agar. The statistical results were presented. **D** Hela, C-33A, and SiHa cells were pretreated with indicated concentration of melatonin for 24 h. Calcein-AM labeled cells (5×10^4) were cultured over fibronectin and the adhesion determined by fluorescence intensity. Results represent the mean ± SEM of data from at least three independent experiments. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, The ANOVA test, followed by Least Significant Difference test, were used to make statistical comparisons

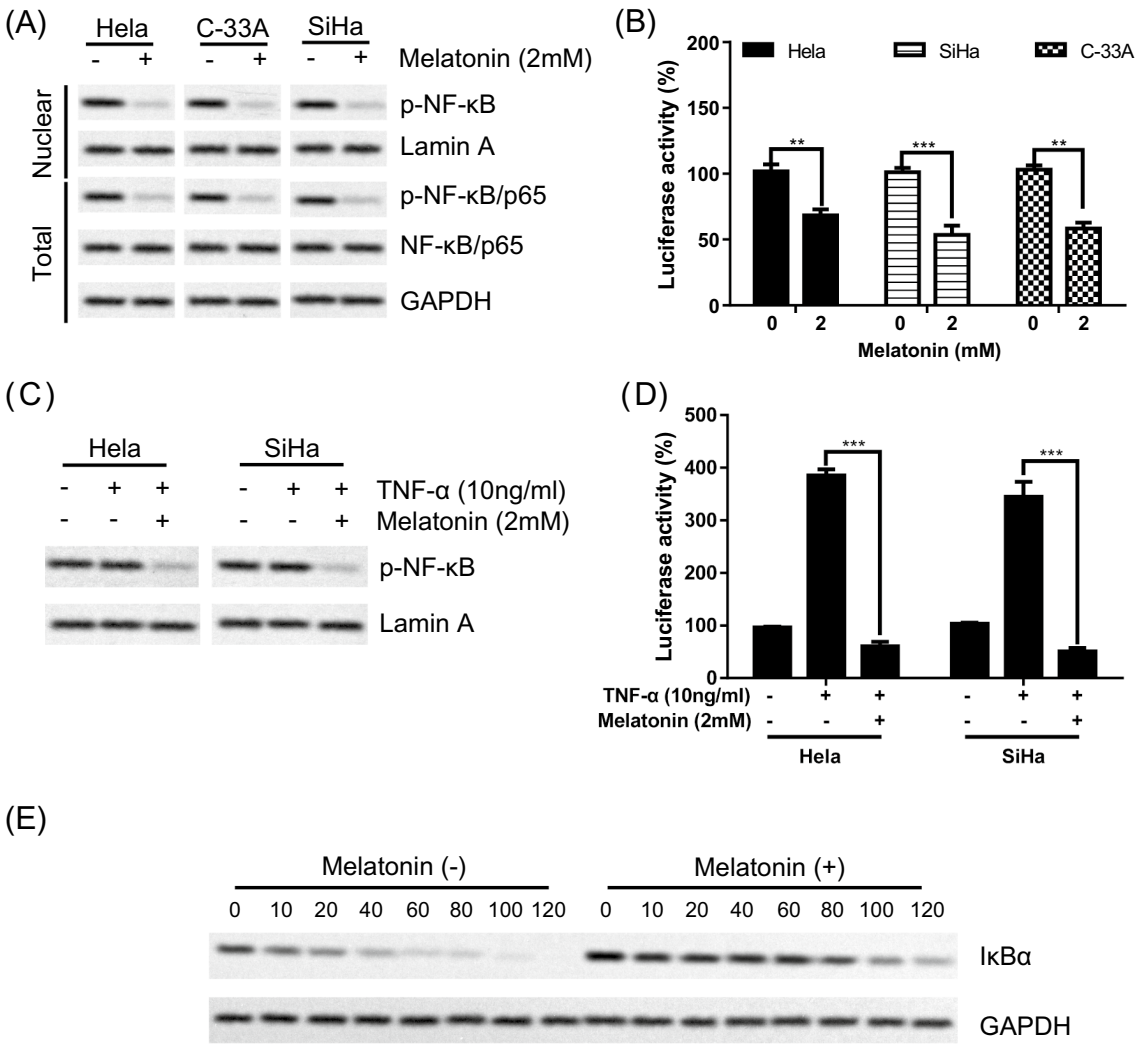
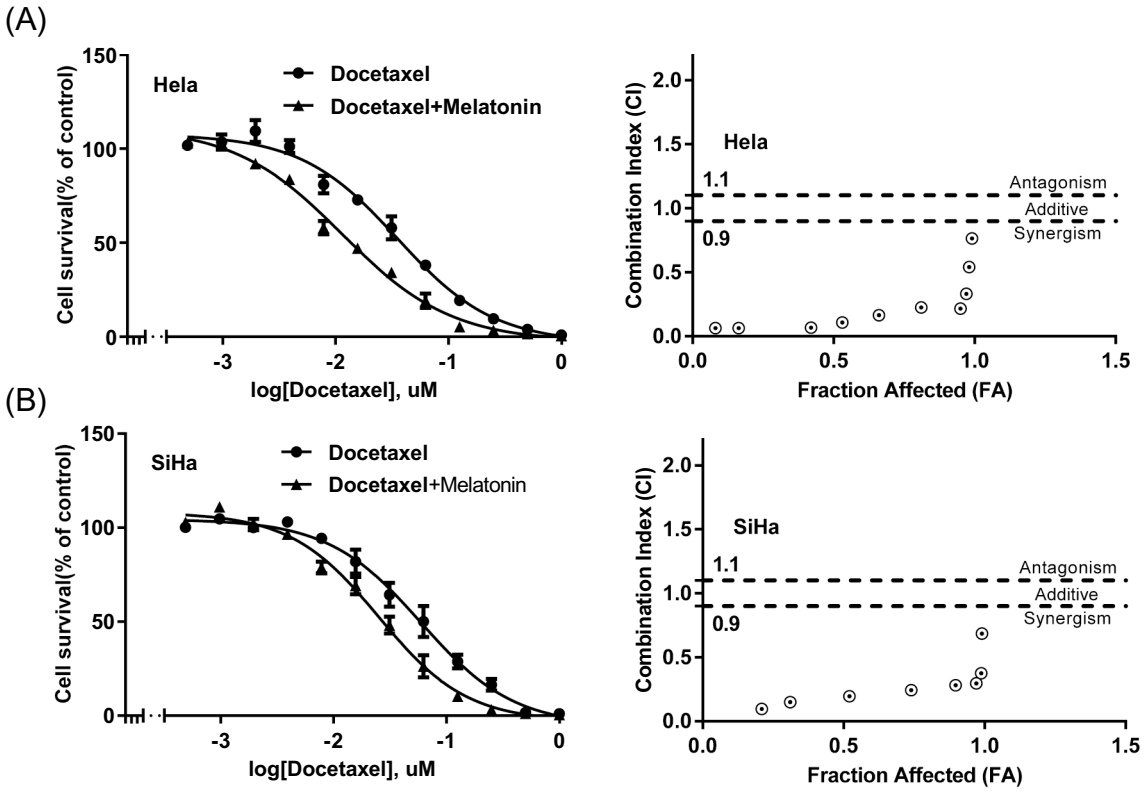


Fig. 2 Melatonin inhibits basal and inducible NF-κB activity by stabilizing IκBα in cervical cancer cells. **A** Hela, C-33A, and SiHa cells (2×10^6) were treated with 2 mM melatonin for 48 h. Cells were subjected for nuclear protein or total protein extraction. Nuclear protein and total protein were analyzed by western blot. **B** Hela, C-33A, and SiHa cells (2×10^6) were treated with 2 mM melatonin for 48 h. Cells were collected and perform luciferase reporter assay. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, Student's t test (**C**). and **D** Triplicate of Hela and SiHa were prepared. Cells were treated with or without 2 mM melatonin for 24 h. Then, cells were treated with or without 10 ng/ml TNF-α for 12 h. Cells were subjected for nuclear protein extraction and western blot analysis (**C**) or luciferase reporter assay (**D**). * $p<0.05$, ** $p<0.01$, *** $p<0.001$, The ANOVA test, followed by Least Significant Difference test, were used to make statistical comparisons.). **E** Hela cells were treated with or without 2 mM melatonin for 24 h. Then, melatonin treated or untreated cells were divided into eight equal parts. These cells were treated with 50ug/ml cycloheximide for indicated time. Cells were subjected for western blot analysis

functions, including antioxidant, immune modulation and anti-aging [15, 16]. Previous studies have elucidated that melatonin suppresses NF-κB pathway via various mechanisms, including inhibition of NF-κB DNA-binding activity [17, 18], promoting the phosphorylation of IKKα/IκBα/p65 [19, 20], promoting the nuclear translocation of NF-κB p50/p65 [19, 20], and enhancing the deacetylation of the RelA/p65 [21]. Additionally, melatonin also participates in regulating endoplasmic reticulum stress by modulating the redox status [22], increasing CHOP expression [23], suppressing PI3K/AKT/mTOR pathway [24]. Currently, melatonin is considered to be an important natural oncostatic agent with low toxicity in multiple cancerous diseases, such as breast cancer, liver cancer and colorectal cancer [25]. However, whether melatonin might function as oncostatic agent in cervical cancer and the underlying mechanisms behind this oncostatic function are still unclear.

In the current study, we evaluated the oncostatic function of melatonin in cervical cancer. Our data might provide


(A)
Hela
Docetaxel
Docetaxel+Melatonin
Cell survival(% of control)
log[Docetaxel], uM
Combination Index (CI)
Fraction Affected (FA)
1.1
0.9
Antagonism
Additive
Synergism
(B)
SiHa
Docetaxel
Docetaxel+Melatonin
Cell survival(% of control)
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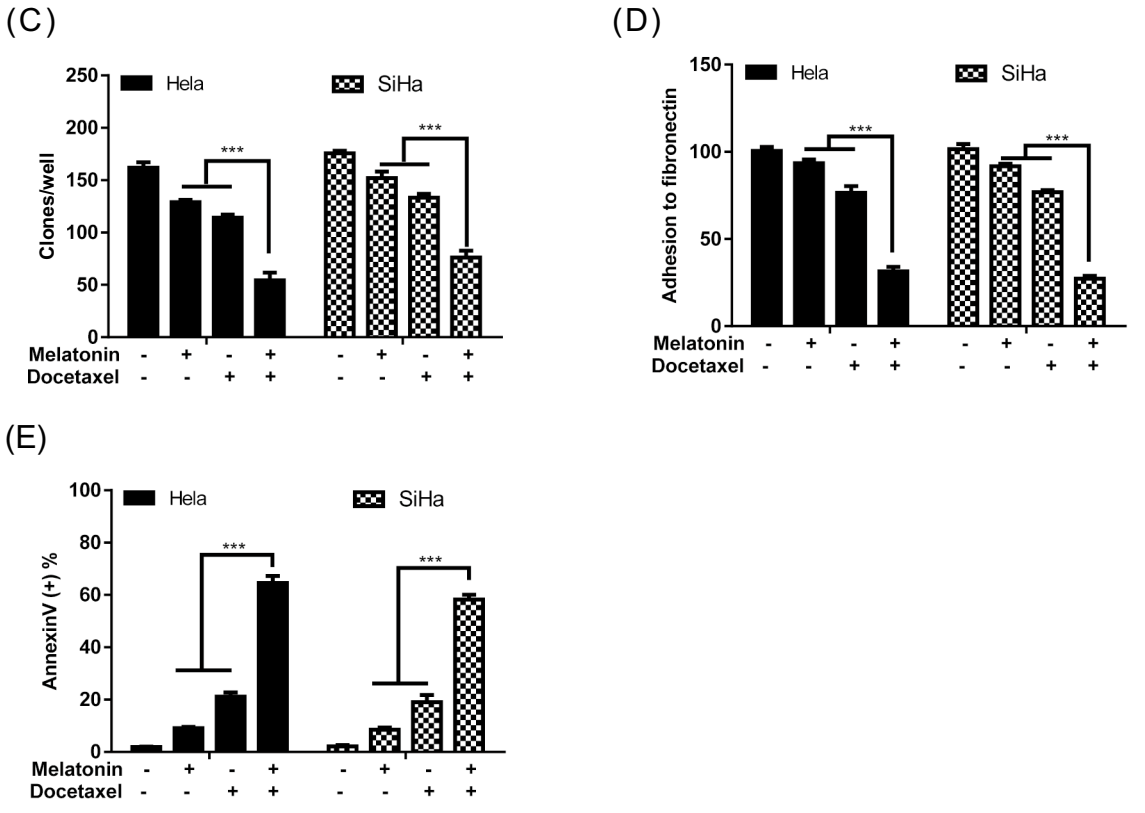
(C)
Hela
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Docetaxel
(E)
Hela
SiHa
AnnexinV (+) %
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Melatonin
Docetaxel

◂**Fig. 3** Synergistic effects of melatonin and bortezomib on cervical cancer cells. **A** and **B** Hela or SiHa cells were divided into two groups. One group of cells was treated with indicated concentration of docetaxel and without melatonin (1 mM). Another group of cells was treated with indicated concentration of docetaxel and melatonin (1 mM). Cell viability was evaluated by MTT assay (left panel). The combination indices were calculated from data obtained in MTT assay. The right panel showed the fraction of Hela or SiHa cells that were affected by the docetaxel and melatonin combinations. **C** Hela or SiHa cells (1×10^3) were cultured in soft agar. Cells were treated with or without docetaxel (10 nM) or melatonin (1 mM). **D** Hela or SiHa cells were pretreated with or without docetaxel (10 nM) or melatonin (1 mM). Calcein-AM labeled cells (5×10^4) were cultured over fibronectin and the adhesion determined by fluorescence intensity. **E** Hela or SiHa cells were treated with or without docetaxel (10 nM) or melatonin (1 mM). Cells were subjected for apoptosis analysis. Bar represent mean ± SEM of three independent experiments * $p<0.05$, ** $p<0.01$, *** $p<0.001$, The ANOVA test, followed by Least Significant Difference test, were used to make statistical comparisons

a rationale for clinical application of melatonin to reduce resistance of docetaxel in cervical cancer cells.

Materials and methods

Reagents and cell culture

Melatonin, cycloheximide, fibronectin, TNF-α, docetaxel, salubrinal, tunicamycin, Calcein-AM and Annexin V-FITC Apoptosis Detection Kit were purchased from Sigma-Aldrich. Dual-Luciferase® Reporter Assay Kit was purchased from Promega. FlowTACSTM Apoptosis Detection Kit was purchased from Trevigen. Antibodies against NF-κB, p-NF-κB (Ser536), Lamin A, IκBα, p-IκBα, CHOP, GPR78, Ki67, and GAPDH were purchased from Cell Signaling Technology. Hela, C-33A, and SiHa cells were obtained from the American Type Culture Collection (ATCC). Cells were cultured in DMEM (Thermo Scientific) supplemented with 10% fetal bovine serum (FBS; Thermo Scientific). All cells were maintained at 37℃ in a humidified 5% CO2 atmosphere.

Cell proliferation and soft agar assays

For the proliferation assays, cells were counted by the trypan blue exclusion method. Clonogenicity of cervical cancer cells was determined by plating 1×10^3 cells in 6-well plates, with a bottom layer of 0.6% agar and a top layer of 0.3% agar containing the cells. Growth media was added on top of the agar and renewed every 3 days. After a 10-day incubation, colonies were visualized using crystal violet staining.

Cell adhesion assays

Cells were labeled by incubation with calcein-AM (1 μg/ml) for 30 min in serum-free media. Cells were washed with PBS three times and resuspended in adhesion media (RPMI-1640, 0.5% BSA, 10 mM HEPES buffer). Cells (100 μl containing 5×10^4 cells) were added to 96-well plates coated with fibronectin, and incubated for 30 min at 37 ℃. Unbound cells were washed out with adhesion media, 100 μl of PBS were added per well and fluorescence was measured using a multiwell plate reader.

Annexin V/PI analysis

Cells treated with the indicated agents and were collected. Cells were resuspended in binding buffer. Annexin-V-FITC was added to the cells followed by the addition of 5 μl/sample PI. The samples were then incubated for 15 min in the dark at 4 °C, and subjected to flow cytometry.

MTT assay

Cells were plated in 96-well plate (4-wells for each dose) at 2×10^3 cells/well in a final volume of 100 μl and treatment with indicated agents. After incubating for indicated time, 20 μl of MTT solution (5 mg/ml) was added to each well. Cells were incubated at 37 °C for 1–4 h. Formazan was solubilized by 200 μl DMSO, and measured using a multiwell plate reader.

Luciferase reporter assay

Cells were transiently transfected with NF-κB reporter, control reporter and/or Renilla luciferase reporter, respectively. After transfection, the cells were incubated in medium with the indicated agents. Cells were collected and lysed with lysis buffer. Luciferase activity was determined by using the Dual-Luciferase Assay System according to the manufacturer's protocol.

Western blot analysis

Cells were harvested and lysed in RIPA buffer. The protein concentration was determined by Bradford method. Equal amounts of protein were subjected to electrophoresis in SDS–polyacrylamide gels and transferred to nitrocellulose membrane. The membranes were blocked, and then incubated with the indicated antibodies at 4℃ overnight. The membranes were washed with TBST, then incubated with appropriate secondary antibodies at RT for 0.5 h. Antibody binding was detected with chemiluminescence kit (Pierce).

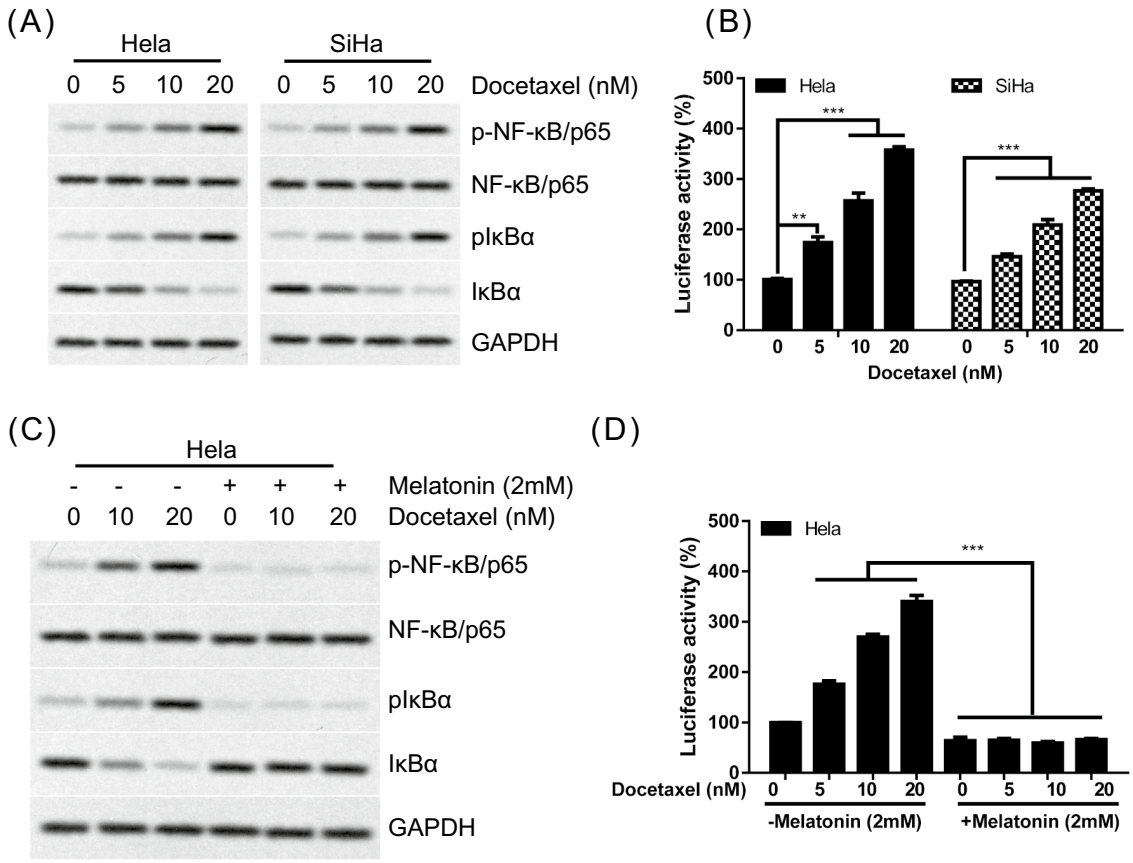


Fig. 4 Melatonin reverses docetaxel induced NF-κB activation in cervical cancer cells. **A** Hela or SiHa cells were treated with indicated concentration of docetaxel for 24 h. Cell were subjected for western blot analysis. **B** Hela or SiHa cells were transfected with NF-κB reporter plasmid and control reporter for 6 h. Then, cells were treated with indicated concentration of docetaxel for 24 h. Cell were subjected for luciferase reporter assay analysis. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, The ANOVA test, followed by Least Significant Difference test, were used to make statistical comparisons. **C** Hela cells were treated with or without indicated concentration of docetaxel or melatonin (2 mM) for 24 h. Cell were subjected for western blot analysis. **D** Hela cells were transfected with NF-κB reporter plasmid and control reporter for 6 h. Then, cells were treated with or without indicated concentration of docetaxel or melatonin (2 mM) for 24 h. Cell were subjected for luciferase reporter assay analysis. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, The ANOVA test, followed by Least Significant Difference test, were used to make statistical comparisons

Combination index (CI) calculation

The data acquired from the MTT assay were used to evaluate the CI. The CI was analyzed with the CompuSyn software using the average fraction of cells that responded to each drug [26].

Statistical analysis

Statistical analyses were performed using the SPSS software, version 16.0 (SPSS Inc.). The unpaired Student's t test was used to perform a statistical comparison between two groups. The ANOVA test, followed by Least Significant Difference test, was used when performing multiple comparison. The level of significance was set at $p<0.05$.

Results

Melatonin suppresses malignant properties of cervical cancer cells

We first examined whether melatonin displayed antiproliferative effects in cervical cancer. Cervical cancer is the most common human papillomavirus (HPV)-related disease. Thus, several cervical cell lines, HeLa (HPV18), SiHa (HPV16), and C33A (HPV −) [27], with different HPV background was chosen in this study. As shown in Fig. 1A, treatment with melatonin (0.5–8 mM) for 48 h induced a dose-dependent reduction of cell viability in Hela, C-33A, and SiHa cells. Apoptosis analysis revealed that melatonin also induced apoptosis in a dose-dependent manner in Hela, C-33A, and SiHa cells (Fig. 1B). Our data

also showed that melatonin was able to effectively suppress cell growth and colony formation of cervical cancer cells in a dose-dependent manner, with > 70% of colonies lost when treated with melatonin in Hela, C-33A, and SiHa cells (Fig. 1C). Additionally, after 24 h of pretreatment with indicated concentration of melatonin, the ability of adhering to fibronectin was markedly inhibited in all three cervical cancer cell lines (Fig. 1D). These data indicated that melatonin could be a novel agent to target cervical cancer cells.

Melatonin inhibits basal and inducible NF-κB activity by stabilizing IκBα in cervical cancer cells

NF-κB signaling pathway plays a key role in the survival and proliferation of many kinds of B-cell tumors, including cervical cancer [3]. We next evaluated the effect of melatonin on NF-κB/p65 activity. During activation, phosphorylation of IκBα by the IKK complex triggers IκBα polyubiquitination and subsequent degradation by the proteasome, which liberates NF-κB/p65 to translocate into nuclear [28]. Our data showed that treatment of 2 mM melatonin induced a remarkable decrease in nuclear levels of NF-κB/p65 with a concomitant suppression of NF-κB/p65 phosphorylation at Ser356 in Hela, C-33A, and SiHa cells (Fig. 2A). Furthermore, the transcription activity of NF-κB/p65 was significantly reduced in Hela, C-33A, and SiHa cells after 2 mM melatonin treatment as determined by luciferase reporter assay (Fig. 2B). Tumor necrosis factor (TNF) is one of the most potent physiological inducers of NF-κB/p65 activation [29]. We investigated whether melatonin might suppress TNF-α induced NF-κB activation. As shown in Fig. 2C, TNF-α indeed induced the nuclear translocation of NF-κB/p65, while treatment of melatonin abolished TNF-α induced NF-κB/p65 nuclear translocation, and further reduced the basal nuclear expression of NF-κB/p65. Consistently, melatonin also abolished TNF-α enhanced NF-κB/p65 transcription activity (Fig. 2D). As the protein stability of IκBα was critical for NF-κB/p65 activation, we evaluated the protein degradation of IκBα. Treatment with 2 mM melatonin significantly reduced the protein degradation of IκBα as compared with the control group (Fig. 2E). These data suggested that melatonin stabilized IκBα to suppress NF-κB activation.

Synergistic effects of melatonin and docetaxel on cervical cancer cells

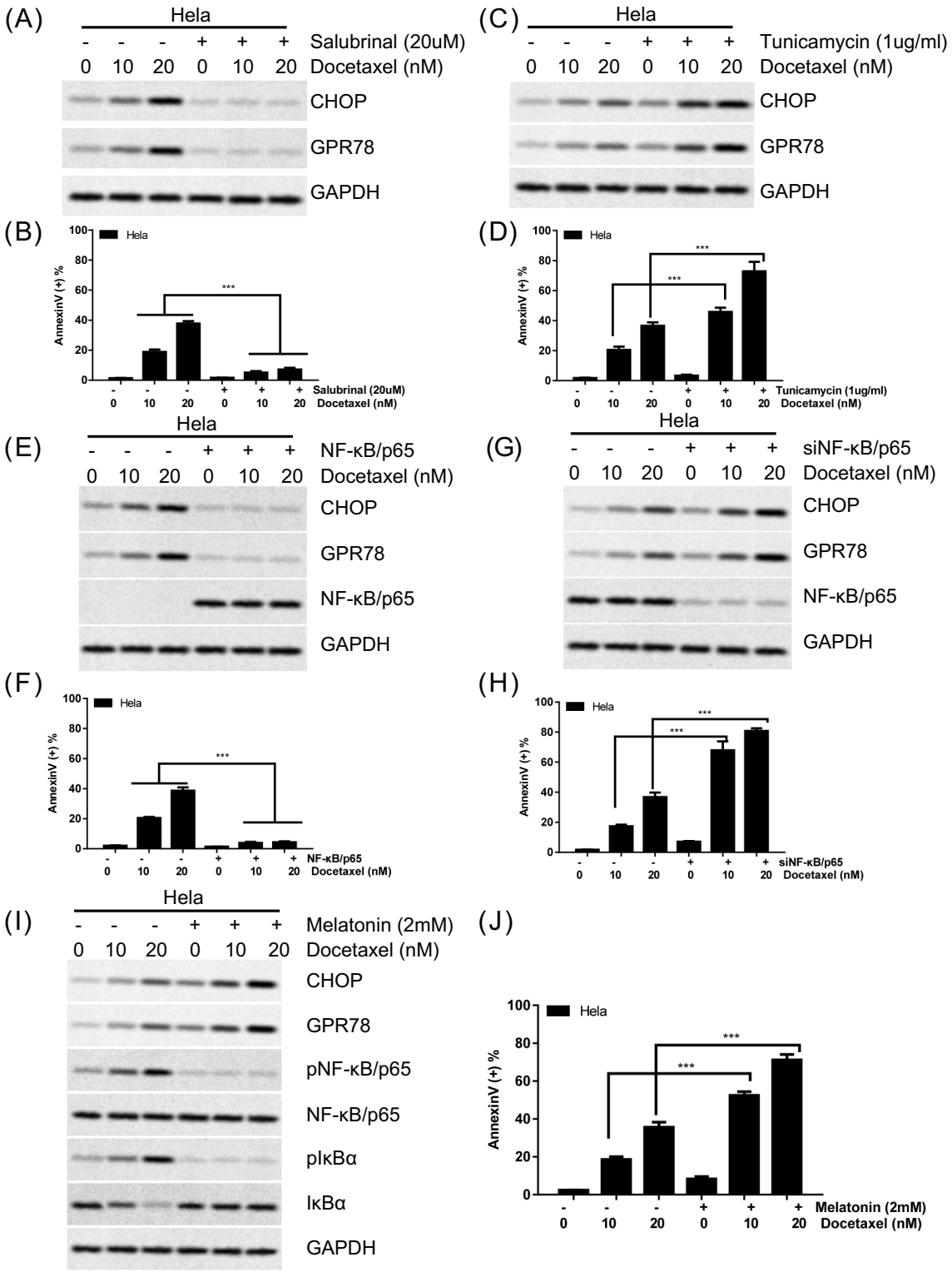
Docetaxel is commonly used to treat cervical cancer. We next determined whether melatonin might interact with docetaxel in cervical cancer cells. The combination of docetaxel and melatonin significantly decreased cell viability as compared with single agent treatment alone (Fig. 3A and B, left panel). We further calculated the drug CI using Calcusyn. The combination of docetaxel and melatonin displayed a synergistic interaction as demonstrated by a consistent CI value < 0.9 (Fig. 3A and B, right panel). Moreover, our data showed that either agent alone slight suppressed cell growth in Hela, and SiHa cells, but the combination of two drugs showed synergistic inhibition effect on colony formation (Fig. 3C). Cell adhesion assay further showed the synergistic effect on adhesion capacity of cervical cancer cells treated with docetaxel plus melatonin (Fig. 3D). Additionally, cell apoptosis analysis also showed a synergistic effect on apoptosis induction when melatonin treatment was combined with docetaxel treatment (Fig. 3E). These results suggested that melatonin enhanced proliferation inhibition, adhesion and cytotoxicity induced by docetaxel in cervical cancer cell lines.

Melatonin reverses docetaxel induced NF-κB activation in cervical cancer cells

We speculated that melatonin might overcome docetaxel induced NF-κB activation and synergistically enhance the anti-cancer effects of docetaxel. Indeed, we observed that the expression of IκBα was downregulated by docetaxel treatment in a dose-dependent manner in Hela and SiHa cells (Fig. 4A). Docetaxel also triggered the phosphorylation of IκBα, which is required for proteasomal degradation of IκBα, and NF-κB in Hela and SiHa cells (Fig. 4A). Consistently, docetaxel markedly increased NF-κB activity in a dose-dependent manner in Hela and SiHa cells (Fig. 4B). We next evaluated whether melatonin might counteract docetaxel induced NF-κB activation. As shown in the Fig. 4C, treatment with 2 mM melatonin effectively abrogated docetaxel induced downregulation of IκBα, and upregulation of IκBα and NF-κB phosphorylation in Hela cells. Additionally, melatonin treatment blocked the docetaxel induced NF-κB transcription activity (Fig. 4D).

Melatonin enhances docetaxel induced endoplasmic reticulum stress and apoptosis by suppressing NF-κB activation in cervical cancer cells

We further explored the mechanism of the synergistic effects of melatonin and docetaxel. We observed that docetaxel treatment induced significant endoplasmic reticulum stress as assessed by the expression of endoplasmic reticulum stress markers, CHOP and GPR78 (Fig. 5A), and apoptosis (Fig. 5B). Alleviating endoplasmic reticulum stress by treating with 20uM salubrinal significantly reduced docetaxel induced endoplasmic reticulum stress (Fig. 5A) and apoptosis (Fig. 5B). On the contrary, enhancing endoplasmic reticulum stress by treating with 1ug/ml tunicamycin substantially aggravated docetaxel induced endoplasmic



reticulum stress (Fig. 5C) and apoptosis (Fig. 5D). Due to the activation of NF-κB by docetaxel treatment, we asked whether docetaxel induced NF-κB activation might affect endoplasmic reticulum stress. Exogenous overexpression of NF-κB markedly reduced docetaxel triggered upregulation of CHOP and GPR78 expression (Fig. 5E). Accordingly, docetaxel induced apoptosis was relieved by NF-κB overexpression (Fig. 5F). We further downregulated NF-κB activation to observe the effect on docetaxel induced endoplasmic reticulum stress and apoptosis. As shown in the Fig. 5G and

◂**Fig. 5** Melatonin enhances docetaxel induced endoplasmic reticulum stress and apoptosis by suppressing NF-κB activation in cervical cancer cells. **A** and **B** Hela cells were treated with or without indicated concentration of docetaxel or salubrinal (20uM) for 24 h. Cell were subjected for western blot analysis (**A**) and apoptosis analysis (**B**). **C** and **D** Hela cells were treated with or without indicated concentration of docetaxel or tunicamycin (1ug/ml) for 24 h. Cell were subjected for western blot analysis (**C**) and apoptosis analysis (**D**). **E** and **F** Hela cells were transfected with plasmid expressed NF-κB or control plasmid for 12 h. Cells were treated with indicated concentration of docetaxel for 24 h. Cell were subjected for western blot analysis (**E**) and apoptosis analysis (**F**). **G** and **H** Hela cells were transfected with siRNA against NF-κB or control siRNA for 24 h. Cells were treated with indicated concentration of docetaxel for 24 h. Cell were subjected for western blot analysis (**G**) and apoptosis analysis (**H**). **I** and **J** Hela cells were treated with or without indicated concentration of docetaxel or melatonin (2 mM) for 24 h. Cell were subjected for western blot analysis (**I**) and apoptosis analysis (**J**). * $p<0.05$, ** $p<0.01$, *** $p<0.001$, The ANOVA test, followed by Least Significant Difference test, were used to make statistical comparisons

H, downregulation of NF-κB expression by siRNA significantly enhanced docetaxel triggered upregulation of CHOP and GPR78 expression and apoptosis. These data suggested that NF-κB activation could be critical for limiting docetaxel induced endoplasmic reticulum stress and apoptosis, while suppression of NF-κB activation by melatonin might overcome these NF-κB caused protective effects. Consistently, melatonin treatment indeed abolished docetaxel induced NF-κB activation (Fig. 5I), and subsequently exacerbated the endoplasmic reticulum stress (Fig. 5I) and apoptosis (Fig. 5J) induced by docetaxel. Our data indicated that melatonin enhanced anti-cancer effects of docetaxel by counteracting NF-κB activation to aggravate endoplasmic reticulum stress and subsequent apoptosis.

Discussion

Docetaxel is one of the most commonly used drugs included in the standard treatment of cervical cancer. However, a portion of patients eventually relapses after initial response. Thus, developing strategies that overcome this docetaxel resistance are urgently needed. In the current study, we utilized melatonin, a natural molecule with low toxicity, to increase docetaxel sensitivity by targeting NF-κB activation to enhance endoplasmic reticulum stress and apoptosis.

Melatonin, a neurohormone produced by the pineal gland, has an important role in influencing a variety of functions, such as the sleep/wake cycle, circadian rhythms, immunomodulation, and regulating antioxidative processes [15]. Melatonin is a low toxicity compound and considered as a dietary supplement for dealing with insomnia, jet lag and other sleep disorders [30]. Current studies have revealed the oncostatic properties of melatonin in a variety of human malignancies at physiological and pharmacological doses [25]. However, the role of melatonin in cervical cancer is remained to be elucidated. The anti-proliferative effects of melatonin on cancer cells rely on its antioxidant, immunostimulating, and apoptotic properties [31]. In this study, we showed that melatonin inhibited proliferation and adhesion, and promoted apoptosis in Hela, C-33A, and SiHa cells in a dose-dependent manner. Our data further revealed that the oncostatic effects of melatonin, at least in part, was attributed to the inhibition of NF-κB pathway activation by stabilizing IκBα.

NF-κB signaling is one of the most critical pathways in cervical cancer pathogenesis [3]. Constitutive NF-κB activity within populations of docetaxel-resistant subclones has been reported [6]. A recent finding indicates that docetaxel triggers an expected increase of phosphorylated IκBα and an unexpected downregulation of total IκBα, followed by constitutive NF-κB activation [32]. Consistently, our data also showed that docetaxel treatment induced constitutive NF-κB activation, indicating that suppression of NF-κB activation could be a constructive strategy to overcome docetaxel resistance. Indeed, several inhibitors of IKKβ kinase, the upstream kinase of IκBα, have been shown efficiently prevented the growth of cancer cells and induced apoptosis [33–35]. Interestingly, our data indicated that melatonin sensitized cervical cancer cells to docetaxel treatment by stabilizing IκBα to suppress NF-κB activation.

ER stress is a double-edged sword for tumors [36]. On the one hand, endoplasmic reticulum stress triggers the UPR to promote the activation of survival or proliferative pathways. On the other hand, a prolonged or severe endoplasmic reticulum stress has a cytotoxic effect and caused cell death. Previous studies have shown the critical role of NF-κB activation in docetaxel resistance [5, 6]. However, the underlying mechanism is unclear. Our data implied that NF-κB activation was important for limiting endoplasmic reticulum stress to avoid triggering the cytotoxic effects and causing cell death. Consistently, a study shows that NF-κB represses GADD153/CHOP promoter activity to reduce endoplasmic reticulum stress and increase cell survival, indicating that a cellular defense against endoplasmic reticulum stress-induced cell death [13]. Importantly, we found that melatonin abrogated the protective effect of NF-κB signaling on endoplasmic reticulum stress by suppressing NF-κB activation, thereby synergistically enhancing endoplasmic reticulum stress to strengthen the oncostatic effects of docetaxel.

In summary, we reported that melatonin synergistically enhanced docetaxel induced oncostatic effects by suppressing NF-κB activation to exacerbate endoplasmic reticulum stress in cervical cancer cells. Our study revealed a novel strategy for improving docetaxel sensitivity in the clinical treatment of cervical cancer patients.

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Authors contribution YS designed, performed experiments, collected, analyzed, interpreted data, wrote the manuscript, and supervised the study; SW performed experiments and analyzed data.

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Data availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no conflict(s) of interest in this research.

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