# Mechanisms of JAK-STAT signaling pathway mediated by CXCL8 gene silencing on epithelial-mesenchymal transition of human cutaneous melanoma cells

XIAORUI HU[1], LILI YUAN[2] and TENG MA[3]

[1]Department of Burn and Plastic Surgery, General Hospital of Ningxia Medical University, Yinchuan, Gansu 750001;
[2]Department of Plastic Surgery, Qingyang People's Hospital of Gansu Province, Qingyang, Gansu 745000;
[3]Department of Traumatic Orthopaedics, General Hospital of Ningxia Medical University, Yinchuan, Gansu 750001, P.R. China

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**Abstract.** Effect of CXCL8 gene silencing-mediated JAK-STAT signaling pathway on epithelial-mesenchymal transition (EMT) of human cutaneous melanoma cells was explored. Eighty patients with cutaneous melanoma were enrolled in the study. Cells were transfected accordingly and divided into five groups: The blank group (human cutaneous melanoma cells), NC group (human cutaneous melanoma cells + blank vector plasmid transfection), CXCL8 siRNA group (human cutaneous melanoma cells + CXCL8 silent expression vector plasmid transfection), AG490 group (human cutaneous melanoma cells + JAK-STAT signal pathway inhibitor transfection), CXCL8 siRNA + AG490 group (human cutaneous melanoma cells + JAK-STAT signaling pathway inhibitor + CXCL8 silent expression vector plasmid transfection). The expression levels of CXCL8, JAK2, STAT3, epithelial cadherin (E-cadherin), neurotrophic cadherin (N-cadherin) and vimentin in tissues and cells were detected by RT-qPCR and western blot analysis. CCK-8 and flow cytometry were used to detect cell proliferation and apoptosis. Compared with adjacent normal tissues, the expression of E-cadherin in human cutaneous melanoma tissues was significantly decreased, whereas the expression of CXCL8, JAK2, STAT3, vimentin and N-cadherin was significantly increased ($P<0.05$). Compared with the blank group, CXCL8 siRNA group and CXCL8 siRNA + AG490 group had significantly lower expression of CXCL8 ($P<0.05$). Compared with the blank group, the expression levels of JAK2, STAT3, vimentin and N-cadherin in CXCL8 siRNA group, AG490 group and CXCL8 siRNA + AG490 group were decreased, the expression of E-cadherin was increased, the cell proliferation ability was decreased and apoptosis was increased ($P<0.05$). Compared with CXCL8 siRNA group, the expression of JAK2, STAT3, vimentin and N-cadherin in CXCL8 siRNA + AG490 group were significantly decreased, the expression of E-cadherin was significantly increased, cell proliferation ability was decreased and apoptosis was increased ($P<0.05$). In conclusion, CXCL8 gene expression silencing may inhibit EMT and cell proliferation while promoting cell apoptosis of human cutaneous melanoma cells by inhibiting the activation of JAK-STAT signaling pathway.

*Correspondence to:* Dr Xiaorui Hu, Department of Burn and Plastic Surgery, General Hospital of Ningxia Medical University, 804 Shengli South Street, Yinchuan, Gansu 750001, P.R. China
E-mail: rdh2on@163.com

*Key words:* CXCL8 gene, JAK-STAT signaling pathway, cutaneous melanoma, proliferation, apoptosis, epithelial-mesenchymal transition

## Introduction

Melanoma originates from neural crest of epiblast and is a malignant melanoma originating from the pigmented areas of the skin (cutaneous melanoma elaborated in this study), mucosa, eye and the central nervous system (1,2). The pathogenesis of melanoma is still unclear. Several studies have reported that melanoma is closely related to factors, such as race, large amount of ultraviolet radiation, abuse of estrogen drugs and immunodeficiency (3-5). Due to various carcinogenic factors, melanoma is one of the most invasive and migratory malignant solid tumors that spreads rapidly and may lead to the death of a great number of patients after several months of diagnosis (6,7). Currently, surgery is still an important treatment for melanoma in patients insensitive to radiotherapy or chemotherapy (8,9). Recent studies have suggested that gene therapy can provide a new direction for the treatment of melanoma, and therefore gene therapy has become a promising research field worldwide (10-12). The pathogenesis of melanoma is a complex multi-step process involving numerous genes (13). The malignant transformation process of normal cells into tumor cells involves various gene changes (14,15). In malignant tumors, the expression of tumor suppressor genes is decreased or the function is absent (16). It is worth noting that the threat of cancer to human life is mainly attributed to malignant growth, due to invasion and metastasis, rather than the growth in the primary site. Melanoma is one of the most invasive and migratory malignant solid tumors (17). Degradation of extracellular matrix and basement membrane is a key step in the complex process of melanoma leaving the primary site, invading the surrounding tissues and passing

through the basement membrane to enter the lymph or blood circulation and transfer to newly diffused tissues (18,19).

Epithelial-mesenchymal transition (EMT) was proposed by Greenburg and Hay in 1982 (20). EMT refers to the phenomenon of epithelial cells transforming into mesenchymal cells under specific physiological and pathological conditions (21,22). Cells may lose their connection and polarity, change in morphology, and their migration ability may be enhanced, thus gaining invasion and metastasis abilities (23,24). It has been reported that although melanocytes are not traditional epithelial components, there is EMT in the process of melanoma invading dermal stroma (25). EMT has been reported in previous studies to be involved in the invasion and metastasis of melanoma (26-28). During EMT, there are obvious differences in the expression of EMT-related molecules (29), whose comparison in melanocytes and different melanoma cell lines (30) has led to the discovery of abnormal expression of epithelial cadherin (E-cadherin), neurotrophic cadherin (N-cadherin) and vimentin in melanocytes (31,32).

JAK/STAT signaling pathway is a widely used signal transduction pathway of hematopoietic growth factor, which mediates a number of physiological processes of cells (33). JAK/STAT signaling pathway may play critical roles in ontogenesis, hematopoietic regulation and immune response (34). Persistent activation of JAK/STAT signaling pathway is related to the occurrence of various human tumors (35,36). Current studies have confirmed that external stimulators are closely associated with the regulation of various cell behaviors and the activation of signal transduction pathways in cells (33,37,38). JAK/STAT signal transduction pathway is one of the most commonly studied pathways and plays an important role in the process of cell signal transduction. JAK is a class of intracytoplasmic non-receptor soluble tyrosine protein kinases, including the four subfamilies of JAK1, JAK2, JAK3 and TYK2 (39). STAT is a cytoplasmic protein that can bind to the DNA of the target gene regulatory region, which is the downstream substrate of JAK (40). STAT family includes seven members, i.e., STAT1-4, STAT5A, STAT5B and STAT6 (41). After tyrosine phosphorylation of JAK family, STATs in cytoplasm are recruited and phosphorylated to form dimers into the nucleus (42). In the nucleus, STATs bind to the promoter of target genes, and then activate gene expression, so as to regulate physiological and pathological reactions of cells, and function significantly in the process of cell growth, activation, differentiation and apoptosis (43). Moreover, AG490 is a selective inhibitor of JAK2 tyrosine phosphorylation (44) which can effectively block the downstream signal transduction and STAT3 activation, and then inhibit corresponding physiological and pathological effects.

The role of JAK/STAT signaling pathway has been recognized in multiple human malignancies. However, there are few studies on the aforementioned targets in cutaneous melanoma. Furthermore, chemokin CXCL8, also known as interleukin-8, is a pro-inflammatory molecule that functions significantly in tumor microenvironment and has been screened to be associated with the development of various tumors, considering its roles in directing neutrophils and oligodendrocytes to combat infection and tumor cells in the progression of metastasis. The present study was carried out to investigate the effect of CXCL8 silencing-mediated activation of JAK-STAT signaling pathway on EMT of human cutaneous melanoma cells. This study is expected to provide potential reference for target gene screening during genetic therapy and updated therapeutic approaches for inhibiting the development of cutaneous melanoma.

## Patients and methods

*Reagents and equipment*. Laboratory reagents: 95% medical ethanol and absolute ethanol (Tianjin Fuyu Fine Chemical Co., Ltd.); xylene (Shanghai YuanMu Biological Technology Co., Ltd.); polyformaldehyde (Sigma-Aldrich; Merck KGaA); hematoxylin and eosin (H&E; Beijing Solarbio Science & Technology Co., Ltd.); protein denaturant (Shanghai Sibas Biotechnology Development Co., Ltd.), primary polyclonal antibody CXCL8 (Abcam), goat anti-rabbit IgG antibody (Wuhan Boster Biological Technology, Ltd.), ECL kit (Amersham; GE Healthcare). DMEM (HyClone; GE Healthcare), PBS and trypsin (both from Beijing Solarbio Science & Technology Co., Ltd.), BCA kit (23250; Thermo Fisher Scientific, Inc.), Annexin V-FITC/PI apoptotic detection kit (Nanjing KeyGen Biotech Co., Ltd.).

Main experimental equipment: Electronic analytical balance (Shanghai Ping Xuan Scientific Instruments, Ltd.); RM2126 paraffin slicing machine (Shanghai Leica Instruments Ltd.); Olympus fluorescent microscope and inverted fluorescent microscope (both from Olympus Corporation); gel imaging system (ChemiDoc MP; Bio-Rad Laboratories, Inc.); pipette (0.5-10 $\mu$l, 20-200 $\mu$l, Research® plus 100-1,000 $\mu$l; Beijing Gilson Science & Technology Co., Ltd.).

*Research subjects*. Melanoma tissues were collected from 80 patients with cutaneous melanoma admitted to the General Hospital of Ningxia Medical University (Yinchuan, China) from January 2016 to January 2018. The collected melanoma tissues served as the experimental group and the adjacent normal tissues as the control group. All cases were confirmed by histopathology. The experimental group consisted of 53 males and 27 females, with an average age of 61.55±9.04 years, and the age at onset ranged from 32 to 80 years. Among the 80 cases, there were 50 cases of superficial diffusion and 30 cases of nodular metastasis, 41 cases of non-lymph node metastasis and 39 cases of lymph node metastasis.

Inclusion criteria of eligible cases: i) patients who were diagnosed with cutaneous melanoma by histopathology; ii) patients with complete medical records; iii) patients with no previous history of radiotherapy and chemotherapy. Exclusion criteria: i) Patients who were not treated in the aforementioned hospital after diagnosis; ii) patients who had a history of treatment in other hospitals; iii) patients with other malignant tumors. Tumor tissues in the center of the lesion and non-tumor tissues adjacent to the cancer (5 cm) were collected and frozen in cryopreservation tubes. Next, the tumor tissues were quickly frozen in liquid nitrogen tanks (-196˚C). The samples were stored in a refrigerator at -80˚C. The study was approved by the Ethics Committee of the General Hospital of Ningxia Medical University. Patients who participated in this research had complete clinical data. Signed informed consents were obtained from the patients or their guardians.

*H&E staining*. During H&E staining, human cutaneous melanoma tissues and adjacent normal tissues were divided into

melanoma group and normal control group, respectively. The main reagent formulations were as follows: i) Hematoxylin staining: Hematoxylin (1 g), ethanol (10 ml), distilled water (200 ml), potassium alum (20 g), HgO (0.5 g); ii) eosin staining: Eosin (2.5-5 g), distilled water (500 ml); iii) hydrochloric acid alcohol differentiation solution: 0.5 ml hydrochloric acid and 100 ml 75% alcohol to mix. a) Tissue sample dewaxing: The tissue sample slices were placed in xylene, soaked fully for 10 min, then replaced in xylene and continuously soaked for 10 min. b) Hydration of tissue samples: The tissue samples soaked in xylene were first immersed in absolute ethanol for 5 min, and then in 95, 85 and 70% ethanol for 5 min for complete hydration. c) Hematoxylin staining, differentiation, and bluing: The dewaxed tissue samples were placed in hematoxylin solution, stained for 5 min, and washed with tap water for 5 min; the stained tissue samples were placed in alcohol hydrochloride differentiation solution for several seconds; the slice was washed for 10 min with tap water, and the nuclear staining was observed under the microscope. d) Eosin staining: Tissue samples were placed in 0.5% eosin solution and stained for 5 min. v) Dehydration: After H&E staining, the tissue samples were put into 95% ethanol I, 95% ethanol II, 100% ethanol I and 100% ethanol II for 2 min, respectively. e) Tissue sample slice air-drying and sealing: The dehydrated tissue sample slice was soaked in xylene twice for 4 min each time, and then the tissue sample slice was dried and sealed with neutral gum. f) Finally, the slices were observed and photographed under a microscope. With the morphological image analysis system, different groups were analyzed (magnification, x400), and the images were collected randomly. The experiment was repeated 3 times.

*RT-qPCR*. Some melanoma tissues and adjacent normal tissues were ground into fine powder by adding liquid nitrogen. Total RNA was extracted using TRIzol® reagent (Sangon Biotech Co., Ltd.), and RNA concentration and purity were determined. Total RNA was reverse transcribed into cDNA (total volume, 20 $\mu$l) using the PrimeScript™ RT reagent kit (Takara Bio, Inc.), according to the manufacturer's protocol. The reaction conditions of the reverse transcription of RNA into cDNA were: Incubation at 25˚C for 10 min, additional incubation at 42˚C for 50 min, and heating at 95˚C for 5 min. cDNA was diluted with DEPC water and blended. Fluorescent RT-qPCR was performed following the manufacturer's protocol. During RT-qPCR double-labeled fluorescent probes (5'-end of the fluorescent reporting group, 3'-end of the fluorescent quenching group) were used, in which the 5'-end was 6-carboxy-fluorescene-labeled fluorescence group and the 3'-end was 6-carboxytetramethyl-rhodamine-labeled fluorescence group (both from Applied Biosystems; Thermo Fisher Scientific, Inc.). The primers of CXCL8, JAK2, STAT3, E-cadherin, N-cadherin, vimentin and β-actin were designed and synthesized by Wuhan Boster Biological Technology, Ltd.: CXCL8 forward (5'-3'), ATGGCT GCTGAACCAGTAGA and reverse (5'-3'), CTAGTCTTC GTTTTGAACAG; JAK2 forward (5'-3'), GGGTGTTCGCGT CGCCACTT and reverse (5'-3'), CAGATCGGGCGACCA GAGCG; STAT3 forward (5'-3'), GCGGCAGTTTCTGGC CCCTT and reverse (5'-3'), CGGGCCACAATCCGGGCAAT; E-cadherin forward primer (5'-3'), ATGCTGATGCCCCCA ATACC and reverse (5'-3'), ATCTTGCCAGGTCCTTTGCT; N-cadherin forward (5'-3'), CCGGAGAACAGTCTCCAACTC and reverse (5'-3'), CCCACAAACAGCACCAGTC; vimentin forward (5'-3'), GGTGCAATCGTGATCTGGGA and reverse (5'-3'), GTCTTTGCTCGAATGTGCGG; and β-actin forward primer (5'-3'), CCCAGCACAATGAAGATCAAGATCAT and reverse (5'-3'), ATCTGCTGGAAGGTGGACAGCGA. The reaction system volume was 25 $\mu$l and was consisted of cDNA (1 $\mu$l), 10X PCR buffer (2.5 $\mu$l), 10 mmol/l dNTPs (2 $\mu$l), PCR upstream primers (1 $\mu$l), PCR downstream primers (1 $\mu$l), Taq DNA polymerase (1 $\mu$l), and deionized water (16.5 $\mu$l). Fluorescent RT-qPCR was performed in ABI PRISM® 7300 system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The reaction conditions were as follows: Pre-denaturation at 95˚C for 5 min, denaturation at 94˚C for 1 min, annealing at 54˚C for 45 sec, extension at 72˚C for 1 min, in a total of 30 cycles, followed by extension at 72˚C for 10 min. β-actin was used as internal reference. All primers were designed and synthesized by Wuhan Boster Biological Technology, Ltd. The $2^{-\Delta\Delta Cq}$ method (45) was used to express the multiplier relationship between the target gene expression in the experimental group and the control group. The experiment was repeated 3 times independently.

*Western blot analysis*. Some melanoma and adjacent normal tissues were ground with liquid nitrogen into a homogeneous fine powder and 1 ml of modified RIPA lysis buffer (Beyotime Institute of Biotechnology) was added for protein extraction. Melanoma and adjacent normal tissues were ground into homogenate in an ice bath. Protein lysate was added to the melanoma tissues at 4˚C for 30 min and shaken every 10 min for the extraction of total protein. According to the manufacturer's instructions of the BCA kit, the content of total protein was determined and frozen at -80˚C. Protein (50 $\mu$g) was collected and mixed with denaturant, boiled for 10 min for denaturation, and then separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After electrophoresis, the protein was transferred from the SDS-PAGE gel to a nitrocellulose membranes using the electrical transfer method. Nitrate cellulose membranes were blocked overnight at 4˚C in PBST (PBS + Tween-20) containing 10% skimmed milk powder. The membranes were rinsed with PBST (5 min for 3 times) and incubated overnight with the primary antibodies: rabbit polyclonal CXCL8 antibody (1:1,000; ab84995), rabbit monoclonal JAK2 antibody (1:1,000; ab108596), rabbit anti-STAT3 antibody (1:100, ab76315), rabbit monoclonal E-cadherin antibody (1:1,000; ab194982), rabbit monoclonal vimentin antibody (1:1,000; ab92547) and rabbit polyclonal N-cadherin antibody (1:1,000; ab76057) (all from Abcam), respectively, followed by PBS washing 3 times at room temperature, 5 min each. Next, the membranes were incubated with goat anti-mouse IgG antibody labeled with horseradish peroxidase (1:2,000; ab6789; Wuhan Boster Biological Technology, Ltd.) for 120 min at room temperature. The reference gene was mouse monoclonal β-actin antibody (1:500; ab8226; Abcam). The membranes were washed 3 times with TBST, and ECL kit was used for luminescence reaction. Protein bands were visualized using Bio-Rad Image Reader imaging analyzer (Bio-Rad Laboratories, Inc.). Quantity One software 4.6.6 software (Bio-Rad Laboratories, Inc.) was used for the analysis of the gray value of the bands. The experiment was repeated three times.

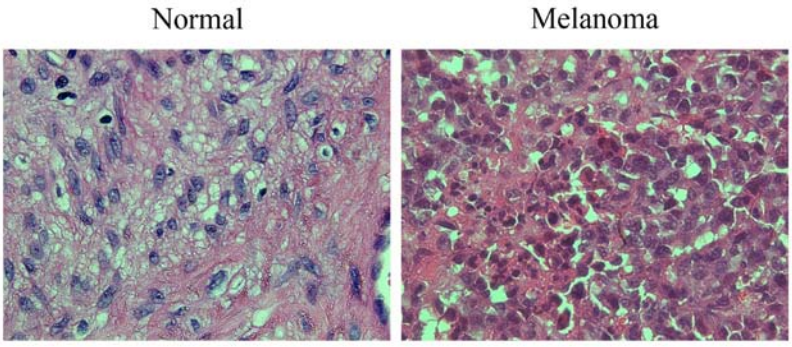


Figure 1. H&E staining for the detection of histopathological changes in tumor and adjacent normal tissues of cutaneous melanoma (x400). Scale bar, 25 $\mu$m. H&E, hematoxylin and eosin.

*Screening of cell lines, cell culture and transfection*. Human melanoma cell lines A375 (cat. no. AC148), SK-MEL-28 (cat. no. AC339802) and SK-MEL-1 (cat. no. AC100491) (Shanghai Institute of Cell Research, Chinese Academy of Sciences) were selected for subculture and were inoculated in culture medium. The cells were cultured in a thermostat at 37˚C and 5% $CO_2$. The medium consisted of 10% fetal bovine serum, RPMI-1640 medium and penicillin-streptomycin. Every 24-48 h, the medium was replaced, digested and passaged with 0.25% trypsin, and the cells in logarithmic phase were selected for the experiment. The expression of CXCL8 in A375, SK-MEL-28 and SK-MEL-1 cells was detected by fluorescent RT-qPCR, and the optimal cell line was screened out for subsequent experiments.

Cells were divided into five groups: Blank group (human cutaneous melanoma cells), NC group (human cutaneous melanoma cells + blank vector plasmid transfection), CXCL8 siRNA group (human cutaneous melanoma cells + CXCL8 silent expression vector plasmid transfection), AG490 group (human cutaneous melanoma cells + JAK-STAT signal pathway inhibitor transfection), CXCL8 siRNA + AG490 group (human cutaneous melanoma cells + JAK-STAT signaling pathway inhibitor + CXCL8 silent expression vector plasmid transfection). Transfection plasmids were purchased from Thermo Fisher Scientific, Inc., and JAK-STAT signaling pathway inhibitor was purchased from Beijing Baioleibo Technology Co., Ltd. Cells were inoculated into 6-well plates 24 h before transfection. When the cell confluency reached ~50-60%, the cells were transfected into a human cutaneous melanoma cell line by Lipo fectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). The cells were transfected for 6 h and then cultured for 48 h, before collected for subsequent experiments.

*CCK-8 assay for the detection of cell proliferation after transfection*. When the cell growth density reached ~80%, all the cells in logarithmic growth phase were washed twice with PBS solution and digested by 0.25% trypsin to form a single cell suspension. After counting, the cells with adjusted density of $3x10^3$ were inoculated into 96-well plates with a volume of 200 $\mu$l/well, with 6 replicates made for each sample. Cells were evenly dispersed by gentle shaking. Subsequently, the 96-well plates were incubated at 37˚C and 5% $CO_2$ saturated humidity. The plates were taken out at 24, 48, 72 and 96 h, respectively, followed by the addition of 10 $\mu$l CCK-8 (Sigma-Aldrich; Merck KGaA) per well for continuous culture for 3 h. The absorbance values (A values) of each well were read at 450 nm by a microplate reader. Each experiment was repeated 3 times. Cell viability curves were plotted with time as abscissa and A value as ordinate.

*Flow cytometry*. For the apoptosis detection by flow cytometry, human cutaneous melanoma cell lines were selected. The experimental groups were the Blank group, NC group, CXCL8 siRNA group, AG490 group and CXCL8 siRNA + AG490 group. i) Cell collection: Adherent cells were digested and directly collected into 5 ml centrifugal tubes. The number of cells per sample was 1 to $5x10^6$/ml. After centrifugation at 800 x g for 5 min, the culture medium was abandoned. ii) Preparation of cell suspension: Cells were washed with PBS and centrifuged at 800 x g for 5 min, and the supernatant was discarded. iii) Annexin V-FITC/PI double labeled staining by using a flow cytometer (Thermo Fisher Scientific, Inc.), with the apoptotic rate analyzed by ModFit LT 3.0 software (Verity Software House, Inc): 500 $\mu$l cells were suspended by a binding buffer, 5 $\mu$l PI and 5 $\mu$l Annexin V-FITC were added to each tube, and the cells were incubated at room temperature in the dark for 15 min. iv) Annexin V-FITC and PI fluorescence were detected by flow cytometry, and apoptosis was detected.

*Statistical analysis*. Statistical data were processed and analyzed by the SPSS 21.0 statistical software (IBM Corp.). Normal distribution and homogeneous tests of variance were performed for all data. The parameters of the experimental measurement data conforming to a normal distribution were expressed as the mean ± standard deviation. Data were analyzed between two groups using the independent samples t-test. One-way analysis of variance (ANOVA) was used for comparisons among groups, followed by the Bonferroni post hoc. $P<0.05$ was considered to indicate a statistically significant difference.

## Results

*H&E staining in human cutaneous melanoma*. H&E staining revealed that compared with the adjacent normal tissues, the cells in the melanoma tissues presented obvious atypia, had oval appearance with different shapes and sizes in most cases, indicating compact arrangement, obvious nucleoli, mitotic figures, large cytoplasm, diffuse distribution, and increased production of melanin granules between and within cells (Fig. 1).

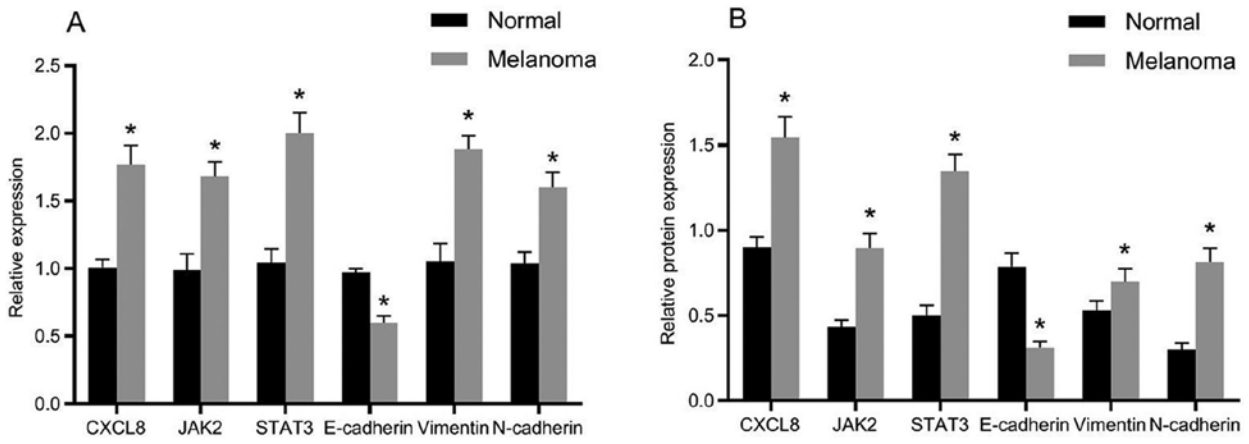


Figure 2. Expression of related genes and proteins in tissues of cutaneous melanoma. (A) Histogram of the mRNA expression of related genes in tissues. (B) Histogram of the protein expression of related proteins in tissues; *P<0.05, compared with adjacent normal tissues.

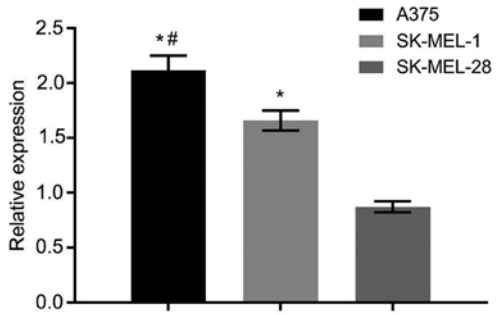


Figure 3. CXCL8 gene expression in different cell lines by fluorescent quantitative PCR. *P<0.05, compared with SK-MEL-28 cell line; #P<0.05, compared with SK-MEL-1 cell line.

*mRNA and protein expression of related genes*. The mRNA and protein expression levels of related genes in normal and melanoma tissues are presented in Fig. 2. Compared with the adjacent normal tissues, the mRNA and protein expression levels of E-cadherin were significantly decreased in melanoma tissues, whereas the mRNA and protein expression levels of CXCL8, JAK2, STAT3, vimentin and N-cadherin were obviously increased. The differences were statistically significant (P<0.05).

*Screening of cell lines*. The expression of CXCL8 gene in A375 human cutaneous melanoma cells was the highest compared with that in SK-MEL-28 and SK-MEL-1 human cutaneous melanoma cells, with statistically significant differences (P<0.05) (Fig. 3). Thus, A375 human cutaneous melanoma cells were screened out for subsequent experiments.

*mRNA and protein expression of related genes after cell transfection*. The mRNA and protein expression levels of related genes after transfection are presented in Fig. 4. The CXCL8 siRNA group and CXCL8 siRNA + AG490 group showed significantly decreased CXCL8 expression compared with the blank group (P<0.05), whereas there was no significant difference in the CXCL8 expression compared with the NC and AG490 groups (P>0.05). Compared with the blank group, the expression levels of JAK2, STAT3, vimentin and

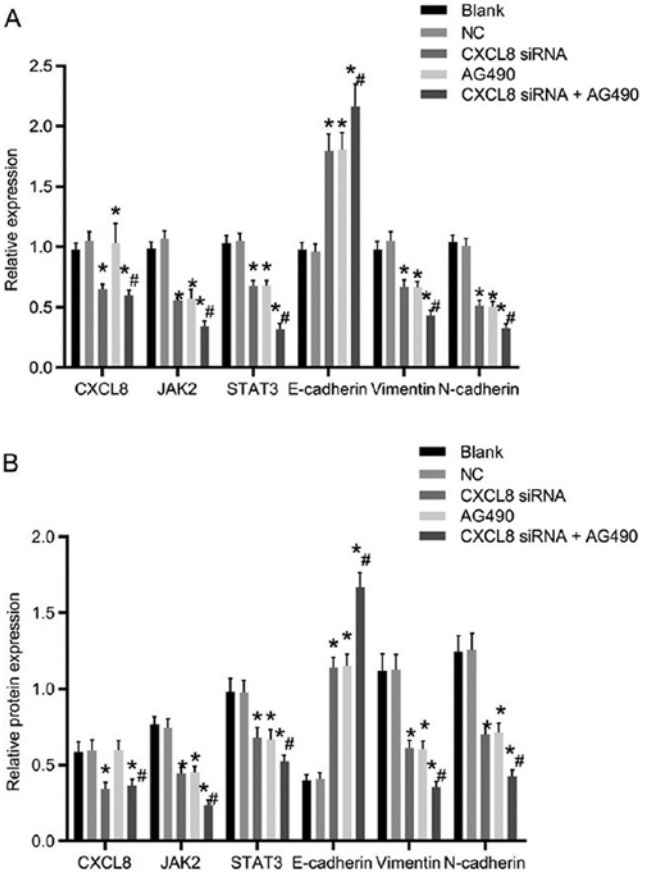


Figure 4. Comparison of the mRNA and protein expression of related genes after transfection in different groups. (A) Histogram of the mRNA expression of related genes in cells. (B) Histogram of the protein expression of related proteins in cells; *P<0.05 compared with the Blank group; #P<0.05 compared with the CXCL8 siRNA group.

N-cadherin in the CXCL8 siRNA group, AG490 group and CXCL8 siRNA + AG490 group were significantly decreased, whereas the expression level of E-cadherin was increased (P<0.05). There was no obvious difference in the expression levels of the related genes compared with those in the CXCL8 siRNA group and AG490 group (P>0.05). Compared with CXCL8 siRNA group, the expression levels of JAK2, STAT3,

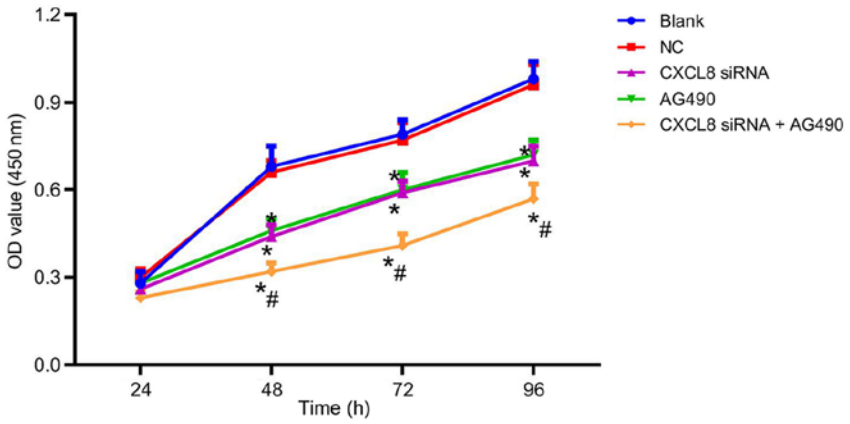


Figure 5. Comparison of cell proliferation in each group after cell transfection. *P<0.05, compared with the blank group, and #P<0.05, compared with the CXCL8 siRNA group.

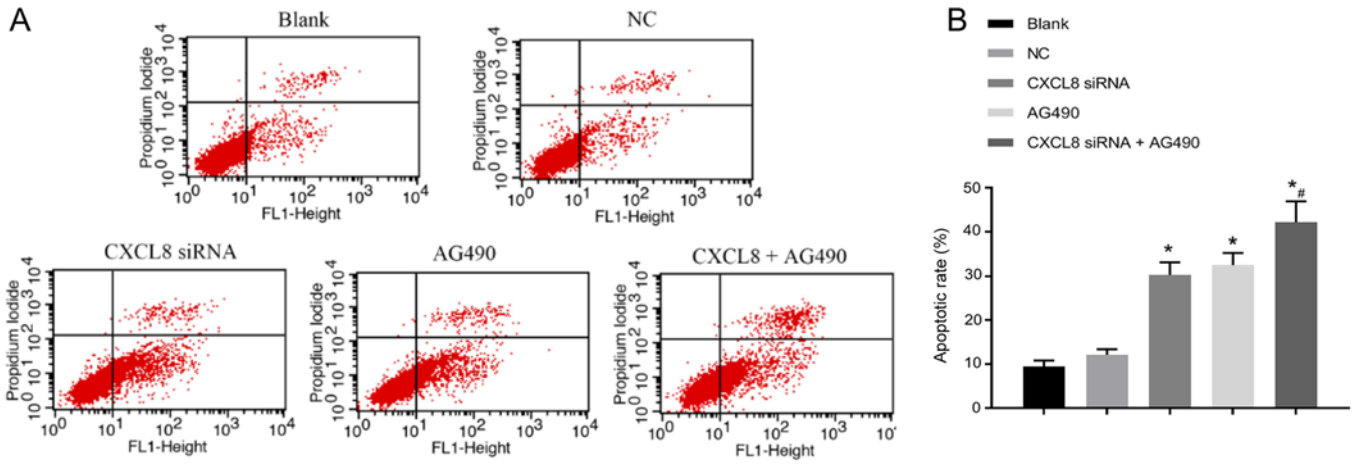


Figure 6. Comparison of cell apoptosis in each group after cell transfection. (A) Flow cytometry results in each group. (B) Comparison histogram of apoptotic rate in each group; *P<0.05, compared with the blank group, and #P<0.05, compared with the CXCL8 siRNA group.

vimentin and N-cadherin in CXCL8 siRNA + AG490 group were obviously decreased, whereas the E-cadherin expression was significantly increased (P<0.05).

*Changes of cell proliferation ability after cell transfection*. After cell transfection, the cell proliferation ability of each group is presented in Fig. 5. There was no obvious difference in cell proliferation among groups at 24 h (P>0.05). At 48, 72 and 96 h, the cell proliferation ability in the CXCL8 siRNA group, AG490 group and CXCL8 siRNA + AG490 group was decreased compared with that in the blank group (P<0.05). There was no significant difference in the cell proliferation of the NC group at the different time-points (P>0.05); and no significant difference was presented between the cell proliferation ability of the CXCL8 siRNA group and AG490 group at the different time-points (P>0.05). Compared with CXCL8 siRNA group, CXCL8 siRNA + AG490 group showed a significant decrease in cell proliferation (P<0.05).

*Changes of cell apoptosis ability after cell transfection*. The apoptotic status is shown in Fig. 6. Compared with the blank group, the apoptotic rate of cells in the CXCL8 siRNA group, AG490 group and CXCL8 + AG490 group was increased (P<0.05). There was no significant difference in cell apoptosis when compared with NC group (P>0.05). In addition, no significant difference was detected in cell apoptosis between CXCL8 siRNA group and AG490 group (P>0.05). Compared with CXCL8 siRNA group, CXCL8 siRNA + AG490 group presented obvious increase in cell apoptosis (P<0.05).

## Discussion

Invasion and migration of malignant tumor cells is a complex multi-step and multi-factor process (46,47). Melanoma is highly malignant and prone to hematogenous and lymphatic metastasis with poor prognosis (1). Early detection and diagnosis are particularly important for the prediction of melanoma prognosis (4). The preferred treatment for malignant melanoma is early surgical excision (8). Chemotherapy and radiotherapy have been used to prevent the recurrence of lesions, remove small foci and metastases, and prevent metastasis after surgical resection (9). However, melanoma is insensitive to current radiotherapy and chemotherapy, and most of the anti-neoplastic drugs currently used in clinic have strong toxicity and side-effects, which seriously affect the quality of life of cancer patients (9). Therefore, searching and

screening for safe, effective, less toxic side-effects of anti-cancer strategies are highly valued.

JAK-STAT signal transduction pathway is a classic signal transduction pathway, which participates in cell proliferation, survival, transformation, migration and other processes (33,34). Among the six family members of STATs, STAT1, STAT3 and STAT5 are the most common (41). It has been reported for numerous tumors that blocking the functions of STAT3 and STAT5 alone can inhibit the growth of tumor cells and induce apoptosis (48). Previous studies have shown that STAT3 is expressed at high levels and is activated in cervical and endometrial cancer tissues (49,50). Blocking the persistent signal pathway of STAT3 is considered to be able to inhibit the growth of cancer cells, which may become a new therapeutic target (51). Pan *et al* (52) have reported that high mRNA expression levels of STAT3 in colon cancer, breast cancer and glioblastoma are related significantly to shorter survival times. In addition, cell proliferation and apoptosis maintain homeostasis under normal conditions, and the mechanism regulating the balance maintains the normal physiological function of the body (53). Once this balance is destroyed, it leads to numerous serious pathological changes, such as tumors, degenerative diseases and autoimmune diseases. Development of tumors are not only related to abnormal proliferation and differentiation of tumor cells, but also to the changes of cell death (54). It has been proven that the reduction of apoptosis can cause tumorigenesis and promote malignant transformation and evolution of cancer cells by escaping (55). Therefore, the strategy of inducing apoptosis of cancer cells has become the focus of cancer treatment in recent decades.

In the present study, H&E staining showed that compared with the adjacent normal tissues, melanoma cells in tissues present obvious atypia, oval appearance with different shapes and sizes in most cases, compact arrangement, obvious nucleoli, mitotic figures, large cytoplasm, diffuse distribution, and increased production of melanin granules between and within cells, showing the biological features of melanoma cells. Subsequent gene expression detection showed that the expression of E-cadherin in melanoma tissues was significantly decreased compared with that of the adjacent normal tissues, whereas the expression levels of CXCL8, JAK2, STAT3, vimentin, and N-cadherin were significantly increased. These results suggest that there may be high CXCL8 expression and activation of JAK-STAT signaling pathway, and presence of EMT in cutaneous melanoma. In the malignant evolution of 90% of epithelial malignant tumors, there may be active downregulation of epithelial cells, decreased polarity of inter-cellular homogeneous adhesion system, disengagement from constraint of inherent organizational structure, acquisition of phenotypes and related gene changes, resulting in cell invasion and migration, with EMT (56,57). During EMT, strong phenotypic characteristics of epithelial cells, such as intercellular adhesion and polar distribution, are replaced by mesenchymal phenotype (decreased adhesion, fibroblast phenotype, increased mobility), which is an important link in malignant evolution and metastasis of cancer cells (58,59). It has been reported that human cancer cells can secrete CXCL8 through autocrine and/or paracrine pathways and interact with chemokine receptors to promote the proliferation and migration of tumor cells (60). Furthermore, important markers of EMT are loss of homogeneous adhesion between epithelial cells (decrease in E-cadherin expression, increase in N-cadherin and vimentin expression) (61,62), resulting in cell adhesion complex disintegration, cell adhesion and cell proliferation disorder, morphological and structural disorders of epithelial cells, loss of cell polarity and contact inhibition, cell growth disorder and infiltration into surrounding tissues (63).

Cell line experiment was carried out, and the highest expression level of CXCL8 gene was found in A375 human cutaneous melanoma cells, when compared with that in SK-MEL-28 and SK-MEL-1 human cutaneous melanoma cell lines. Thus, A375 cells were screened out for subsequent experiments. The results for the mRNA and protein expression levels of related genes after transfection were: Compared with the blank group, CXCL8 siRNA group and CXCL8 siRNA + AG490 group showed evidently decreased CXCL8 expression. The expression levels of JAK2, STAT3, N-cadherin and vimentin in CXCL8 siRNA group, AG490 group and CXCL8 siRNA + AG490 group were decreased, whereas the expression of E-cadherin was increased. The aforementioned trends are especially significant in CXCL8 siRNA + AG490 group. In subsequent research of biological characteristics, cell proliferation test results showed that at 48, 72 and 96 h, CXCL8 siRNA group, AG490 group and CXCL8 siRNA + AG490 group had reduced cell proliferation, with more evident decrease of cell proliferation in CXCL8 siRNA + AG490 group. The results indicated that the silenced expression of CXCL8 can inhibit the proliferation of cancer cells, while JAK-STAT signaling pathway activation may promote the proliferation of cancer cells. Apoptotic status of each group further revealed that compared with the blank group, the apoptotic rate in the CXCL8 siRNA group, AG490 group and CXCL8 + AG490 group was increased, and it was in particular significantly increased in the CXCL8 siRNA + AG490 group. These results suggest that suppressed expression of CXCL8 can promote apoptosis of cancer cells, whereas activation of JAK-STAT signaling pathway can inhibit apoptosis of cancer cells.

In conclusion, silencing of CXCL8 gene expression may inhibit EMT and cell proliferation while promoting cell apoptosis of human cutaneous melanoma cells by inhibiting JAK-STAT signaling pathway activation. In clinical practice, the study of the association of CXCL8 and JAK-STAT signaling pathway with EMT and apoptosis of cutaneous melanoma will have essential impact on the diagnosis, treatment and drug screening of cutaneous melanoma, especially with the discovery of more signal transduction substrates, activators and inhibitors.

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## Availability of data and materials

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

## Authors' contributions

XH wrote the manuscript, analyzed and interpreted the patients' data. LY and TM performed H&E staining, PCR, western blot analysis, CCK-8 assay and flow cytometry, and were responsible for the statistical analysis. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

The study was approved by the Ethics Committee of the General Hospital of Ningxia Medical University (Yinchuan, China). Patients who participated in this research had complete clinical data. Signed informed consents were obtained from the patients or their guardians.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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