NUSANTARA BIOSCIENCE
Vol. 17, No. 1, pp. 178-184
May 2025

ISSN: 2087-3948
E-ISSN: 2087-3956
DOI: 10.13057/nusbiosci/n170117

Melatonin partly reverses differentiation and apoptosis resistance caused by chemical hypoxia

XIAO LIU[1,2], HUI LIU[2], YINLIANG QI[2], SUMEI ZHANG[3,♥], YINGCAI MA[1,4,♥♥]
[1]Suzhou Medical College of Soochow University. No. 199 Ren-Ai Road, Suzhou 215123, Jiangsu, China
[2]Department of Hyperbaric Oxygen, The Second People's Hospital of Hefei, Hefei Hospital Affiliated to Anhui Medical University. Hefei 230011, Anhui, China
[3]Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Anhui Medical University. No. 81 Meishan Road, Hefei 230032, Anhui, China. ♥email: zhangsumei@ahmu.edu.cn
[4]Digestive Department of Qinghai Provincial People's Hospital. Xining 810007, Qinghai, China. ♥♥email: mayingcai0271@sina.com

Manuscript received: 23 January 2025. Revision accepted: 30 May 2025.

Abstract. *Liu X, Liu H, Qi Y, Zhang S, Ma Y. 2025. Melatonin partly reverses differentiation and apoptosis resistance caused by chemical hypoxia. Nusantara Bioscience 17: 178-184.* Hypoxia, a condition characterized by a deficiency of oxygen reaching tissues, is a key feature of malignant solid tumors. Tumor cells under a hypoxic microenvironment exhibit more malignant phenotypes to adapt to the hypoxic environment for survival by regulating gene expression involved in cell proliferation, differentiation, invasion, migration, angiogenesis, and also chemoresistance. Melatonin is a hormone that has been proven to induce apoptosis and differentiation in various types of malignant cells. The present study aims to investigate the role and potential molecular mechanism of melatonin in mediating chemical hypoxia-induced differentiation and apoptosis resistance. Cobalt chloride ($CoCl_2$) was used to generate an in vitro hypoxia culture model, as verified by the increased expression of hypoxia-inducible factor-1α. The cells treated with melatonin and $CoCl_2$ were collected to determine differentiation and apoptosis levels through LDH (Lactate Dehydrogenase) activity measurement and Hoechst staining. GRP78, p53, and endocan levels were detected using Western blot and ELISA (Enzyme Linked Immunosorbent Assay) to elucidate the potential molecular mechanisms by which melatonin induces differentiation and resistance to apoptosis in response to chemical hypoxia. The significant elevation of a hypoxia marker protein successfully demonstrated ($CoCl_2$-induced hypoxia). The chemical hypoxia environment resulted in a less differentiated phenotype and a lower apoptotic ratio in gastric cancer cells. Melatonin converted the differentiation and apoptosis resistance phenotypes in $CoCl_2$-treated gastric cancer cells. Changes in the expression of endocan, LDH, p53, and GRP78 were observed. Melatonin-regulated differentiation and apoptosis-related genes partly reverse differentiation and apoptosis resistance caused by chemical hypoxia induced by $CoCl_2$. The study provides a new perspective on the anti-tumor effect of melatonin.

Keywords: $CoCl_2$, gastric cancer, GRP78, Lactate Dehydrogenase, p53

Abbreviations: LDH: Lactate Dehydrogenase, MLT: Melatonin

INTRODUCTION

Malignant tumor cells are more proliferative than normal cells. The surrounding blood vessels are compressed due to the abnormal proliferation and rapid growth of the tumor, resulting in lower oxygen pressure in the tumor compared to normal tissues (< 7.5 mmHg vs. > 40 mmHg). This phenomenon is known as hypoxia (Lugano et al. 2020). Thus, hypoxia is one of the most important features of malignant solid tumors (Jing et al. 2019). To adapt to a hypoxia environment, genes to regulate differentiation, proliferation, mobility, and angiogenesis were differentially expressed in cancer cells under the hypoxia microenvironment, which made the cells more malignant and more likely to resist chemotherapy (Paredes et al. 2021). Lots of research has indicated that the poor prognosis of solid tumors is considered to be related to the adaptive resistance of tumor cells caused by local hypoxia in tumor tissues (Zheng and Gao 2019).

Gastric cancer is one of the most frequent malignancies worldwide, with high morbidity as well as high mortality (Machlowska et al. 2020) with 968350 newly diagnosed cases and a total of 659,853 deaths per year according to Global Cancer Statistics (GLOBOCAN) 2022 (Bray et al. 2024). Although a variety of treatments such as surgery, chemotherapy, and radiotherapy, have been applied to the treatment of gastric cancer, the prognosis for patients remains poor (Shi and Gao 2016). Especially in patients with metastatic, disseminated, or advanced gastric cancer, there is still a low 5-year survival rate, and the median survival is less than 2 years even after palliative chemotherapy (Guan et al. 2023). Hypoxic condition in gastric cancer tissues results in higher malignant phenotypes and constitute one of the main causes leading to drug resistance and the failure of clinical therapy (Rankin and Giaccia 2016). Thus, more effective therapeutic strategies against gastric cancer by targeting or reversing hypoxia with lower toxicity are still urgently needed.

Melatonin (N-acetyl-5-methoxytryptamine, MLT) is a kind of indole neurohormone mainly synthesized by the pineal gland and has been proven to have a wide range of biological effects (Minich et al. 2022). It has been shown to possess differentiation inducer and chemotherapeutic potential in human cancers, including gastric cancer, by modulating multiple signal transduction pathways associated with cell apoptosis, cell growth, and migration and invasion capabilities (Woo et al. 2015; Cheng et al. 2023). However, few studies have focused on whether melatonin could reverse the increased malignant phenotypes caused by hypoxia microenvironment. The present study was designed to explore whether melatonin can attenuate the higher malignancy caused by chemical hypoxia in gastric cancer cells and to investigate the possible molecular mechanisms by which melatonin exerts this effect.

Cell dedifferentiation or blocked differentiation is a key biological characteristic in the process of malignant tumor development and progression in addition to malignant proliferation (Marsafy and Larghero 2023). LDH (Lactate Dehydrogenase) is an important enzyme known to play a crucial role in regulating gastric cell differentiation, with upregulated activities in gastric cancer cells and decreased activity during the process of normal gastric cell differentiation. LDH was shown to have high activity in low-differentiated cells. These indicated that LDH functions as a dedifferentiation factor in the stomach and is recognized as a marker enzyme for gastric cancer cell differentiation level (Jin et al. 2020). The secreted proteoglycan, endocan, was reported to be positively correlated with gastric cancer differentiation level (Zhang et al. 2012). Exogenous endocan promoted the differentiation of gastric cancer cells (Zhang et al. 2012). Under a chemical hypoxic condition mimicked by $CoCl_2$, gastric cancer cells exhibited a poorer differentiation state, as indicated by increased Lactate Dehydrogenase (LDH) activity and a lower level of secreted endocan in the culture medium's supernatants. This study confirmed that endocan and LDH are involved in the reversal effect of melatonin on dedifferentiation caused by chemical hypoxia in gastric cancer cells. Cell apoptosis is co-mediated by various apoptotic pathways. For example, P53 is a proapoptotic protein that activates the intrinsic apoptosis pathway (Ozaki and Nakagawara 2011), and GRP78 is an endoplasmic reticulum stress-associated apoptotic protein (Zhang et al. 2020).

Cobalt chloride ($CoCl_2$), a recognized chemical hypoxia inducer (Chen et al. 2018), was used in the present study to treat human gastric cancer cells to generate an in vitro hypoxia model verified by elevated hypoxia marker protein, hypoxia-inducible factor-1α (HIF-1α) (Gao et al. 2023). The effects of melatonin on differentiation and apoptosis of $CoCl_2$ treated gastric cancer cells, and also the potential molecular mechanisms were investigated here. We found that melatonin treatment attenuated the more malignant behavior caused by $CoCl_2$ in gastric cancer cells at least partly by altering the expression of differentiation and apoptosis-related proteins.

MATERIALS AND METHODS

Materials

The present research was conducted at the Laboratory of the Department of Biochemistry and Molecular Biology, Anhui Medical University (AHMU), Hefei, China. The human gastric cancer cell line SGC7901 was obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). High glucose DMEM (Dulbecco's modified Eagle's medium) was obtained from Gibco (Carlsbad, CA, USA). FBS (Fetal bovine serum) was from Clark Bioscience. Melatonin (MLT), DMSO (Dimethyl Sulfoxide), and MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) were purchased from Sigma Aldrich (Merck KGaA, Darmstadt, Germany). Cobalt chloride ($CoCl_2$) was obtained from Tianjin Guangfu Technology (Tianjin, China). Primary antibodies against HIF-1α, GRP78, p53, and β-actin were from Santa Cruz (Dallas, TX). All corresponding secondary antibodies were from Thermo Fisher Scientific.

Cell culture

SGC7901 cells were cultured in high-glucose DMEM supplemented with 10% Fetal Bovine Serum (FBS) and antibiotics (40 U/mL penicillin and 100 U/mL streptomycin) in an incubator with 5% CO2 and 100% humidity at 37°C under corresponding conditions.

Cell proliferation assessment

SGC7901 cells grown in 96-well plates (5×10^3 cells/well) were treated with $CoCl_2$ at different final dilutions (0, 100, 200, 300, 400, 500, and 600 μM) for 48 h to select a suitable concentration to induce cytochemical hypoxia without affecting cell viability. MTT was added to each well, followed by a 4 h incubation at 37°C. Formazan crystals formed in wells were dissolved in 100 μL DMSO for 10 min at 37°C, and the absorbance was measured at 570 nm using an absorbance microplate reader (Thermo Scientific). The growth inhibition ratio of each group was calculated as: Inhibition ratio (%) = $[1 - (\text{mean OD}_{\text{treated groups}} - \text{mean OD}_{\text{blank controls}}) / (\text{OD}_{\text{control groups}} - \text{OD}_{\text{blank controls}})]$.

After the appropriate concentration of $CoCl_2$ was determined, cells were treated as follows: (i) Normoxia group: Cells cultured routinely; (ii) hypoxia group: Cells treated with $CoCl_2$; (iii) Melatonin group: Cells cultured in 10^{-4} M melatonin without being exposed to $CoCl_2$; and (iv) Hypoxia induction group: Melatonin in the concentration of 10^{-4} M was given simultaneously to treat $CoCl_2$-exposed gastric cancer cells.

Quantification of endocan

Endocan concentration in the culture supernatants was measured using a commercial ELISA Kit (CUSABIO) according to the manufacturer's instructions. Briefly, a 100 mL standard or supernatant sample from each group was incubated in the ELISA plate at 37°C for 2 h, followed by Biotin-antibody binding and HRP-avidin incubation. TMB substrate and stop solution were added in sequence, and the absorbance of each well was read using a microplate reader at 450 nm (Thermo Scientific).

Lactate Dehydrogenase (LDH) assay

After the various treatments mentioned above, a culture medium was collected for the determination of LDH activity using the microplate method. According to the instructions of the LDH assay kit, LDH activity was calculated as $(OD_{\text{of the unknown sample}} - OD_{\text{of the blank control}}) / (OD_{\text{of the standard sample}} - OD_{\text{of the blank control}}) \times$ the concentration of the standard (0.2 μmol/mL) × 1000 based on the absorbance values read at 450 nm.

Hoechst 33258 staining to detect cell apoptosis

SGC7901 cells grown on sterilized coverslips were fixed and stained with Hoechst 33258 for 5 minutes at 37°C after 48 hours of different treatments. The stained cells were visualized and pictured under a fluorescence microscope.

Western blot analysis

SGC7901 cells treated for 48 h, as mentioned above, were lysed in RIPA (Radio Immunoprecipitation Assay) buffer and centrifuged to collect the supernatant. The total protein concentration was determined by BCA assay. An equal amount of total protein of the cell lysate from each group was separated by SDS-PAGE and transferred onto the PVDF membrane. Expression levels of target proteins were detected by incubating specific antibodies against HIF-1α (1:500), GRP78 (1:500), p53 (1:500), or β-actin (1:1000), followed by HRP-conjugated secondary antibodies and visualization using enhanced chemiluminescence. Western blot data were quantified using Quantity One software. β-actin was accepted as a reference gene.

Data analysis

Each experiment was repeated three times, and all data were expressed as mean ± standard deviation. Data analysis was performed with SPSS 17.0 Software (IBM SPSS, USA). The differences among the groups were analyzed using one-way Analysis of Variance (ANOVA) and Least Significant Difference (LSD). $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

CoCl$_2$ simulated an anoxic microenvironment successfully

To select a suitable concentration of CoCl$_2$ that effectively induces hypoxia without significant cytotoxicity, an MTT assay and Western blot were performed to assess cell viability and HIF-1α levels, respectively. HIF-1α is used here as a marker of hypoxia to confirm if CoCl$_2$ treatment could result in a chemically hypoxic microenvironment in cells. CoCl$_2$ at 100 μM could successfully induce HIF-1α expression in SGC7901 cells, but did not cause decreased cell viability (Figures 1 and 2). Therefore, CoCl$_2$ at 100 μM was used as an optimal dose to generate a hypoxic state in SGC7901 cells in further experiments.

Melatonin could promote SGC7901 cell apoptosis and differentiation under a chemically hypoxic environment

LDH and endocan are known molecular differentiation-related markers of gastric cancer cells. Secreted endocan concentration and LDH activity in culture supernatant were detected to evaluate the differentiation level of cells in each group (Figures 3 and 4). The concentration of secreted endocan was obviously reduced, and LDH activities were increased in the cells exposed to CoCl$_2$ when compared to normoxic cells, which mean that gastric cancer cells under chemical hypoxic environment show lower differentiation degree. Melatonin significantly attenuates CoCl$_2$-induced lower differentiation, as evidenced by a higher secreted endocan level and lower LDH activity in the cell culture medium, compared to chemically hypoxic cells treated with CoCl$_2$ alone.

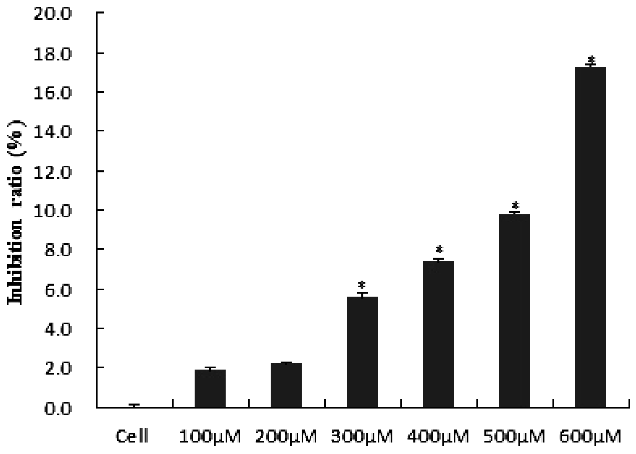

Figure 1. The effect of CoCl$_2$ on the viability of SGC7901 cells was assessed using the MTT assay. SGC7901 cells were treated with CoCl$_2$ at various concentrations (0, 100, 200, 300, 400, 500, and 600 μM). n = 3. Data were analyzed using ANOVA with LSD test for multiple groups comparisons. *p < 0.01 compared to control cells

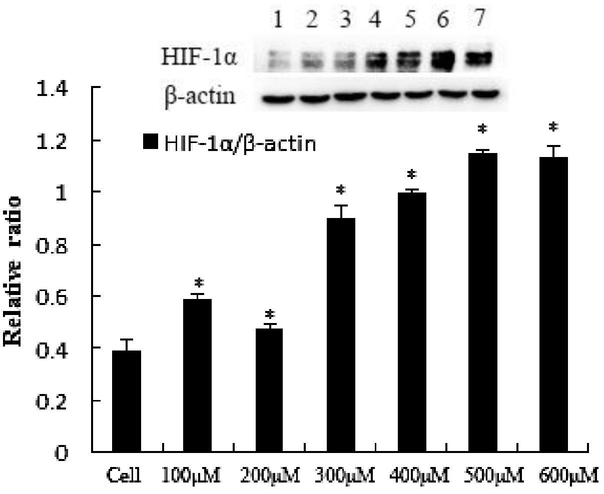

Figure 2. The expression of HIF-1α after CoCl$_2$ treatment at different concentrations was detected by western blot. 1: Cell control; 2: 100 μM CoCl$_2$; 3: 200 μM CoCl$_2$; 4: 300 μM CoCl$_2$; 5: 400 μM CoCl$_2$; 6: 500 μM CoCl$_2$; 7: 600 μM CoCl$_2$. n = 3. Data were analyzed using ANOVA with LSD test for multiple groups comparisons. *p < 0.01 compared to control cells

Hoechst staining was used to stain the cell nucleus, allowing for the observation of apoptotic cells, which could be distinguished from uniformly stained non-apoptotic cells. Apoptosis resistance was observed in $CoCl_2$-induced hypoxic cells, characterized by the presence of few apoptotic cells, as indicated by nuclear shrinkage and apoptotic corpuscles detected by Hoechst staining throughout the entire field of view. Melatonin could promote SGC7901 cell apoptosis under both normoxic and hypoxic states, as shown in Figure 5, by which the reversal role of melatonin on apoptosis resistance caused by $CoCl_2$-induced hypoxia was ascertained.

GRP78 and p53 are involved in apoptosis resistance in hypoxic gastric cancer cells

GRP78 and p53 are famous genes that mediate the endoplasmic reticulum apoptotic pathway and p53-dependent mitochondrial apoptosis, respectively. The Western blot assay revealed an obvious upregulation of GRP78 and a downregulation of p53 in $CoCl_2$-treated cells (Figure 6). Treatment with melatonin attenuated the upregulation of GRP78 and downregulated p53 in cells under a chemically hypoxic environment induced by $CoCl_2$.

Discussion

Gastric cancer has become a public health problem worldwide as a major gastrointestinal malignancy with high mortality and incidence (Siegel et al. 2023). In China, gastric cancer is the third leading cause of cancer mortality, with a mortality rate the second highest among all the malignancies (Yang et al. 2023). The causes and pathogenic factors of gastric cancer occurrence are very complex, including dietary habits, the surrounding environment, *Helicobacter pylori* infection, and so on (Whitmire and Merrell 2019). Many gastric cancers are diagnosed in the middle or late stages due to the complex pathogenesis and the difficulty in early diagnosis. At the same time, its poor response or resistance to chemotherapy drugs in advanced and disseminated gastric cancer patients is a tough problem for effective clinical treatment of gastric carcinoma (Shi and Gao 2016).

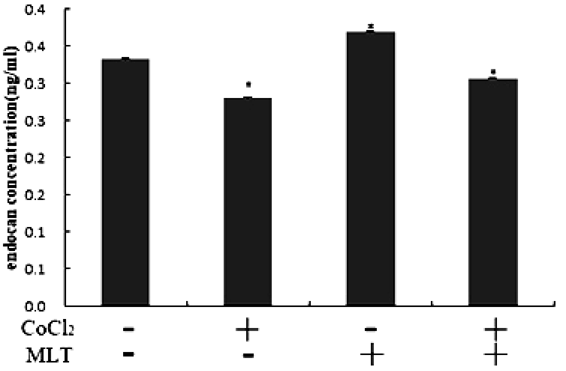

Figure 3. The concentration of endocan in the cultural supernatant of the SGC7901 cells was detected by ELISA assay. $^*p<0.05$ compared to cell control, $^\#p<0.05$ compared to $CoCl_2$ treated cells

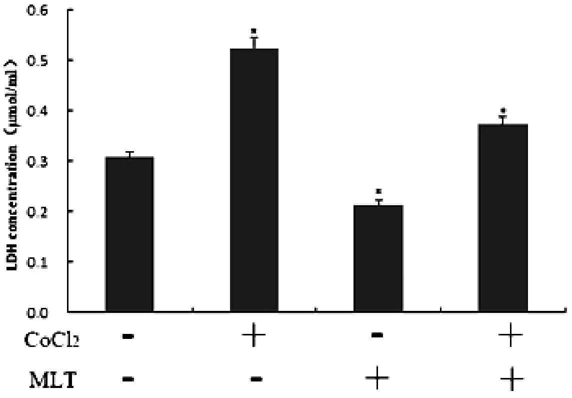

Figure 4. The activities of LDH in SGC7901 cells. $^*p<0.05$ compared to cell control, $^\#p<0.05$ compared to $CoCl_2$ treated cells

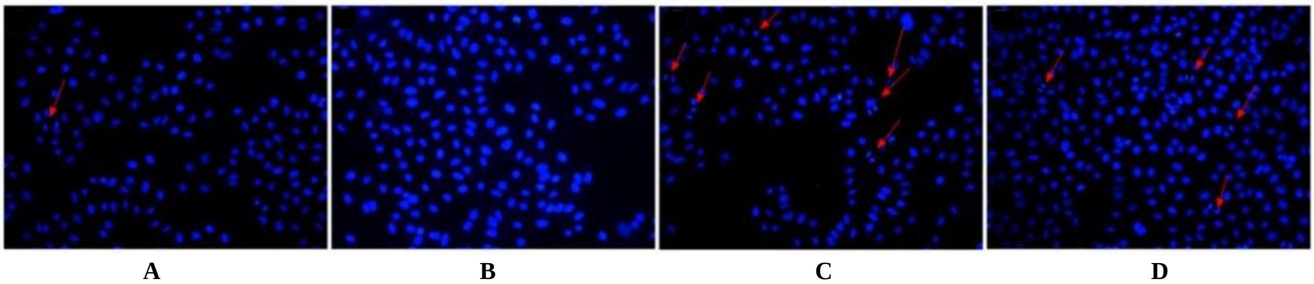

Figure 5. The morphology of apoptosis was detected by Hoechst staining. The arrows point to typical apoptotic cells, characterized by nuclear shrinkage, pyknosis, or karyorrhexis. A: Cell control; B: $CoCl_2$; C: MLT; D: $CoCl_2$+MLT. (×200)

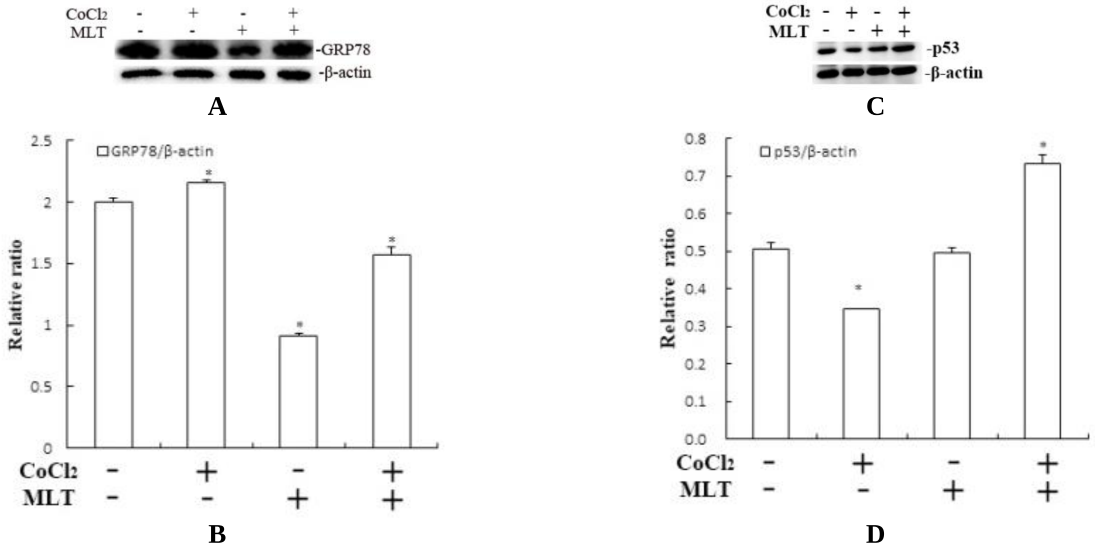

Figure 6. Western blot assay was performed to detect the expression level of GRP78 and p53. A. Western blot bands of GRP78; B. The relative gray values of GRP78; C. Western blot bands of p53; D. The relative gray values of p53. $^*p<0.05$ compared to cell control, $^\#p<0.05$ compared to $CoCl_2$ treated cells

Studies have found that malignant cells in a hypoxic environment, caused by excessively rapid growth and insufficient blood supply, are prone to develop resistance to chemotherapy drugs due to the adaptive changes of tumor cells to hypoxia (Kopecka et al. 2021; Paredes et al. 2021; Wei et al. 2021). Under a hypoxic microenvironment, malignant cells adapt to the hypoxic environment by regulating the expression levels of genes that contribute to cell proliferation, apoptosis, differentiation, and resistance to chemotherapy (Borsi et al. 2015). Thus, cancer cells under hypoxia exhibit higher malignant phenotypes, including resistance to apoptosis, more vigorous proliferation, increased neovascularization, lower differentiation levels, and a greater likelihood of developing local invasion and distant metastasis. This adaptation was related to hypoxia-induced expression of HIF-1α in solid tumors. HIF-1α is known as a transcription factor that affects the expression of more than hundreds of downstream target genes related to hypoxia adaptation and tumor growth and thereby increasing tumor proliferation, invasion, and metastasis ability (Feng et al. 2019). Thus, finding safe and effective agents that can alleviate or overcome the more malignant phenotypes and chemotherapy resistance caused by hypoxia is especially important.

The application of melatonin provides a new direction in drug therapy for malignant tumors (Cheng et al. 2023). Melatonin is an indole heterocyclic compound acting as a kind of natural hormone secreted mainly by the human pineal gland (Kubatka et al. 2018). The therapeutic properties of melatonin on malignant tumors have been confirmed both in clinical and basic research (Li et al. 2015). Melatonin has significant value in preventing the occurrence of tumors and delaying their growth and progression. It delayed the malignant transformation of cells to modulate cancer initiation and promoted tumor cell differentiation and apoptosis to restrain cancer promotion through receptor-dependent or receptor-independent mechanisms (Hsieh et al. 2020). Other scientists and our group have shown that MLT inhibited malignant behaviors by different signaling pathways to regulate numerous gene expressions involved in apoptosis, proliferation, differentiation, angiogenesis, and so on (Cheng et al. 2019). It was reported that melatonin can decrease HIF-1α, thereby preventing the formation of vasculogenic mimicry and the Epithelial-Mesenchymal Transition (EMT) in cancer cells under hypoxic conditions, and is considered beneficial in cancer treatment (Maroufi et al. 2020). In the present study, we attempted to demonstrate that melatonin exerts therapeutic sensitization by alleviating the apoptosis and differentiation inhibition of tumor cells caused by hypoxia.

$CoCl_2$ has almost the same biochemical response as physiological hypoxia in gastric cancer cells (Rath et al. 2016), which is suitable for inducing cell hypoxia models. Hence, we chose $CoCl_2$ to simulate the hypoxic environment of gastric cancer cells *in vitro*, and increased HIF-1α level was used as an indicator of successfully induced hypoxia. The HIF-1α level detected by Western blot assay revealed that the hypoxia culture model in vitro was successfully established by $CoCl_2$ treatment at all concentrations used (100, 200, 300, 400, 500, and 600 μM). MTT assay showed that $CoCl_2$ did not conspicuously inhibit the viability of SGC7901 cells at 100 μM. These results could suggest that $CoCl_2$ at 100 μM induced a successful chemical hypoxia model with no obvious cytotoxicity. In the subsequent experiments of the present study, $CoCl_2$ at 100 μM was used as the optimal dose to induce chemical hypoxia in cells.

Melatonin at 10^{-4} M (Zhang et al. 2013) was used to treat gastric cancer cells with or without exposure to $CoCl_2$. Hypoxic cells exhibited a decreased differentiation level (Figure 4) and lower levels of apoptosis (Figure 5) compared to the control cells. MLT promoted the differentiation of hypoxic SGC7901 cells, as indicated by decreased LDH activity and increased endocan levels in the culture medium. Malignant cells are more likely to obtain energy through anaerobic glycolysis instead of aerobic oxidation (Shafaee et al. 2015). As an important enzyme catalyzing glycolysis, the serum level of LDH directly or indirectly reflects the strength of glycolysis, thereby predicting the ability of oncocyte proliferation and tumor development. This provides a theoretical basis for ascertaining the degree of malignancy and evaluating tumor prognosis. It has been reported that the serum level of LDH is significantly increased in tumor patients, and those with higher LDH have more poorly prognosis (Liu et al. 2017). Thus, LDH is a well-known enzyme that negatively correlates with the differentiation level of gastric cancer cells.

Endocan, initially named endothelial cell-specific molecule-1 (ESM-1), is a secreted proteoglycan (Lassalle et al. 1996) involved in molecular interactions. Endocan has a wide range of biological activities that are crucial for biological processes of tumorigenesis or metastasis, such as cell adhesion, migration, proliferation, and angiogenesis (Lin et al. 2017). Previously, our team reported that endocan levels were positively correlated with differentiation levels of gastric cancer and colorectal carcinoma (Zhang et al. 2012). Over-expression of functional endocan promoted the differentiation-induced cell apoptosis, but reduced the migration of gastric cancer cells, with the opposite effect when endocan was knocked down (Sumei et al. 2016). The apoptosis-associated protein p53 and caspase 3, together with migration-associated protein MMP-9, were proved to participate in an endocan-mediated anti-carcinogenic role, and melatonin promoted differentiation of gastric cancer cells at least partly through upregulating endocan expression (Zhang et al. 2012). We found that MLT increased endocan secretion (Figure 3) in hypoxic SGC7901 cells, suggesting that endocan may also be involved in MLT-induced cell differentiation and apoptosis in hypoxic gastric cancer cells.

Furthermore, apoptosis-associated proteins crucial to different apoptotic pathways, such as p53 and GRP78, were detected using Western blot. P53 is a famous tumor suppressor gene involved in various signaling pathways to regulate cell proliferation and cell death (Tanikawa et al. 2017). As reported in numerous studies, increased expression of p53 protein is vital for apoptosis induction (Nakanishi et al. 2014; Tanikawa et al. 2017; Vaddavalli and Schumacher 2022). Western blot analysis revealed that p53 expression was decreased in $CoCl_2$-treated cells, indicating that hypoxia exposure downregulated p53 expression, which is involved in the apoptosis resistance induced by hypoxia. Also, abnormal expression of GRP78 is involved in tumor occurrence and progression (La et al. 2018). Increased GRP78 in tumor tissues promoted cell migration and proliferation and was correlated with poor survival in many cancer types (Niu et al. 2015). Moreover, GRP78 promoted therapeutic resistance in cancer (Clarke et al. 2019). Mechanically, GRP78 prevented cell apoptosis by suppressing caspase-7 activation (Zhang et al. 2020). Reducing GRP78 expression in tumor cells or suppressing its anti-apoptotic properties has profound significance for apoptosis induction and migration inhibition, and is considered an effective approach to reversing resistance to anti-cancer treatments. The lacking of oxygen in malignant cells induces GPR78 expression through the endoplasmic reticulum stress pathway (Dauer et al. 2019). We found an increased expression of GRP78 in gastric cancer cells under hypoxia. This indicates that both the GRP78-mediated endoplasmic reticulum stress apoptotic pathway and the p53-dependent mitochondrial apoptosis pathway are involved in hypoxic apoptosis resistance. Melatonin worked to upregulate p53 and down-regulate GRP78 when combined with $CoCl_2$. Thus, it can be seen that melatonin can mitigate the abnormal expression of p53 and GRP78 in hypoxic gastric cancer cells exposed to $CoCl_2$, thereby attenuating the apoptosis resistance caused by hypoxia.

In conclusion, melatonin significantly alleviates the reduced apoptosis and differentiation levels caused by chemical hypoxia by regulating p53-dependent mitochondrial apoptosis and GRP78-mediated endoplasmic reticulum stress apoptotic pathway. We revealed a possibility that melatonin serves as an effective adjuvant therapy for treating gastric cancer by overcoming higher malignancy and chemotherapy resistance caused by a hypoxic microenvironment in tumor tissues. The results obtained from the present study provide clues for further exploration on the intimate molecular mechanisms in subsequent studies.

ACKNOWLEDGEMENTS

This study was supported by the Natural Science Foundation of Anhui Province (1708085MH212), the National Natural Science Foundation of China (81201907), the Science Research Project of Anhui Medical University (2019xkj083 and 2023xkj227), and the Natural Science Research Project of Anhui Educational Committee (KJ2020A0143 and 2023AH050564). The authors declare no competing interests.

REFERENCES

Borsi E, Terragna C, Brioli A, Tacchetti P, Martello M, Cavo M. 2015. Therapeutic targeting of hypoxia and hypoxia-inducible factor 1 alpha in multiple myeloma. Transl Res 165 (6): 641-650. DOI: 10.1016/j.trsl.2014.12.001.

Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. 2024. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 74 (3): 229-263. DOI: 10.3322/caac.21834.

Chen R, Xu J, She Y, Jiang T, Zhou S, Shi H, Li C. 2018. Necrostatin-1 protects C2C12 myotubes from $CoCl_2$-induced hypoxia. Intl J Mol Med 41 (5): 2565-2572. DOI: 10.3892/ijmm.2018.3466.

Cheng J, Yang HL, Gu CJ, Liu YK, Shao J, Zhu R, He YY, Zhu XY, Li MQ. 2019. Melatonin restricts the viability and angiogenesis of vascular endothelial cells by suppressing HIF-1/ROS/VEGF. Intl J Mol Med 43 (2): 945-955. DOI: 10.3892/ijmm.2018.4021.

Cheng L, Li S, He K, Kang Y, Li T, Li C, Zhang Y, Zhang W, Huang Y. 2023. Melatonin regulates cancer migration and stemness and enhances the anti-tumour effect of cisplatin. J Cell Mol Med 27 (15): 2215-2227. DOI: 10.1111/jcmm.17809.

Clarke WR, Amundadottir L, James MA. 2019. CLPTM1L/CRR9 ectodomain interaction with GRP78 at the cell surface signals for survival and chemoresistance upon ER stress in pancreatic adenocarcinoma cells. Intl J Cancer 144 (6): 1367-1378. DOI: 10.1002/ijc.32012.

Dauer P, Sharma NS, Gupta VK, Durden B, Hadad R, Banerjee S, Dudeja V, Saluja A, Banerjee S. 2019. ER stress sensor, glucose regulatory protein 78 (GRP78) regulates redox status in pancreatic cancer thereby maintaining "stemness". Cell Death Dis 10 (2): 132. DOI: 10.1038/s41419-019-1408-5.

Feng B, Zhu Y, Sun C, Su Z, Tang L, Li C, Zheng G. 2019. Basil polysaccharide inhibits hypoxia-induced hepatocellular carcinoma metastasis and progression through suppression of HIF-1α-mediated epithelial-mesenchymal transition. Intl J Biol Macromol 137: 32-44. DOI: 10.1016/j.ijbiomac.2019.06.189.

Gao HY, Nepovimova E, Heger Z, Valko M, Wu QH, Kuca K, Adam V. 2023. Role of hypoxia in cellular senescence. Pharmacol Res 194: 106841. DOI: 10.1016/j.phrs.2023.106841.

Guan WL, He Y, Xu RH. 2023. Gastric cancer treatment: Recent progress and future perspectives. J Hematol Oncol 16 (1): 57. DOI: 10.1186/s13045-023-01451-3.

Hsieh MJ, Lin CW, Su SC, Reiter RJ, Chen AWG, Chen MK, Yang SF. 2020. Effects of miR-34b/miR-892a upregulation and inhibition of ABCB1/ABCB4 on melatonin-induced apoptosis in VCR-resistant oral cancer cells. Mol Ther Nucleic Acids 19: 877-889. DOI: 10.1016/j.omtn.2019.12.022.

Jin HF, Wang JF, Shao M, Zhou KL, Ma X, Lv XP. 2020. Down-regulation of miR-7 in gastric cancer is associated with elevated LDH-A expression and chemoresistance to cisplatin. Front Cell Dev Biol 8: 555937. DOI: 10.3389/fcell.2020.555937.

Jing XM, Yang FM, Shao CC, Wei K, Xie MY, Shen H, Shu YQ. 2019. Role of hypoxia in cancer therapy by regulating the tumor microenvironment. Mol Cancer 18 (1): 157. DOI: 10.1186/s12943-019-1089-9.

Kopecka J, Salaroglio IC, Perez-Ruiz E, Sarmento-Ribeiro AB, Saponara S, De Las Rivas J, Riganti C. 2021. Hypoxia as a driver of resistance to immunotherapy. Drug Resist Updat 59 100787. DOI: 10.1016/j.drup.2021.100787.

Kubatka P, Zubor P, Busselberg D, Kwon TK, Adamek M, Petrovic D, Opatrilova R, Gazdikova K, Caprnda M, Rodrigo L, Danko J, Kruzliak P. 2018. Melatonin and breast cancer: Evidences from preclinical and human studies. Crit Rev Oncol Hematol 122: 133-143. DOI: 10.1016/j.critrevonc.2017.12.018.

La X, Zhang L, Li H, Li Z, Song G, Yang P, Yang Y. 2018. Ajuba receptor mediates the internalization of tumor-secreted GRP78 into macrophages through different endocytosis pathways. Oncotarget 9 (21): 15464-15479. DOI: 10.18632/oncotarget.24090.

Lassalle P, Molet S, Janin A, Heyden JV, Tavernier J, Fiers W, Devos R, Tonnel AB. 1996. ESM-1 is a novel human endothelial cell-specific molecule expressed in lung and regulated by cytokines. J Biol Chem 271 (34): 20458-20464. DOI: 10.1074/jbc.271.34.20458.

Li WM, Fan MD, Chen YN, Zhao Q, Song CY, Yan Y, Jin Y, Huang ZM, Lin CJ, Wu JS. 2015. 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. Cell Physiol Biochem 37 (6): 2323-2338. DOI: 10.1159/000438587.

Lin LY, Yeh YC, Chu CH, Won JGS, Shyr YM, Chao Y, Li CP, Wang SE, Chen MH. 2017. Endocan expression is correlated with poor progression-free survival in patients with pancreatic neuroendocrine tumors. Medicine (Baltimore) 96 (41): e8262. DOI: 10.1097/MD.0000000000008262.

Liu L, He Y, Ge G, Li L, Zhou P, Zhu Y, Tang H, Huang Y, Li W, Zhang L. 2017. Lactate Dehydrogenase and creatine kinase as poor prognostic factors in lung cancer: A retrospective observational study. PLoS ONE 12 (8): e0182168. DOI: 10.1371/journal.pone.0182168.

Lugano R, Ramachandran M, Dimberg A. 2020. Tumor angiogenesis: Causes, consequences, challenges and opportunities. Cell Mol Life Sci 77 (9): 1745-1770. DOI: 10.1007/s00018-019-03351-7.

Machlowska J, Baj J, Sitarz M, Maciejewski R, Sitarz R. 2020. Gastric cancer: Epidemiology, risk factors, classification, genomic characteristics and treatment strategies. Intl J Mol Sci 21 (11): 4012. DOI: 10.3390/ijms21114012.

Maroufi NF, Amiri M, Dizaji BF, Vahedian V, Akbarzadeh M, Roshanravan N, Haiaty S, Nouri M, Rashidi MR. 2020. Inhibitory effect of melatonin on hypoxia-induced vasculogenic mimicry via suppressing Epithelial-Mesenchymal Transition (EMT) in breast cancer stem cells. Eur J Pharmacol 881: 173282. DOI: 10.1016/j.ejphar.2020.173282.

Marsafy SE, Larghero J. 2023. Cancer cell de-differentiation: Plasticity-driven stratagem for tumor metastasis and recurrence. Curr Stem Cell Res Ther 18 (1): 54-61. DOI: 10.2174/1574888x17666220608101852.

Minich DM, Henning M, Darley C, Fahoum M, Schuler CB, Frame J. 2022. Is melatonin the "next vitamin d"?: a review of emerging science, clinical uses, safety, and dietary supplements. Nutrients 14 (19): 3934. DOI: 10.3390/nu14193934.

Nakanishi A, Kitagishi Y, Ogura Y, Matsuda S. 2014. The tumor suppressor PTEN interacts with p53 in hereditary cancer (Review). Intl J Oncol 44 (6): 1813-1819. DOI: 10.3892/ijo.2014.2377.

Niu ZY, Wang MY, Zhou L, Yao LT, Liao Q, Zhao YP. 2015. Elevated GRP78 expression is associated with poor prognosis in patients with pancreatic cancer. Sci Rep 5: 16067. DOI: 10.1038/srep16067.

Ozaki T, Nakagawara A. 2011. Role of p53 in cell death and human cancers. Cancers (Basel) 3 (1): 994-1013. DOI: 10.3390/cancers3010994

Paredes F, Williams HC, San Martin A. 2021. Metabolic adaptation in hypoxia and cancer. Cancer Lett 502: 133-142. DOI: 10.1016/j.canlet.2020.12.020.

Rankin EB, Giaccia AJ. 2016. Hypoxic control of metastasis. Science 352 (6282): 175-180. DOI: 10.1126/science.aaf4405.

Rath S, Anand A, Ghosh N, Das L, Kokate SB, Dixit P, Majhi S, Rout N, Singh SP, Bhattacharyya A. 2016. Cobalt chloride-mediated protein kinase Cα (PKCα) phosphorylation induces hypoxia-inducible factor 1α (HIF1α) in the nucleus of gastric cancer cell. Biochem Biophys Res Commun 471 (1): 205-212. DOI: 10.1016/j.bbrc.2016.01.140.

Shafaee A, Dastyar DZ, Islamian JP, Hatamian M. 2015. Inhibition of tumor energy pathways for targeted esophagus cancer therapy. Metabolism 64 (10): 1193-1198. DOI: 10.1016/j.metabol.2015.07.005.

Shi WJ, Gao JB. 2016. Molecular mechanisms of chemoresistance in gastric cancer. World J Gastrointest Oncol 8 (9): 673-681. DOI: 10.4251/wjgo.v8.i9.673.

Siegel RL, Miller KD, Wagle NS, Jemal A. 2023. Cancer statistics, 2023. CA Cancer J Clin 73 (1): 17-48. DOI: 10.3322/caac.21763.

Sumei Z, Shaolong C, Xiang W, Yinliang Q, Qing Z, Yuan W. 2016. Endocan reduces the malign grade of gastric cancer cells by regulating associated protein expression. Tumour Biol 37 (11): 14915-14921. DOI: 10.1007/s13277-016-5398-y.

Tanikawa C, Zhang Y, Yamamoto R, Tsuda Y, Tanaka M, Funauchi Y, Mori J, Imoto S, Yamaguchi R, Nakamura Y, Miyano S, Nakagawa H, Matsuda K. 2017. The transcriptional landscape of p53 signalling pathway. EBioMedicine 20: 109-119. DOI: 10.1016/j.ebiom.2017.05.017.

Vaddavalli PL, Schumacher B. 2022. The p53 network: Cellular and systemic DNA damage responses in cancer and aging. Trends Genet 38 (6): 598-612. DOI: 10.1016/j.tig.2022.02.010.

Wei F, Wang D, Wei JY, Tang NW, Tang L, Xiong F, Guo C, Zhou M, Li XL, Li GY, Xiong W, Zhang SS, Zeng ZY. 2021. Metabolic crosstalk in the tumor microenvironment regulates antitumor immunosuppression and immunotherapy resisitance. Cell Mol Life Sci 78 (1): 173-193. DOI: 10.1007/s00018-020-03581-0.

Whitmire JM, Merrell DS. 2019. Helicobacter pylori genetic polymorphisms in gastric disease development. Adv Exp Med Biol 1149: 173-194. DOI: 10.1007/5584_2019_365.

Woo SM, Min KJ, Kwon TK. 2015. Melatonin-mediated Bim up-regulation and cyclooxygenase-2 (COX-2) down-regulation enhances tunicamycin-induced apoptosis in MDA-MB-231 cells. J Pineal Res 58 (3): 310-320. DOI: 10.1111/jpi.12217.

Yang WJ, Zhao HP, Yu Y, Wang JH, Guo L, Liu JY, Pu J, Lv J. 2023. Updates on global epidemiology, risk and prognostic factors of gastric cancer. World J Gastroenterol 29 (16): 2452-2468. DOI: 10.3748/wjg.v29.i16.2452.

Zhang R, Hao J, Guo KW, Liu WX, Yao F, Wu QM, Liu C, Wang Q, Yang XZ. 2020. Germacrone inhibits cell proliferation and induces apoptosis in human esophageal squamous cell carcinoma cells. BioMed Res Intl 2020 (1): 7643248. DOI: 10.1155/2020/7643248.

Zhang S, Qi Y, Zhang H, He W, Zhou Q, Gui S, Wang Y. 2013. Melatonin inhibits cell growth and migration, but promotes apoptosis in gastric cancer cell line, SGC7901. Biotech Histochem 88 (6): 281-289. DOI: 10.3109/10520295.2013.769633.

Zhang S, Zuo L, Gui S, Zhou Q, Wei W, Wang Y. 2012. Induction of cell differentiation and promotion of endocan gene expression in stomach cancer by melatonin. Mol Biol Rep 39 (3): 2843-2849. DOI: 10.1007/s11033-011-1043-4.

Zheng J, Gao P. 2019. Toward normalization of the tumor microenvironment for cancer therapy. Integr Cancer Ther 18: 1534735419862352. DOI: 10.1177/1534735419862352.