Melatonin induces the apoptosis and inhibits the proliferation of human gastric cancer cells via blockade of the AKT/MDM2 pathway

JUN SONG[1,3,7], SAI-JUN MA[2], JIAN-HUA LUO[3,7], HUI ZHANG[4], RI-XIONG WANG[5], HUI LIU[6,7], LI LI[1,3,7], ZHI-GUANG ZHANG[1,3,7] and RUI-XIANG ZHOU[3,6,7]

[1]Department of Cell Biology and Genetics, [2]Clinical Laboratory, Second Inpatient Department, Fuzhou General Hospital; [3]Key Laboratory of Ministry of Education for Gastrointestinal Cancer; [4]Fujian Center for Safety Evaluation of New Drugs, Fujian Medical University; [5]Department of Chemotherapy, The First Affiliated Hospital, Fujian Medical University; [6]Department of Human Anatomy, Histology and Embryology; [7]School of Basic Medical Sciences, Fujian Medical University, Fuzhou, Fujian 350108, P.R. China

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Abstract. Globally, gastric cancer (GC) is one of the most common types of cancer and the third leading cause of cancer-related death. In China, gastric and liver cancers have the highest mortality rates. Melatonin, also known as *N*-acetyl-5-methoxytryptamine, is a hormone that is produced by the pineal gland in animals and regulates sleep and wakefulness. Melatonin has been shown to inhibit various carcinomas, including GC. There are many different hypotheses to explain the anticancer effects of melatonin, including stimulation of apoptosis, inhibition of cell growth, regulation of anticancer immunity, induction of free-radical scavenging, and the competitive inhibition of estrogen. However, the underlying mechanism by which these effects are elicited remains elusive. The aim of the present study was to investigate the effects of melatonin on human GC cells and determine the underlying molecular mechanism. We treated SGC-7901 GC cells with melatonin and analyzed the resulting protein changes using protein chip technology. Several proteins related to cell apoptosis and proliferation were identified and further tested in SGC-7901 GC cells. We found that melatonin induced cell cycle arrest and the downregulation of CDC25A, phospho-CDC25A (at Ser75), p21 ($p21^{Cip1}$/$p21^{Waf1}$) and phospho-p21 (at Thr145). Melatonin also induced upregulation of Bax, downregulation of Bcl-xL, an increase in cleaved caspase-9 level and activation of caspase-3, which confirmed the involvement of the mitochondria in melatonin-induced apoptosis. Upstream regulators of the above proteins, MDM2, phospho-MDM2 (at Ser166) and AKT, phospho-AKT (at Thr308) were all attenuated by melatonin, which led to an increase in p53. The present study demonstrated that the oncostatic effects of melatonin on SGC-7901 GC cells are mediated via the blockade of the AKT/MDM2 intracellular pathway.

Introduction

Gastric cancer (GC) is currently the fifth most common cancer worldwide (1). As reported in the World Health Organization's World Cancer Report, there were an estimated 22,220 new cases of GC (7% of all new cancer diagnoses) and 10,990 deaths from GC (9% of all cancer-related deaths) globally in 2014. In China, gastric and liver cancers have the highest mortality rates; the incidence of new cases of GC is second only to that of liver cancer (2).

Melatonin (*N*-acetyl-5-methoxytryptamine) biosynthesis is initiated by the uptake of the essential amino acid tryptophan by the pineal gland. Melatonin possesses diverse physiological functions, including regulation of circadian rhythms, controlling the maturation of the reproductive system and promoting skeletal growth; melatonin also has antitumor, immunomodulatory, antioxidant and free-radical scavenging activities (3,4). Many tissues and organs have the ability to synthesize melatonin in addition to the pineal gland, including the retina, striatum, spleen, liver and gastrointestinal tract (5). In the gastrointestinal tract, for example, the amount of melatonin produced far exceeds that produced by other organs: Approximately 400 times higher than that in the pineal gland, with concentrations 10-100 times higher than those found in plasma (6). Cells of gastrointestinal tissues not only have the ability to secrete melatonin but also express melatonin receptors. Melatonin exerts important protective

Correspondence to: Professor Rui-Xiang Zhou, Department of Human Anatomy, Histology and Embryology, School of Basic Medical Sciences, Fujian Medical University, 1 Xueyuan Road, Fuzhou, Fujian 350108, P.R. China
E-mail: zhourx@mail.fjmu.edu.cn

Abbreviations: GC, gastric cancer; MLT, melatonin

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and regulatory effects via autocrine and paracrine activities in the gastrointestinal tract. A previous study found that melatonin enhances the immune system of the gut (7), regulates fecal water content, reduces peristalsis, and prevents gut damage due to digestive enzymes, hydrochloric acid (6) and exogenously administered drugs (8).

In previous studies, melatonin was found to inhibit various carcinomas, such as GC (9-13), liver (14-17), breast (18-21), oral (22,23) and prostate cancer (24-26). Melatonin exerts anticancer effects by promoting cellular apoptosis, inhibiting cell growth, regulating anticancer immunity, scavenging free radicals and competitively inhibiting estrogen. However, the mechanism by which melatonin exerts these effects is unclear. Our previous studies found that melatonin exhibited effective anticancer effects that were mediated by the stimulation of apoptosis, inhibition of cell growth, and reduction in the number of $CD^{4+}CD^{25+}$ regulatory T cells in mouse GC cells and *in vivo* (27,28). We also found that the nuclear receptor RORγ is involved in the effects of melatonin on human GC cells (12,13). A recent study demonstrated that melatonin induces AGS cell apoptosis via the activation of JNK and p38, and the suppression of NF-κB (13). To further elucidate the effects of melatonin on human GC cells and the molecular mechanism involved, we selected the human GC cell line SGC-7901 and analyzed the resulting changes in proteins using protein chip technology. Several proteins related to apoptosis and cell proliferation were identified and further tested in SGC-7901 cells, including AKT, MDM2, CDC25A, p53, p21, Bcl-xL and Bax. Both CDC25A and p21 are known to regulate cell cycle progression via their interactions with cyclin/CDK complexes. Bcl-xL and Bax are both mitochondrial proteins that control the release of cytochrome *c* and are involved in mitochondrial apoptosis. AKT, MDM2 and p53 are upstream regulators of the mitochondrial apoptosis pathway. The aim of the present study was to elucidate the mechanism by which melatonin elicits its anticancer effects in GC cells.

Materials and methods

Cell culture and reagents. Human GC cell line SGC-7901 was purchased from the Chinese Academy of Sciences, Shanghai Institute for Biological Science (Shanghai, China). SGC-7901 cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with 10% fetal bovine serum (both from GE Healthcare Life Sciences, HyClone Laboratories, Logan, UT, USA). The cultures were maintained at 37˚C in 5% CO_2. Melatonin (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) was dissolved in ethanol prior to use. The final concentration of ethanol in the culture medium never exceeded 1%.

Cell viability assays. After exposure of GC cells to various concentrations of melatonin for various times, we assessed cell viability using the MTS assay (CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay; Promega, Madison, WI, USA), which is based on the mitochondrial conversion of MTS (a tetrazolium salt) into a water-soluble, colored, formazan precipitate that can be quantified by spectrophotometry. SGC-7901 cells were seeded at a density of $1x10^4$ cells/ml in 96-well plates. After 24 h of culture, the cells were treated with 0 (1% ethanol as control was added), 1, 2, 3, 4 or 5 mM melatonin for 24, 48 or 72 h. Absorbance of cells at 490 nm was measured using a microplate reader (Synergy HT; BioTek Instruments Inc., Winooski, VT, USA) when MTS was added 2 h later. Effects on SGC-7901 cell viability was measured by determining the percentage of viable cells relative to the control: % cell inhibition = $[1 - (OD_{mt} - OD_{blank})]/(OD_c - OD_{blank}) \times 100\%$, where OD_{mt} is the average OD value of the melatonin-treated samples, OD_c is the average OD value of the control samples, and OD_{blank} is the average OD value of the blank samples without cells.

Cell morphology at the microscopic and ultramicroscopic levels. SGC-7901 cells were seeded at a density of $2x10^5$ cells/ml in 6-well plates. After 24 h of culture, the cells were treated with 3 mM melatonin or 1% ethanol (control) for 24 h. Cell morphology was observed with an inverted microscope (Primo Vert; Carl Zeiss Microscopy GmbH, Jena, Germany) and an electron microscope (EM208; FEI, Hillsboro, OR, USA).

Cell cycle analysis. After treatment with melatonin, cells were collected and stained with propidium iodide (PI; BD Pharmingen; BD Biosciences, Franklin Lakes, NJ, USA). Single-cell suspensions were sorted by flow cytometry, which revealed the distribution of cells in the three major phases of the cycle (G_0/G_1 vs. S vs. G_2/M).

Analysis of apoptosis. We use the TUNEL assay (DeadEnd™ Fluorometric TUNEL System; Promega) to analyze cell apoptosis *in situ*. This assay measures nuclear DNA fragmentation of apoptotic cells tagged with fluorescein-12-dUTP, which can be visualized by fluorescence microscopy. SGC-7901 cells were cultured on coverslips and treated with either 3 mM melatonin or 1% ethanol for 24 h. After three washes with phosphate-buffered saline, the cells were fixed in 4% paraformaldehyde and incubated at 4˚C for 25 min. Fixed cells were permeabilized with 0.2% Triton X-100 solution for 5 min. The cells were then incubated with a nucleotide mixture and rTdT buffer solution, and incubated at 37˚C for 60 min to allow the tailing reaction to occur in the dark. After terminating the reaction in 2X SSC, the cells were counterstained with PI for 15 min at room temperature. Positive apoptotic cells were identified under 5 random fields of view by fluorescence microscopy (Axio Observer A1; Carl Zeiss Microscopy GmbH, Jena, Germany).

To analyze the rate of cellular apoptosis, we used the FITC Annexin V apoptosis detection kit I (BD Pharmingen; BD Biosciences, Franklin Lakes, NJ, USA), following the manufacturer's instructions. Briefly, SGC-7901 cells were treated with 3 mM melatonin for 24 h and collected. In cells that have undergone apoptosis, phosphatidylserine (PS), which is usually located in the inner leaflet of the plasma membrane, is translocated to the outer leaflet of the plasma membrane. Once on the outer surface of the membrane, PS is bound by FITC-labeled Annexin V and detected by flow cytometry.

Protein extraction and western blot analysis. Melatonin-and vehicle-treated SGC-7901 cells were lysed in cell lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China) supplemented with a protease inhibitor cocktail and phosphatase

inhibitors (Roche, Basel, Switzerland). Protein concentrations were measured using the enhanced BCA protein assay kit (Beyotime Institute of Biotechnology). Protein extracts (40 μg) were subjected to 12 or 15% polyacrylamide gel electrophoresis. The proteins in the gels were transferred to polyvinylidene difluoride membranes, which were then blocked in Tris-buffered saline containing 0.5% bovine serum albumin. Blocked membranes were incubated with primary antibodies: anti-MDM2 (cat. no. ab137413; dilution, 1:1,000), anti-phospho-MDM2 (at Ser166; cat. no. ab170880; dilution, 1:50,000), anti-CDC25A (cat. no. ab140247; dilution, 1:200), anti-phospho-CDC25A (at Ser75; cat. no. ab47279; dilution, 1:1,000), anti-p21 (cat. no. ab109199; dilution, 1:5,000), and anti-phospho-p21 (at Thr145; cat. no. ab47300; dilution, 1:1,000) were purchased from Abcam (Cambridge, UK); anti-AKT (cat. no. 4685S; dilution, 1:1,000), anti-phospho-AKT (at Thr308; cat. no. 4056S; dilution, 1:1,000), anti-Bcl-xL (cat. no. 2764S; dilution, 1:1,000), anti-Bax (cat. no. 5023P; dilution, 1:1,000), anti-caspase-9 (cat. no. 9508S; dilution, 1:1,000) and anti-GAPDH (cat. no. 2118S; dilution, 1:1,000) were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA) and anti-p53 was purchased from Medical and Biological Laboratories Co., Ltd., Nagoya, Japan (cat. no. K0181-3; dilution, 1:5,000). Proteins were detected by the addition of alkaline phosphatase-conjugated secondary antibody, goat anti-rabbit IgG (cat. no. ab98505; dilution, 1:5,000; Abcam) or goat anti-mouse IgG (cat. no. sc-2008; dilution, 1:1,000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Target proteins were visualized by the addition of CDP-Star reagents (Roche Diagnostics, Mannheim, Germany). The bands were detected using an ImageQuant LAS 4000 mini (GE Healthcare, Chicago, IL, USA). Band intensities were quantified using ImageJ2x software (National Institutes of Health, Bethesda, MD, USA) and the relative intensities to the internal GAPDH control were calculated.

Analysis of caspase-3 activity. SGC-7901 cells were seeded at a density of $1x10^4$ cells/ml in 96-well plates. After 24 h of culture, the cells were treated with 0 (1% ethanol as control) or 3 mM melatonin for 24 h. Melatonin- and vehicle-treated SGC-7901 cells were added together with 100 μl of Caspase-Glo® 3/7 Reagent according to the caspase-Glo® 3/7 assay kit (Promega Corp., Madison, WI, USA) manufacturer's instructions. This assay provides a luminogenic caspase-3/7 substrate that is released following caspase cleavage, and the subsequent production of light can be detected by a microplate luminometer (Orion microplate luminometer; Berthold Detection Systems GmbH, Pforzheim, Germany).

Data analysis. The data represent the means ± standard deviations (SD) from at least three independent experiments. One-way ANOVA and the Student's paired t-test were used to determine statistical significance. P<0.05 was considered to indicate a statistically significant difference. All analyses were performed using SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA).

Results

Melatonin inhibits the proliferation of SGC-7901 cells. We assessed the cell viability using the MTS assay. Results demonstrated that cell growth was inhibited after melatonin exposure in a dose- and time-dependent manner (Fig. 1). Based on these results, we selected 3 mM melatonin and 24 h of exposure for the follow-up experiments; these parameters reflect a 50% inhibition of cell viability. Cells treated with higher concentrations of melatonin for longer times were not suitable for use in subsequent experiments. The morphology of treated SGC-7901 cells was assessed microscopically (Fig. 2). At the microstructural level, melatonin-treated cells appeared thinner and more elongated than control cells, with twisted cytoplasmic extensions and a greater number of floating dead cells. At the ultrastructural level, apoptotic cells were evident, surface microvilli were absent, plasmolysis and nuclear pyknosis were present, mitochondria exhibited vacuolation, and apoptotic bodies appeared in the cultures of melatonin-treated cells.

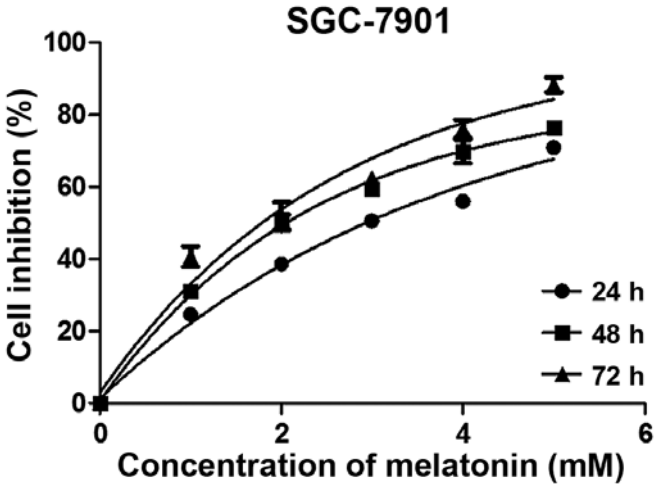

Figure 1. Percentage of cell inhibition as determined by MTS assay. SGC-7901 cells were treated with 0 (1% ethanol was added as control) or 1, 2, 3, 4 or 5 mM melatonin for 24, 48 or 72 h.

Melatonin induces cell cycle arrest and apoptosis in SGC-7901 cells

Melatonin arrests SGC-7901 cells in the G_1/S phase of the cell cycle. We assessed the distribution of SGC-7901 cells in various phases of the cell cycle by flow cytometry. The analysis showed that the proportion of cells in the G_0/G_1 phase increased from 59.357±1.518 to 68.583±0.649% (P<0.05) in the melatonin-treated SGC-7901 cells compared to the controls (Fig. 3). Consistent with this finding, the proportion of cells in the S and G_2/M phases decreased relative to the controls: As shown in Fig. 3, the proportion of S-phase cells decreased from 21.177±1.322 to 16.590±1.874% (P<0.05), and the proportion of G_2/M-phase cells decreased from 20.450±1.868 to 14.727±1.194% (P<0.01). All differences were statistically significant. These results indicate that melatonin effectively arrested SGC-7901 cells in the G_1/S phase of the cell cycle.

Melatonin induces apoptosis in SGC-7901 cells. We used two methods to determine whether melatonin stimulates the apoptosis of SGC-7901 cells. First, we used the TUNEL assay to measure apoptotic SGC-7901 cells *in situ*. As shown in Fig. 4, apoptosis could be observed in the SGC-7901 cells

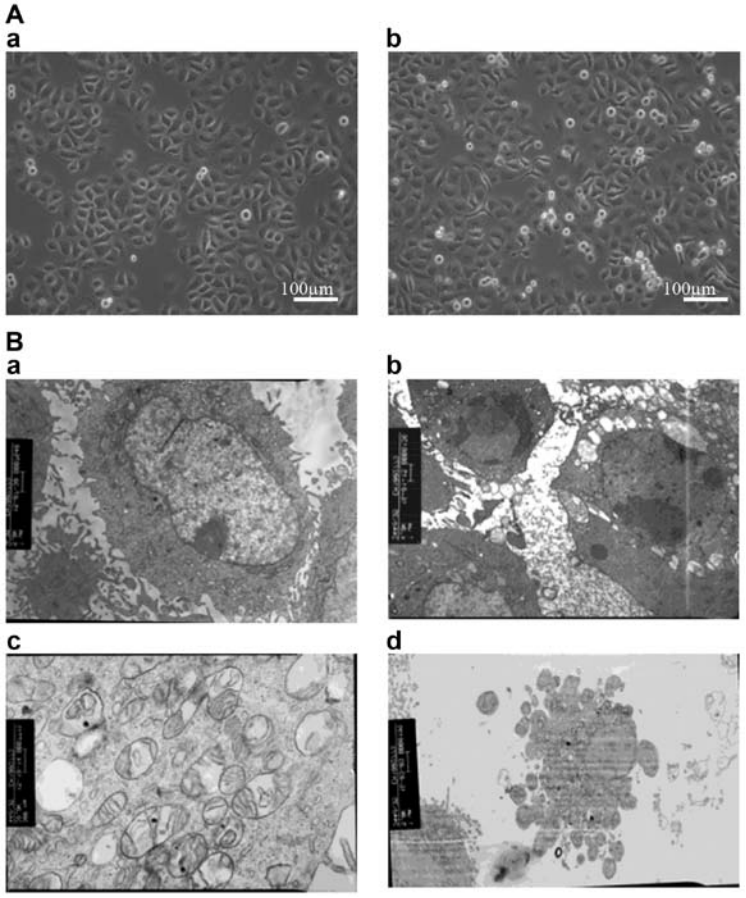

Figure 2. Microscopic examination of SGC-7901 cells treated with 3 mM melatonin for 24 h compared to control cells. (A) Control cells viewed under an inverted microscope (a); melatonin-treated SGC-7901 cells viewed under an inverted microscope (b). (B) Control cells viewed by electron microscopy (a); apoptotic melatonin-treated SGC-7901 cells viewed by electron microscopy (b); vacuolation of mitochondria in apoptotic SGC-7901 cells (c); apoptotic bodies after melatonin treatment (d).

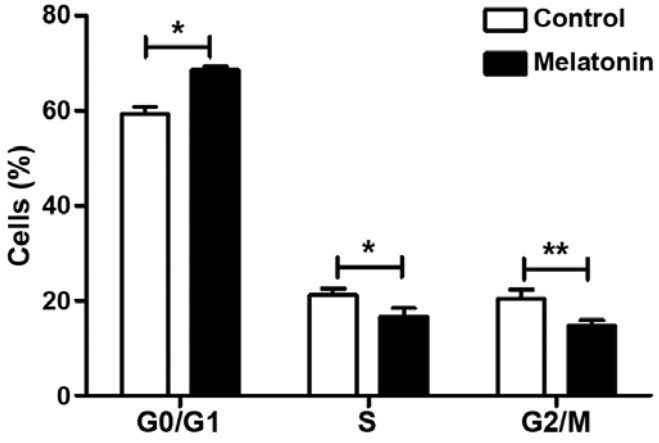

Figure 3. Cell cycle analysis of melatonin-treated SGC-7901 cells by flow cytometry. *P<0.05, **P<0.01 vs. control.

treated with melatonin but not in the controls. We then used flow cytometry to determine the percentage of apoptotic cells using the FITC Annexin V apoptosis detection kit I. The percentages of melatonin-treated cells in the early and late stages of apoptosis were found to increase compared with the controls (Fig. 5). Early apoptotic cells increased from 0.97±0.31 to 7.25±3.00 (P<0.05), late apoptotic cells increased from 1.23±0.53 to 10.22±2.22 (P<0.05), and the total number of apoptotic cells increased from 2.20±0.81 to 17.48±4.98 (P<0.05). All differences were statistically significant.

Melatonin affects proteins associated with the cell cycle and apoptosis. CDC25A and p21 are known regulators of cell cycle progression that interact with cyclin/CDK complexes. Melatonin reduced the expression of CDC25A and the level of CDC25A phosphorylation at Ser75 in SGC-7901 cells compared to the controls (Fig. 6A). Moreover, we found that both the level of p21 and its phosphorylation at Thr145 were decreased (Fig. 6B). We examined changes in apoptosis-associated proteins Bcl-xL, Bax, caspase-9 and caspase-3. In SGC-7901 cells treated with melatonin, western blot analysis showed evidence of a reduced expression level of Bcl-xL and a significantly increased expression level of Bax compared with controls (Fig. 6C). Melatonin treatment also increased cleaved caspase-9 levels and caspase-3 activity (Fig. 6G and H).

Melatonin affects the expression of upstream regulators MDM2, p53 and AKT. MDM2, p53 and AKT are upstream regulators of the apoptosis- and cell cycle-related proteins mentioned above. To further understand the molecular

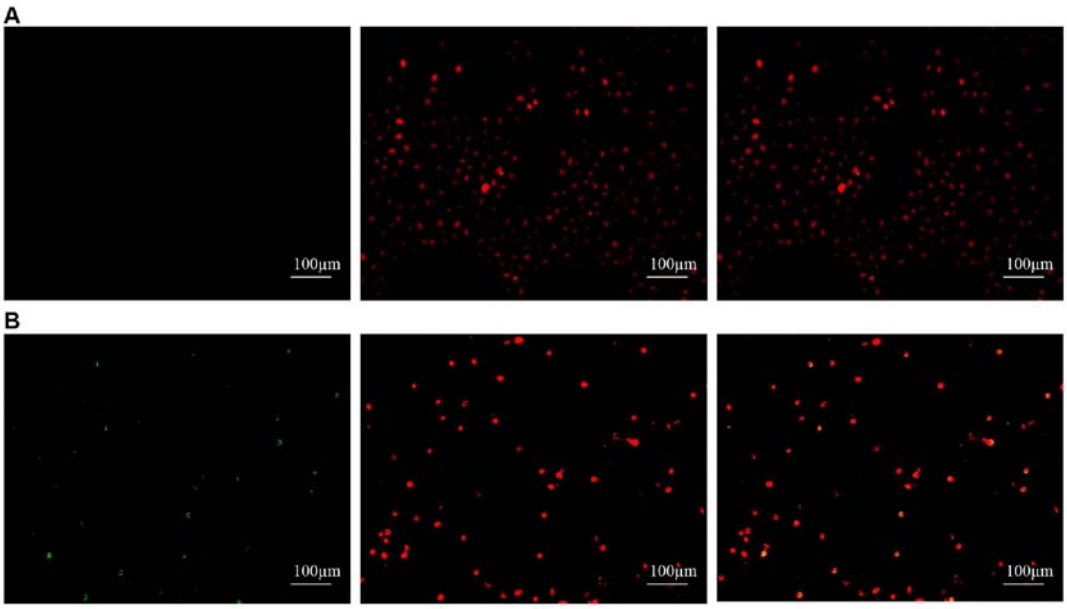

Figure 4. Analysis of apoptosis in SGC-7901 cells treated with 3 mM melatonin for 24 h compared to control cells treated with 1% ethanol by fluorescence microscopy and TUNEL assay. Positive apoptotic cell nuclei appear green and yellow. (A) Control group. (B) Melatonin-treated group.

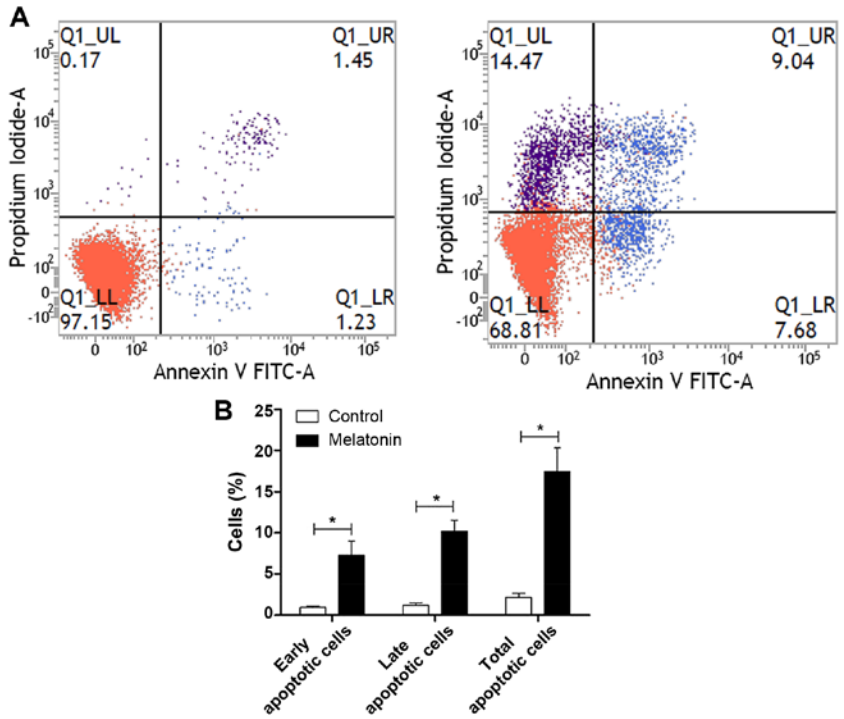

Figure 5. Analysis of apoptosis in SGC-7901 cells treated with 3 mM melatonin for 24 h by flow cytometry. (A) Representative spectra. LL, intact cells; LR, early apoptotic cells; UR, late apoptotic cells; UL, necrotic cells. (B) Bar graph representation of the percentage of apoptotic cells. *P<0.05 vs. control.

mechanism underlying melatonin-induced cell apoptosis and inhibition of cell proliferation, we evaluated the expression levels of MDM2, p53 and AKT in SGC-7901 cells after melatonin exposure. Western blot analysis showed that the expression levels of MDM2 and phospho-MDM2 (at Ser166) were decreased compared with the controls (Fig. 6D), whereas the expression level of p53 was increased compared with the controls (Fig. 6F). Levels of both AKT and phospho-AKT (at Thr308) were decreased after melatonin treatment of SGC-7901 cells compared to the controls (Fig. 6E).

Discussion

The results of the present study confirmed that melatonin arrests SGC-7901 GC cells in the G1/S phase of the cell cycle and promotes apoptosis via a mitochondrial apoptosis pathway, involving a key factor, MDM2. We detected increased expression levels of MDM2, phospho-MDM2 (at Ser166), and the upstream regulator AKT in response to melatonin. Murine double 2 min, also HDM) (MDM2) was originally identified as an amplified oncogene on double-minute chromosomes

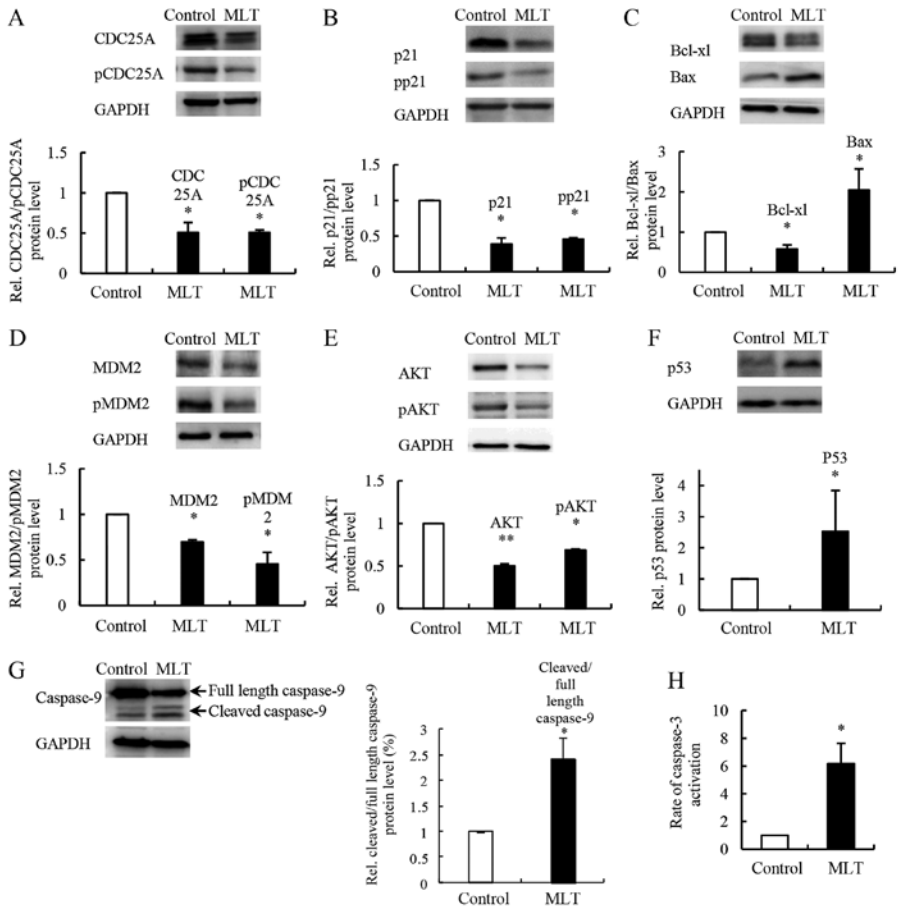

Figure 6. Analysis of expression and activation of proteins associated with cell proliferation and apoptosis. (A-G) Western blot analysis. Equal loading of proteins is illustrated by the GAPDH band. (H) Caspase-3 activity. Data are expressed as means ± SD. *P<0.05, **P<0.01 vs. control. Data shown are representative of three independent experiments. MLT, melatonin; GAPDH glyceraldehyde 3-phosphate dehydrogenase.

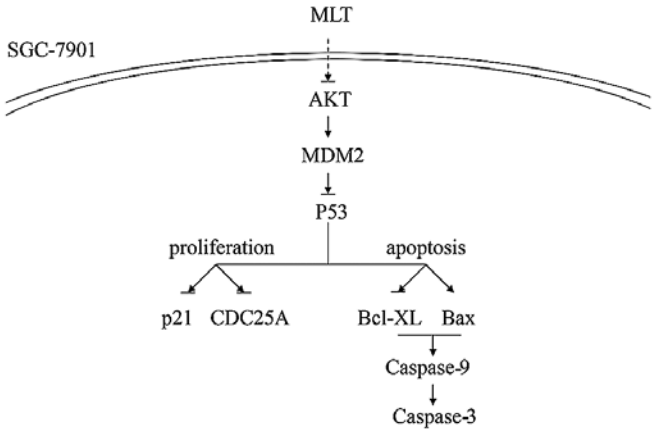

Figure 7. The signaling pathway involved in the induction of apoptosis and inhibition of cellular proliferation by melatoninin SGC-7901 cells. MLT, melatonin.

in transformed mouse fibroblasts (13). While it is involved in the regulation of gene expression as E3 ubiquitin-protein ligase, MDM2 itself is overexpressed in various human cancers (30,31), including GC (32-36). MDM2 is an important negative regulator of the p53 tumor suppressor. Overexpression of MDM2 promotes tumor development by suppressing the function of p53 (37). At the protein level, MDM2 increases both the polyubiquitination of p53, which drives its proteasomal degradation, and the monoubiquitination of p53, which exposes a nuclear export signal, leading to the cytoplasmic translocation of p53 (38,39) and inhibition of its interaction with DNA (40-43). MDM2 also directly inhibits p53 transcription by binding to the transactivation domain of the p53 gene at the transcriptional level (44,45). Furthermore, MDM2 inhibits p53 mRNA translation by binding the 5'-UTR of p53 mRNA, likely through interactions with ribosomal protein L26, a positive regulator of p53 expression (46). The phosphorylated form of MDM2 at Ser166 is a substrate for AKT that is activated when its Thr308 is phosphorylated by PDPK1, which increases the nuclear localization of MDM2 and the subsequent degradation of p53 (47-49). In our experiments, melatonin exposure caused the downregulation of AKT, phospho-AKT (at Thr308), MDM2, and phospho-MDM2 (at Ser166), and the upregulation of p53, suggesting that melatonin inhibits cancer cell growth by attenuating AKT activity, which leads to the inactivation of MDM2.

However, the presence of p53 mutations in cancer diminishes the ability of p53 to inhibit tumor growth. Many studies have argued that there are p53-independent effects of MDM2 (50,51). MDM2 could influence the activities of other transcription factors, such as p73 (52-55), p65 (56), Smad proteins (57,58), cyclin D1, c-Jun, c-Myc and pRb (E2F1) (59,60). Moreover, MDM2 has been shown to influence

chromatin modifications by interacting directly with chromatin (61-63). A p53 mutation was reported in SGC-7901 cells, but its oncostatic function was not blocked completely (64). However, this infers that other components may be involved in the melatonin-induced inhibition of GC cell growth.

In the present study, melatonin was found to affect other components of the mitochondrial apoptosis pathway downstream of MDM2 and AKT. Melatonin treatment caused a decrease in Bcl-xL levels and an increase in Bax levels, which were consistent with the results of our previous studies concerning the inhibitory effect of melatonin on the proliferation of mouse precancerous cells (28). The changes in Bcl-xL and Bax as well as the upregulation of cleaved caspase-9 and activated caspase-3 indicated that melatonin induced apoptosis via a mitochondrial apoptosis pathway. Cell cycle-related proteins CDC25A and p21 were also affected by melatonin treatment. CDC25A is a dual-specificity phosphatase that activates the G1/S-phase cyclin-dependent kinases CDK4 and CDK2, which are required for cell cycle progression (65). CDC25A was also found to suppress apoptosis by inhibiting ASK1 activity (66). The degradation of CDC25A by ubiquitination is blocked by the phosphorylation of CDC25A at Ser75 (67). Furthermore, CDC25A is overexpressed in a variety of tumor types, such as lung, breast, prostate and GC and is correlated with poor prognosis (68). Melatonin induces the downregulation of CDC25A and phospho-CDC25A (Ser75), causing cell cycle arrest in the G1/S phase. Surprisingly, levels of p21, a cyclin/CDK complex inhibitor, were decreased in SGC-7901 cells after melatonin treatment which was different from previous studies in mouse precancerous cells (28). However, some researchers have shown increases in p21 levels in response to mitogenic signals. The binding of p21 to cyclin/CDK forms a complex that stimulates cell cycle progression (69). Martin *et al* (70) also found increased p21 expression levels in C6 glioma cells treated with melatonin. Their results are consistent with those of our experiments. Moreover, Rother *et al* (71) proposed that p53 suppresses CDC25A expression, independent of p21, therefore, the role of p21 is complex and may vary in different conditions.

In conclusion, the downregulation of AKT, MDM2, and changes in the above-mentioned factors suggest that the AKT/MDM2 pathway is involved in the mechanism by which melatonin inhibits the growth of SGC-7901 GC cells (Fig. 7). Melatonin induces apoptosis and inhibits the proliferation of SGC-7901 via downregulation of AKT and MDM2, inducing an increase in p53. Previous studies suggest that p53 activates the expression of the Cdk inhibitor p21, resulting in CDC25A decrease, and cell cycle arrest in the G1/S phase. However, we found a decrease in p21 indicating that the CDC25A decrease is independent of p21. Further research should be conducted concerning the role of p21 in the effect of melatonin against GC SGC-7901 cell growth. There are other points needed to be tested in future studies. Firstly, the flow cytometric analysis showed that the proportion of cells in the G_0/G_1 phase was increased and in the S, G_2/M phases was decreased relative to the controls. It would be better if the G_0 and G_1 phase of SGC-7901 cells are differentiated further, since there would be a few early apoptotic cells induced by the melatonin mixing with the cells of G_0 phase. The conclusion that melatonin induces cell cycle arrest in G_1/S would be confirmed to a higher degree removing these potential apoptotic cells. Secondly, this study analyzes only one type of human GC cell line. We are currently in the selection of more cell lines and a PDX model to confirm the anticancer effect of melatonin. The experiments are still underway and the results need further verification.

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Availability of data and materials

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

Authors' contributions

RXZ was involved in the study concept and design, supervision and provided final approval of the version to be published. JS was involved in the drafting of the manuscript, the analysis and interpretation of the data, performed experiments and obtained funding. SJM, JHL and HZ were involved in performing experiments, analysis and interpretation of the data. RXW, HL, LL and ZGZ assisted with the experimental design, data interpretation, acquisition of funding. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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