The expression of Lin28 in gastric cancer cells treated with an anticancer bioactive peptide combined with oxaliplatin in vitro and in vivo

Xian Li
Affiliated Hospital of Inner Mongolia Medical University
Lihua Kang
Inner Mongolia Medical University
Jiaqi Peng
Inner Mongolia Medical University
Wenyan Han
Affiliated Hospital of Inner Mongolia Medical University
Xiulan Su (xlsu@hotmail.com)
Affiliated Hospital of Inner Mongolia Medical University

Research Article

Keywords: gastric cancer, anticancer bioactive peptides, oxaliplatin, Lin28 expression, chemotherapeutic drugs

Posted Date: May 4th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1484455/v3

Abstract

Background: Gastric cancer has become one of the major diseases threatening human health. This study aimed to investigate the effect and mechanism of an anticancer bioactive peptide (ACBP) combined with oxaliplatin (OXA) on MKN-45, SGC7901, and NCI-N87 differentiated human gastric cancer cells and GES-1 immortalized human gastric mucosal epithelial cells. And the therapeutic effect and action mechanism of short-term intermittent ACBP combined with OXA on nude mice with human gastric cancer were investigate.

Methods: The half-maximal inhibitory concentrations of these agents in these cells were measured by an MTT assay, and cell morphological changes were observed by H&E staining. The expression of Lin28, miR-107, miR-609 and Let-7 in these four cell lines was determined by q-PCR after drug treatment. Lin28 protein expression in these four cell lines treated with these drugs was measured by western blotting. Furthermore, activity and quality of life were observed daily in all tumor-bearing nude mice, and the expression of Lin28 in tumor tissue was determined by immunohistochemistry and RT-PCR.

Results: The results showed that ACBP inhibited the proliferation of MKN-45, SGC7901, and NCI-N87 gastric cancer cells in a dose-dependent manner and weakly suppressed the proliferation of GES-1 cells. Moreover, the inhibitory effect on proliferation was stronger in poorly differentiated gastric cancer cells. ACBP, OXA and the combination upregulated Lin28 gene expression in MKN-45 cells and downregulated it in SGC7901 and GES-1 cells. ACBP and the combination therapy downregulated Let-7 expression in MKN-45 cells and upregulated Let-7 expression in SGC7901 cells. The combination of ACBP with OXA has significant anticancer sensitization on oxaliplatin, and also significantly improves the quality of life of tumor-bearing nude mice and reduces the toxic side effects of chemotherapeutic drugs on nude mice.

Conclusion: ACBP alone and in combination with oxaliplatin influenced the expression of tumor stem cell marker gene Lin28 and regulated the expression of microRNAs specifically regulated by Lin28. In addition, the anticancer effects and attenuated sensitization effects of ACBP may be related to the Lin28/miRNA-107 signaling pathway, acting by inhibiting the proliferation of cancerous stem cells.

1. Introduction

At present, malignant tumors are the main public health problem worldwide. According to the latest cancer statistics from 2015, the incidence of gastric cancer in China is second only to that of lung cancer [1]. Gastric cancer has become one of the major diseases threatening human health. There are no obvious symptoms in the early stage of gastric cancer, and most patients with confirmed disease have advanced-stage disease without the option of surgical eradication. Chemotherapy still plays an important role in the treatment of gastric cancer, but resistance arising during treatment leads to failure of chemotherapy or cessation of treatment. In addition, chemotherapeutic drugs have insurmountable toxic side effects, such as bone marrow suppression, gastrointestinal reactions, and neurotoxicity. It is urgent to explore new anticancer drugs with low toxicity, minimal side effects and therapeutic efficiency.

Oxaliplatin (OXA) is a 3rd-generation platinum chemotherapeutic agent acting on DNA. Although its effect on bone marrow suppression is less severe than that of cisplatin, bone marrow suppression, gastrointestinal reactions, and neurotoxicity are still inevitable. Anticancer bioactive peptides (ACBP) are a class of polypeptides that have a variety of special physiological functions and are naturally found in animals, plants and other organisms. Research shows that bioactive peptides inhibit bacterial growth, hypertension, thrombosis, and cancer and perform other functions. Bioactive peptides have not only anticancer activity but also low toxicity [2, 3], compensating for the limitations of traditional radiotherapy and chemotherapy. Exploring bioactive peptides with anticancer activity is a new strategy to develop novel low-toxicity and targeted agents for tumor prevention and treatment. Bioactive peptides are expected to become a new adjuvant for the prevention and treatment of cancer [4–6].

Anticancer bioactive peptide (ACBP) were obtained by our team from goat organs (livers or spleens) [3]. ACBPs are polypeptides with a medium to low molecular weight (~ 8000 Da). Studies from the Preliminary Clinical Research Center confirmed the potential role of ACBPs in inhibiting tumor cell proliferation in mice with leukemia and in vitro studies of human nasopharyngeal cancer. In addition, ACBPs have considerable anticancer properties and minimal toxic effects [7, 8]. Previous research results show that ACBP have a strong inhibitory effect on the proliferation of human gastric cancer cells [3]. We have also performed many relevant mechanistic studies to confirm that its cancer suppression mechanism is associated with induction of tumor cell apoptosis and regulation of the cell cycle. Recent studies have shown abnormal expression of microRNAs that regulate the malignant biological behavior of tumor stem cells (CSCs) by influencing the expression of the corresponding target genes in a variety of tumors. Research on microRNAs has gained increasing attention, and some tumor-related microRNAs have been confirmed. Based on a literature analysis and our previous research, the tumor stem cell marker gene Lin28 was studied.

Lin28 is a highly conserved protein in the RNA-binding protein family, whose members have a molecular weight of less than 30 kDa [9]. The Lin28 protein structure contains a cold shock domain (CSD) [10] and a zinc finger domain (ZKD). Its RNA binding function is a key regulator of growth and development [11]. The population of mouse stem cells decreased significantly after Lin28 knockout, and these mice could not survive after birth [12]. Lin28 is highly expressed in mouse and human embryonic stem cells (mESCs and hESCs, respectively) and in developing tissues, and its expression gradually decreases with tissue differentiation. Lin28 is essential in embryonic cell growth and development [12]. Regarding the biological function of Lin28, it has been found that Lin28 is involved in the regulation of cell development, glucose metabolism, differentiation, tissue regeneration, and tumor formation processes [13, 14].

Studies have shown that Lin28 plays an important role mainly by inhibiting the expression of its target microRNA and acts as an oncogenic protein that is overexpressed in tumors and promotes the proliferation and malignant progression of tumor cells. Lin28 overexpression has been identified in ovarian cancer [15], liver cancer [16] and gastric cancer [17], and the Lin28 expression level is closely related to the survival of patients with malignant tumors; high Lin28 expression may indicate poor prognosis. The present research suggests that Lin28 is a potential target for tumor treatment.

MicroRNAs regulate protein expression at the transcriptional level by binding to the 3'UTR of the target gene and play an important role in a variety of physiological processes, including development, apoptosis, and metabolism. Lin28 can regulate its expression by interacting directly with its target microRNA. Lin28 has been shown to regulate the expression of miR-107, Let-7, miR-370 [18], miR-664, miR-485-3p, and miR-495 [19].

Mir-107 is one of the downstream target genes of Lin28. Lin28A and its homologous gene Lin28B inhibit the expression of Let-7, and Let-7 downregulates the expression of Lin28. High expression of Lin28 in gastric cancer cells reduces Let-7 expression in gastric cancer tissues, thus promoting the proliferation, invasion and metastasis of gastric cancer cells [17]. MiR-609 is a microRNA that was confirmed by early gene screening in gastric cancer tissue. The relationship between miR-609 and Lin28 has not been reported, and the specific role and mechanism of miR-609 are unknown and need further study. This paper initially explores the effect of an ACBP on miR-609 and the relevance of miR-609 to Lin28 expression.

Based on our previous work and a literature analysis, we propose the hypothesis that ACBP may regulate the expression of the Lin28 gene and its targeting microRNA. The mechanism of action of ACBP in inhibiting the proliferation of gastric cancer cells may involve Lin28/microRNA networks. In this study, the influence of ACBP on Lin28, miR-107, Let-7 and miR-609 expression was analyzed by q-PCR and at the protein level, and the half-maximal inhibitory concentration of ACBP was determined. It is hoped that this study provides a scientific theory and data to explore new strategies for low toxicity and efficient tumor treatment and to explain the mechanism of action of the ACBP in depth.

In addition, This study will explore the effect of ACBP and combined with OXA on the quality of life of Dutch gastric cancer MKN-45 cells, and explore the role of ACBP on MKN-45 cells nude mice by detecting the expression of lin28 gene, initially clarify the mechanism of ACBP and provide new methods and new ideas for the comprehensive treatment of tumors.

2. Materials And Methods

2.1 Cell lines

The poorly differentiated human gastric adenocarcinoma cell line MKN-45, the highly differentiated human gastric adenocarcinoma cell line SGC7901, the human gastric adenocarcinoma cell line NCI-N87 and the immortalized, nontumor human gastric mucosal epithelial cell line GES-1 were donated by Professor Ke Yang of the Cancer College of Peking University and by Professor Deng Dajun.

2.2 Cell culture

MKN-45 cells were cultured in DMEM high-glucose medium containing 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal bovine serum. SGC 7901 cells, NCI-N 87 cells, and GES-1 cells were cultured in RPMI-1640 medium containing 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal bovine

serum. MKN-45, SGC7901, NCI-N87 and GES-1 cells in the logarithmic growth phase were collected. The resulting cell suspensions were added to a 96-well plate (4×10^3 cells per well) at 37°C in a 5% CO_2 incubator. The designated cells were treated with media that contained 5, 10, 15, 20, and 25 μg/mL ACBP. Another group of plated cells was treated with 5, 10, 15, 20, and 25 μg/mL oxaliplatin. After 48 h of culture, 20 μL of MTT solution was added to each well for 4 h. After 4 h, the wells were washed carefully (to avoid removal of the purple crystals) and 150 μl of DMSO was added; only 150 μl DMSO was added to the blank well. Afterward, the absorbance at 570 nm was measured according to the manufacturer's instructions. Each concentration of each agent was tested in triplicate. The average absorbance values were calculated. The formula for calculating the cell growth inhibition rate is as follows: IR= $((OD_{control}-OD_{experiment})/OD_{control}) \times100\%$.

2.3 H&E staining

Coverslips were first placed in a 6-well plate, and cells were seeded in the 6-well plate at 1×10^6 cells per well. After 24 h, the drugs were added at concentrations corresponding to the IC_{50} for each drug in each cell line when used alone or at 50% of these concentrations when used in combination, and drug-free medium was added to the blank control wells. After 48 h, H&E staining was performed as follows: remove the primary medium and fix the cells for 20 min with 95% ethanol, wash 2 times with PBS for 1 min each, stain with hematoxylin for 3 min and wash with running water, differentiate with 1% acid alcohol for 5 s, stain with eosin for 1 min, dry naturally and seal the coverslips with neutral gum, and observe the staining.

2.4 RNA Isolation and RT-PCR

Total RNA was extracted from tumor samples using TRIzol reagent according to the manufacturer's instructions. After spectrophotometric quantification, 1 mg of total RNA was used to synthesize first-strand cDNA with a RevertAid H Minus First Strand cDNA Synthesis Kit (Fermentas, USA). Colorimetric analysis was conducted using a Beckman Coulter DU 800 UV spectrophotometer. The OD ratio at 260 nm/280 nm was measured. Specifically, a ratio from 1.8 to 2.0 shows that the purity of the RNA is high. Gel electrophoresis was used to confirm the purity of the extracted RNA. The extracted tumor mRNA was typed with BstU-I. The digestion products were resolved using 2% agarose gel electrophoresis with ethidium bromide staining and detected under UV light. The bands were visualized using a digital camera and BioCapt software (Vilbert-Lourmant). Real-time RT–PCR was carried out in an Mx3000P real-time PCR system (Stratagene, USA). To correct for experimental variations between samples, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the internal control. Primers were designed and synthesized by Sangon Biotech Company (Shanghai, China), as shown in Table 1. The expression levels of Lin28, miR-107, Let-7, U6 and miR-609 (Santa Cruz, CA, USA) in tumor tissue were measured. The thermal cycling program for PCR was set as follows: (1) initial denaturation at 95°C for 5 min; (2) 35 cycles of denaturation at 95°C, annealing at 60°C, and elongation at 60°C for 30 s; and (3) a final extension step at 60°C for 40 s. RT–PCR was performed using the Taq-Man Gene Expression Assay protocol (ABI, USA).

2.5 Western Blot Analysis

Protein was extracted with TRIzol (CW0580/A, CWBIO, China) supplemented with protease inhibitors (Roche). Protein concentrations were quantified by using a BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). Equal amounts of samples were lysed with 1 × SDS loading buffer (50 mM Tris-Cl (pH 6.8), 100 mM DTT, 2% SDS, 10% glycerol and 0.1% bromophenol blue) as the whole-cell sample and were subjected to SDS–PAGE. All proteins were transferred to polyvinylidine difluoride (PVDF) membranes. Immunoblotting was carried out with primary antibodies at a dilution of 1:1000, including antibodies specific for Lin28A (orb651287, Biorbyt, Britain) and the internal reference protein β-actin (4970, Cell Signaling Technology, USA). Then, membranes were incubated with labeled goat immunoglobulin (IgG) (A21020, Abbkine) at a dilution of 1:10000 as the secondary antibody. Bands were detected with an ECL chemiluminescence detection kit (E412-01/02, Vazyme, China), and band intensities were quantified by using Image LabTM Software (Bio–Rad, Shanghai, China).

2.6 Nude mice and cell lines

The study was approved by the Ethics Committee of The Affiliated Hospital of Inner Mongolia Medical University. Here, the nude mice model was established by MKN-45 cell according to the ethics committee. Human gastric cancer cell strain MKN-45 was provided by the Clinical Medical Research Center of the Affiliated Hospital of Inner Mongolia Medical University.

The balb/c-Nu nude mice, animal grade: SPF grade, animal sex and number: females, a total of 65 animals were purchased from Beijing Vitonolihua Experimental Animal Technology Co., Ltd.

2.7 Established a model of subcutaneous graft tumors in nude mice

A subcutaneous graft model of human gastric cancer cell line MKN-45 nude mice was established. Routine culture of MKN-45 cell was adjusted to 1×10^7/ml. The MKN-45 gastric cancer cells were seeded with a 1mL disposable syringe, at 0.1mL / only dose, and in the right axillary subcutaneous area (1×10^7/mL/only). On tumor-bearing mice were placed in UV-irradiated, clean animal chambers, and the survival and axillary tumor growth of nude mice were regularly observed.

After the successful preparation of the tumor-bearing nude mouse model, 32 nude mice of about 4–5 mm^3 were randomly divided into four groups: saline group (NS), anti-cancer bioactive peptide group (ACBP), oxaliplatin group (OXA), and ACBP + OXA group (MIX), 8 in each group. Drug administration: the NS group gave 0.9% sodium chloride solution with 0.5ml intraperitoneal injection; The ACBP group was administered intraperitoneally with 0.5 mL of the anticancer bioactive peptide; Oxaliplatin was administered intraperitoneally at 10 mg/kg in the OXA group; The MIX group received 0.5mL of the anticancer bioactive peptide combined with oxaliplatin at 10mg/kg intraperitoneally and administered every Monday and Thursday. The experiments were carried out in strict accordance with the rearing conditions of nude mice. Tumor weight was calculated every 3 days, and the maximum tumor length and

shortest transverse diame ter perpendicular to it in each group. Tumor volume (v) = $ab^2/2$, here, a is tumor body maximum length, b is shortest cross diameter.

Two weeks later, nude mice were sacrificed by cervical vertebra dislocation, tumor weaving for its mass and recorded. The tumor bodies were divided into triplicate in liquid nitrogen, fixed in 10% neutral formaldehyde solution and − 80°C freezer. Tumor suppressor rate (TSR) was calculated, TSR= ($W_{\text{control group}}$ - $W_{\text{experimental group}}$) / $W_{\text{control group}}$ ×100%.

2.8 Immunohistochemistry

The expression of Lin28 protein in the transplanted tumor tissue was determined by immunohistochemistry. Tumor tissue fixed in 10% neutral formaldehyde was sliced after paraffin-embedded, and protein expression of graft Lin28 gene of 10% neutral formaldehyde was fixed by S-P immunohistochemistry and scored using a conventional semi-quantitative scoring method. Semi-quantitative scoring method as follow: Under optical microscopy, the Lin28 protein was expressed within the cytoplasm, presenting yellow, tan, or brown particles. The staining intensity seen under the meat glasses was colorless, buff, tan, and tan as 0, 1, 2 and 3, respectively. At high magnification of 400 x, 10 fields were averaged and the percentage of positive cells was calculated and scored according to the proportion of positive tumor cells, < 5%, 6–25%, 26–50%, 51–75%, and > 75% were scored at 0, 1, 2, 3, 4 points, respectively. The staining intensity was multiplied by the positive percentage integral, 0 as (-),1–4 as (+), 5–8 as (++), ≥ 9 as (+++). The double-blind method was evaluated by the teachers using the pirker scoring standard after training.

2.9 RT-PCR

The expression of the tumor-related genes was determined by RT-PCR. Total RNA was extracted from tumor tissue using the TRIzol reagent according to the manufacturer's instructions. After spectrophotometric quantification, 1 mg of total RNA was used to synthesize firststrand cDNA with the Revert Aid H Minus First Strand cDNA synthesis kit (Fermentas). Real-time RT-PCR reactions were carried out on an Mx3000P real-time PCR system (Stratagene). To correct for the experimental variations between samples, GAPDH was used as the internal control. Primers were designed and synthesized by the Sangon Biotech Co., Ltd., as shown in Table 2. The expression of Lin28 (sc13156) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) in the tumor tissue was detected. The PCR program was as follows: i) initial denaturation at 95˚C for 5 min;) 35 cycles of denaturation at 95˚C, annealing at 60˚C, and elongation at 60˚C for 30 sec; and) a final extension at 60˚C for 40 sec. RTPCR was performed using the TaqMan Gene Expression Assay protocol (Applied Biosystems). The mRNA from tumor tissue was typed with BstUI, and the digestion products were resolved by 2% agarose gel electrophoresis with ethidium bromide staining and UV light detection. The results were documented by digital camera and stored as computer files in BioCapt software (VilbertLourmant, Marne LaValle, France).

2.6 Statistical Analysis

The data are presented as the mean ± standard deviation (SD) values. Student's t-test and one-way analysis of variance (ANOVA) were performed to determine P values using SPSS version 18.0 software. All experiments were repeated at least three times unless otherwise indicated. P values < 0.05 were considered statistically significant.

Table 1
The primer sequences used for real-time PCR

Primer name	Primer sequence (5'-3')
Lin28-F	CGGGCATCTGTAAGTGGTTC
Lin28-R	CAGACCCTTGGCTGACTTCT
GAPDH-F	GAGTCCACTGGCGTCTTCA
GAPDH-R	GGGGTGCTAAGCAGTTGGT
miR-107-F	CAGACGACCATCAGAGCATTGTACAGGG
miR-107-RT	GCACTTCAGTGTCGTGGTCAGTGACGGCAATTTGAAGTGCTGATA
Let-7-F	CAGACGACCATCAGAGAGGTAGTAGGTTGC
Let-7-RT	GCACTTCAGTGTCGTGGTCAGTGACGGCAATTTGAAGTGCAATGC
miR-609-F	CAGACGACCATCAGAGGGTGTTTCTCTC
miR-609-RT	GCACTTCAGTGTCGTGGTCAGTGACGGCAATTGAAGTGCAGAGATGA
U6-F	CTCGCTTCGGCAGCACAT
U6-R	GAACGCTTCACGAATTTGCGT

Table 2
Primer sequences of Lin28.

target gene	Primer sequences
Lin28	Forward
	5'- CGGGCATCTGTAAGTGGTTC
	Reverse 5'-CAGACCCTTGGCTGACTTCT
GAPDH	Forward
	5'- TCCACCACCCTGTTGCTGTA
	Reverse 5'-ACCACAGTCCATGCCATCAC

3. Results

3.1 Analysis of the MTT assay results

MKN-45, SGC 7901, NCI-N 87, and GES-1 cells were treated with ACBP and OXA at gradient concentrations (5, 10, 15, 20, and 25 μg/ml) and evaluated by an MTT assay. When MKN-45 cells were exposed to these concentrations of OXA for 48 h, the respective survival rates of the MKN-45 cells were decreased to 29.8%, 43.6%, 59.1%, 65.8%, and 66.8%. The half-maximal inhibitory concentration (IC_{50}) of OXA in MKN-45 cells after 48 h was 11.7 μg/mL. When MKN-45 cells were exposed to these concentrations of ACBP for 48 h, the respective survival rates of the MKN-45 cells were decreased by 1.5%, 22.7%, 44.2%, 51.1%, and 59.3%. The IC_{50} of ACBP in MKN-45 cells was 18.8 μg/mL at 48 h. ACBP had a definite inhibitory effect on MKN-45 cell proliferation in a concentration-dependent manner (Fig. 1A).

When SGC7901 cells were exposed the abovementioned concentrations of OXA for 48 h, the respective survival rates of the SGC 7901 cells were decreased by 48.9%, 51.4%, 61.2%, 70.5%, and 72.3%. The IC50 of OXA in SGC 7901 cells was 6.6 μg/ml after 48 h. When SGC 7901 cells were exposed to the abovementioned concentrations of ACBP for 48 h, the respective survival rates of the SGC 7901 cells decreased by 25.1%, 37.6%, 52.2%, 75.0%, and 78.6%. The IC_{50} of ACBP in SGC 7901 cells was 11.9 μg/ml at 48 h. Thus, ACBP suppressed SGC 7901 cell proliferation in a concentration-dependent manner (Fig. 1B).

When NCI-N87 cells were exposed the abovementioned concentrations of OXA for 48 h, the respective survival rates of the NCI-N87 cells were decreased by 27.3%, 35.4%, 41.2%, 48.6%, and 50.6%. The IC_{50} of OXA in NCI-N87 cells was 24 μg/ml at 48 h. When NCI-N87 cells were exposed the abovementioned concentrations of ACBP for 48 h, the respective survival rates of the NCI-N87 cells were decreased by 31.2%, 34.2%, 41.3%, 44.6%, and 52.6%. The IC50 of ACBP in NCI-N87 cells was 24.3 μg/ml at 48 h. This suggests that ACBP suppresses NCI-N87 cell proliferation over a range of increasing concentrations (Fig. 1C).

When GES-1 cells were exposed the abovementioned concentrations of OXA for 48 h, the respective survival rates of the GES-1 cells were decreased by 53.6%, 62.7%, 66.5%, 69.5%, and 71.5%, respectively. The OXA was 3.6 μg/ml for the IC_{50} of GES-1 cells at 48 h. When GES-1 cells were exposed the abovementioned concentrations of ACBP for 48 h, the respective survival rates of the GES-1 cells was decreased by 12.6%, 14.9%, 16.1%, 19.8%, and 26.3%. Due to the weak inhibitory effect of ACBP on GES-1 cells, its half-maximal inhibitory concentration was not estimated. Compared with the positive drug OXA, ACBP weakly inhibited GES-1 cell proliferation, a result that was also observed in GES-1 immortalized gastric mucosal epithelial cells. This suggests that OXA has toxic effects on gastric mucosal epithelial cells, while ACBP has very weak toxic effects on gastric mucosal epithelial cells (Fig. 1D).

In conclusion, ACBP inhibited MKN-45, SGC7901, and NCI-N87 gastric cancer cell proliferation in a dose-dependent manner, but its inhibitory effect on the proliferation of GES-1 immortalized gastric mucosal epithelial cells was weak, suggesting that ACBP has a definite targeted inhibitory effect on tumor cells.

3.2 Evaluation of MKN-45, SGC7901, NCI-N87 and GES-1 cells after drug treatment by H&E staining

To further observe the changes in MKN-45 cell morphology after drug treatment, cells were seeded in a 6-well plate containing coverslips, and cells on climbing slides were treated separately with serum-free culture medium, 11.7 μg/ml OXA, 18.8 μg/ml ACBP, and 5.85 μg/ml OXA combined with 9.4 μg/ml ACBP. The staining results showed that the number of cells was decreased significantly, the cell morphology was rounded and apoptotic, and some cells showed core consolidation and enhanced nuclear basophilism in the ACBP group compared with the control group. The OXA and combination treatment groups exhibited sparse cell coverage, a rounded cell morphology, core consolidation, enhanced nuclear staining, nuclear fragmentation and nuclear lysis (Fig. 2- MKN-45).

To further observe the changes in SGC7901 cell morphology after drug treatment, SGC7901 cells were treated separately with serum-free medium, 6.6 μg/ml ACBP, 11.9 μg/ml OXA, and 3.3 μg/ml OXA with 5.95 μg/ml ACBP. The staining results showed that more cells were in the proliferation and division stage in the ACBP and OXA groups. After treatment with the combination of ACBP and OXA, the cell number was significantly decreased, and the cell shape was rounded, with core consolidation and enhanced staining (Fig. 2- SGC7901).

To further observe the changes in NCI-N87 cell morphology after drug treatment, NCI-N87 cells were treated separately with serum-free culture medium, 24 μg/ml OXA, 24.3 μg/ml ACBP, and 12 μg/ml OXA combined with 12.15 μg/ml ACBP. The staining results showed that some cells were rounded and the cell number was decreased in the ACBP group compared with the control group. In the OXA group, the number of cells was significantly decreased, the cell shape was rounded, the cytoplasm was dense, some cells had core consolidation, and core staining was enhanced. However, in the combination group, the cell shape was rounded, the cell number was reduced, core consolidation was observed, core staining was enhanced, cell shrinkage occurred, and the cytoplasm was dense (Fig. 2- NCI-N87).

To further observe the changes in the morphology of GES-1 cells after drug treatment, GES-1 cells were treated separately with serum-free medium, 3.6 μg/ml OXA, 25 μg/ml ACBP, and the combination of 12.5 μg/ml OXA with 1.8 μg/ml ACBP (because ACBP had a weak effect on GES-1 cells, the IC_{50} could not be estimated; therefore, 25 μg/ml was used as the ACBP treatment concentration). The staining results showed that there were no differences in cell morphology or cell number between the control group and ACBP group. In the OXA group, the number of cells was decreased significantly, some cells acquired a rounded morphology, some cells exhibited shrinkage, and the cytoplasm was dense. In addition, in the combination group, the number of cells was significantly reduced, the cell shape became rounded, and some cells developed core consolidation, enhanced nuclear staining, nuclear fragmentation, and nuclear lysis (Fig. 2- GES-1).

3.3 The expression of Lin28, miR-107, miR-609 and Let-7 in MKN-45 cells

To study the effects of OXA, ACBP and the combination on the expression of Lin28, miR-107, miR-609 and Let-7, MKN-45 cells were treated separately with serum-free medium, 11.7 μg/ml ACBP, 18.8 μg/mL OXA, and 5.85 μg/ml OXA combined with 9.4 μg/ml ACBP. After 48 h, cells were collected, and RNA was extracted. The relative expression levels of Lin28, miR-107, miR-609 and Let-7 were determined by q-PCR.

The relative expression levels of Lin28 in each treatment group show that for the comparisons of the OXA, ACBP and combination groups with the control group, the P values were 0.00, 0.015, and 0.005, respectively, and these differences were statistically significant ($P < 0.05$). For the comparisons of the OXA group with the ACBP and combination groups, the p values were 0.005 and 0.015, respectively, and these differences were statistically significant ($P < 0.05$). The difference between the ACBP group and the combination group was not statistically significant ($P > 0.05$) (Fig. 3- MKN-45-A).

The relative expression levels of miR-107 in each treatment group showed that the differences between the OXA and ACBP groups and the control group were not statistically significant ($P > 0.05$) from the. The miR-107 level in the combination group was significantly different from that in the control group ($P < 0.05$). The miR-107 levels in the ACBP and combination groups were not significantly different ($P > 0.05$) compared with that in the OXA group. The miR-107 level in the combination group was significantly different from that in the ACBP group ($P > 0.05$) (Fig. 3- MKN-45-B).

The relative expression levels of miR-609 in each treatment group showed that the levels in the OXA and combination groups were significantly different from that in the control group ($P < 0.05$). The difference was not statistically significant between the ACBP group and the control group ($P > 0.05$). The differences between the ACBP and combination groups and the OXA group were not statistically significant ($P > 0.05$). The difference between the ACBP group and the combination group was not statistically significant ($P > 0.05$) (Fig. 3- MKN-45-C).

Regarding the expression of Let-7, the difference between the OXA group and the control group was not statistically significant. The expression of Let-7 in the ACBP and combination groups was significantly different from that in the control group ($P < 0.05$). Moreover, compared with the levels in the ACBP and OXA groups, the level in the combination group was significantly different ($P < 0.05$) (Fig. 3- MKN-45-D).

In conclusion, in mkn45 cells, lin28 expression was upregulated in the ACBP, OXA and combination groups and was upregulated more significantly in the OXA group. MiR-107 expression was reduced in the combination group and was not affected by OXA or ACBP treatment alone. MiR-609 expression was altered in the OXA and combination groups, while ACBP had no effect on miR-609 expression. Let-7 expression was downregulated in the ACBP and combination groups, while there was no significant change in the OXA group.

3.4 The expression of Lin28, miR-107, miR-609 and Let-7 in SGC7901 cells

To study the effects of OXA, ACBP and the combination treatment on Lin28, miR-107, miR-609 and Let-7 expression in SGC7901 cells, these cells were treated separately with serum-free medium, 6.6 μg/ml OXA, 11.9 μg/ml ACBP, and 3.3 μg/ml OXA combined with 5.95 μg/ml ACBP. After 48 h, cells were collected, and RNA was extracted. The relative expression levels of Lin28, miR-107, miR-609 and Let-7 were determined by q-PCR.

Regarding the relative expression levels of Lin28 in the ACBP, OXA and combined groups, the results showed that the levels in the ACBP and OXA groups were significantly different from that in the control group ($P < 0.05$). The level in the combination group was significantly different from that in the ACBP group ($P < 0.05$). Moreover, the level in the combination group was not significantly different from that in the control group ($P > 0.05$). Compared with the level in the OXA group, the levels in ACBP and combination group were not significantly different ($P > 0.05$) (Fig. 3- SGC7901-A).

Regarding the relative expression level of miR-107, the results showed that the levels in the ACBP and OXA groups were significantly different from those in the control group ($P < 0.05$). However, the difference between the combination group and either monotherapy group was not statistically significant ($P > 0.05$). Compared with that in the OXA group, the relative expression level of miR-107 in the ACBP and combination groups was significantly different ($p < 0.05$). Compared with that in the ACBP group, the level in the combination group was significantly different ($p < 0.05$) (Fig. 3- SGC7901-B).

Regarding the relative expression level of miR-609, the results showed that the levels in the ACBP, OXA and combination groups showed no significant differences compared with that in the control group ($P > 0.05$). Compared with that in the OXA group, the level in the ACBP group showed no significant difference ($P > 0.05$). Compared with those in the OXA and ACBP groups, the level in the combination group was significantly different ($p < 0.05$) (Fig. 3- SGC7901-C).

Regarding the relative expression level of miR-609, the results showed that the levels in the ACBP, OXA and combination groups were significantly different from that in the control group ($P < 0.05$). However, the level in the ACBP group was not significantly different from that in the OXA group ($P > 0.05$). Compared with the levels in the OXA and ACBP groups, the level in the combination group was significantly different ($P < 0.05$) (Fig. 3- SGC7901-D).

In conclusion, in SGC7901 cells, Lin28 expression was downregulated in the ACBP and OXA groups, while the combination treatment had no effect on Lin28 expression. ACBP downregulated miR-107 expression, while OXA upregulated miR-107 expression, and the combination treatment had no effect. Neither OXA, ACBP nor the combination had an effect on miR-609 expression. Both OXA, ACBP and the combination upregulated Let-7 expression.

3.4 The expression of Lin28, miR-107, miR-609 and Let-7 in NCI-N87 cells

To investigate the effect of OXA, ACBP and the combination on Lin28, miR-107, miR-609 and Let-7 expression in NCI-N87 cells, NCI-N87 cells were treated separately with serum-free medium, 24 μg/ml ACBP, 24.3 μg/ml OXA, and 12 μg/ml OXA in combination with 12.15 μg/ml ACBP. After 48 h of treatment, cells were harvested, and RNA was extracted to determine the relative expression levels of Lin28, miR-107, miR-609 and Let-7 by q-PCR. Compared with that in the control group, Lin28 expression in the OXA, ACBP and combination groups was significantly different ($p < 0.05$). Compared with that in the OXA group, Lin28 expression in the ACBP group was not significantly different ($P > 0.05$). Compared with that in the OXA and ACBP groups, Lin28 expression in the combination group was significantly different ($p < 0.05$) (Fig. 3- NCI-N87-A).

Regarding miR-107 expression, the levels in the OXA, ACBP and combination groups were not significantly different ($P > 0.05$) from that in the control group. Compared with that in the OXA group, the levels in the ACBP and combination groups were not significantly different ($P > 0.05$). However, the combination group showed no significant difference ($P > 0.05$) compared with the ACBP group (Fig. 3- NCI-N87-B).

Regarding miR-609 expression, compared with that in the control group, miR-609 expression in the OXA, ACBP and combination groups was not significantly different ($P > 0.05$). Compared with that in the ACBP group, the levels in the OXA and combination groups were not significantly different ($P > 0.05$). Compared with the OXA group, the combination group showed a significant difference ($p < 0.05$) (Fig. 3- NCI-N87-C).

For Let-7 expression, compared with the control group, Let-7 expression in the OXA, ACBP and combination groups was not significantly different ($P > 0.05$). Compared with the ACBP group, the OXA and combination groups showed no significant difference ($P > 0.05$) (Fig. 3- NCI-N87-D).

In conclusion, in NCI-N87 cells, lin28 expression was upregulated in the ACBP and OXA groups and downregulated in the combination group. There were no significant differences in miR-107, miR-609 and Let-7 expression in the OXA, ACBP and combination groups compared with the control group.

3.5 The expression of Lin28, miR-107, miR-609 and Let-7 in GES-1 cells

To investigate the effect of OXA, ACBP and the combination on Lin28, miR-107, miR-609 and Let-7 expression in GES-1 cells, GES-1 cells were treated separately with serum-free medium, 3.6 μg/ml OXA, 25 μg/ml ACBP, and 1.8 μg/ml OXA in combination with 12.5 μg/ml ACBP. After 48 h of treatment, cells were harvested, and RNA was extracted to determine the relative expression levels of Lin28, miR-107, miR-609 and Let-7 by q-PCR.

Compared with that in the control group, Lin28 expression in the OXA, ACBP and combination groups was significantly different ($P < 0.05$). Compared with the OXA group and ACBP group, the combination group exhibited significant differences ($P < 0.05$) (Fig. 3- GES-1-A).

Compared with that in the control group, miR-107 expression in the ACBP and combination groups was significantly different ($P < 0.05$). Compared with the OXA group and ACBP group, the combination group exhibited a significant difference ($P < 0.05$) (Fig. 3- GES-1-B).

Compared with that in the OXA group, the expression of miR-609 in ACBP showed a significant difference ($P < 0.05$). The combination group showed a significant difference compared with the ACBP group ($P < 0.05$). Moreover, the expression of Let-7 in the combination and OXA groups was significantly different from that in the ACBP group ($P < 0.05$) (Fig. 3- GES-1-C, D).

In conclusion, the expression of Lin28 in GES-1 cells in the ACBP, OXA and combination groups was reduced, and the reduction was more significant in the OXA group. miR-107 expression was elevated in the ACBP group compared with the control group but was decreased in the combination group and exhibited no significant change in the OXA group. miR-609 expression was significantly reduced in the OXA, ACBP and combination groups compared with the control group. Let-7 expression was increased in the ACBP group compared with the control group but was decreased in both the OXA and combination groups.

3.6 Lin28 protein expression in MKN-45 and SGC7901 cells after drug treatment

To investigate the effect of OXA, ACBP and the combination on Lin28 protein expression in MKN-45 and SGC7901 cells, after 48 h, total protein was extracted, and the relative expression level of the Lin28 protein was determined by western blot analysis. The Lin28 protein in each treatment group showed a band at 26 kD, and β-actin showed a band at 42 kD.

The relative expression levels of the Lin28 protein in each treated group of MKN-45 cells and SGC7901 cells show that in MKN-45 cells, lin28 protein expression was downregulated in the OXA, ACBP and combination groups. Lin28 protein expression in SGC7901 cells was upregulated both in the OXA and combination groups, and Lin28 protein expression in SGC7901 cells was slightly upregulated in the ACBP group (Fig. 4- A, B and C).

3.7 Lin28 protein expression in NCI-N87 and GES-1 cells after drug treatment

To investigate the effect of OXA, ACBP and the combination on Lin28 protein expression in NCI-N87 and GES-1 cells, after 48 h, total protein was extracted, and the relative expression level of the Lin28 protein was determined by western blot analysis. The results showed that the Lin28 protein was visible as a band at 26 kD, whereas this band was unclear in GES-1 cells. The relative expression levels of the Lin28 protein in each treated group of NCI-N87 cells showed that in NCI-N87 cells, Lin28 protein expression was downregulated in the ACBP and combination groups but was upregulated in the OXA group (Fig. 4- D and E).

3.8 Effect of mouse living status and feeding, and tumor growth

From the Figure.5, during the experiment, nude mice in the ACBP and MIX group and NS group showed large feeding and activity, and increased body quality, but the difference was not statistically significant ($P > 0.05$). In the OXA group and the NS group, the diet and activity volume changed little, and the body quality was reduced, but the difference was not significant ($P > 0.05$). However, compared to the status of OXA, there were large diet and activity, and increased body quality in the ACBP and MIX groups ($P < 0.05$). The ACBP, OXA and MIX had mild tumor mass compared to control NS and showed statistical difference in tumor quality ($P < 0.05$), no statistical difference between ACBP, OXA ($P > 0.05$) and significant difference between MIX and NS ($P < 0.05$).

3.9 Immunohistochemical results

In the Figure.6, Immunohistochemistry results showed that Lin28 protein exxpressuin are decreased significantly difference in the ACBP and OXA groups, MIX, compared with NS group ($P < 0.05$), but there was no difference between ACBP and OXA ($P > 0.05$). In addition, the expression of Lin28 in ACBP and OXA shows significantly difference compared with MIX ($P < 0.05$).

3.10 The tumor-related Lin28 gene expression

The expression of Lin28 in each group was detected by quantitative PCR (Figure.7), which showed that decreased expression in ACBP and OXA groups compared with NS groups ($P < 0.05$); but there was no statistical difference between ACBP and OXA groups ($P > 0.05$). while the expression of Lin28 in ACBP and OXA shows significantly difference compared with MIX ($P < 0.05$). (Figure.7A).

3.11 miRNA107 expression

The expression of miRNA-107 in each group was detected by quantitative PCR, which showed that increased expression in ACBP, OXA and MIX compared with NS ($P < 0.05$); but there was a statistical difference between ACBP and OXA groups ($P < 0.05$), while higher expression of miRNA-107 in ACBP than OXA group. Furthermore, the expression of miRNA-107 in ACBP and OXA shows significantly difference compared with MIX ($P < 0.01$) (Figure.7B).

4. Discussion

According to the 2015 statistics, there were approximately 4292000 new cancer cases and 2814000 cancer deaths in 2015 [20]. Gastric cancer had the second-highest incidence [20]. Exploring and developing new anticancer drugs with low toxicity and therapeutic efficiency is a hot topic worldwide. Antitumor peptides have become a hot topic of tumor therapy research in the 21st century. At the cellular, molecular and animal levels, ACBPs isolated from animal organs have been shown to enhance immune function, exhibit targeted antitumor effects, and inhibit the colony formation of gastric cancer stem cells.

The mechanism involves the induction of tumor cell apoptosis and interference with tumor DNA synthesis to inhibit tumor cell proliferation. A previous Dutch study of ACBP and cisplatin in a nude mouse model of human gastric cancer found that ACBP and cisplatin inhibited Bcl-2 protein expression and induced BAX, caspase 3 and caspase 8 protein expression.

In addition, anticancer bioactive peptide-3 (ACBP-3) was found to inhibit gastric cancer by inhibiting the proliferation of gastric cancer stem cells. ACBP-3 can accelerate the time-dependent apoptosis of SC-derived cells to induce cancer cell death and exert its anticancer role [21]. Via in vitro cell experiments, we studied the effects of an ACBP on the expression of the stem cell marker gene Lin28, which is involved in the control of tumorigenesis and tumor development, and investigated its regulation of microRNA expression and mutual relationships to explore the role of ACBP in biological treatment and inhibition of tumor cell proliferation, aiming to provide rich research data and a scientific basis. Lin28 is a well-established stem cell marker gene that is highly expressed in multiple tumor cells, and its high expression predicts short survival times and poor prognosis [22]. Furthermore, it was shown that treatment with Lin28, Oct4, Sox2 and Nanog induced the transformation of fibroblasts into pluripotent stem cells [23, 24]. While Lin28 expression is high in mesenchymal cells and only slightly detectable in epithelial cells, high Lin28 expression promotes epithelial-to-mesenchymal transition (EMT). However, EMT is closely related to in situ tumor invasion and distant metastasis. Lin28 promotes the transformation of epithelial cells to stromal cells and can promote tumor metastasis.

The lin28 protein is present in the cytoplasm and nucleus, and it has been shown that Lin28 can regulate microRNAs such as miR-107 and Let-7 [25]. Lin28 can bind to the terminal loop of Let-7 miRNA family precursors to inhibit processing by Drosha and Dicer and thus inhibit precursor processing into a mature microRNA [26, 27]. MiR-107 is involved in a variety of important biological pathways and cellular stress responses, and its abnormal expression can lead to the development of multiple diseases, including tumors, and affect prognosis. MiR-107 plays an inhibitory role in lung and head and neck squamous cancers while promoting pancreatic, liver, esophageal and colorectal cancers. Furthermore, the role of miR-107 in breast and gastric cancers is still controversial. Thus, the specific role of miR-107 in cancer has not been determined, and the specific mechanism of action is complex and diverse, requiring further research.

To investigate the effect of ACBP, OXA and the combination on the Lin28, miR-107, Let-7 and miR-609 gene and Lin28 protein expression levels in four cell lines, we performed q-PCR and western blot analyses. The results showed that Lin28 gene expression was upregulated in MKN-45 cells treated with OXA, ACBP and the combination and was most significantly upregulated in the OXA group. However, Lin28 protein expression was downregulated in the OXA, ACBP and combination groups. It has been shown that Lin28 is a stem cell marker gene that acts as an oncogene [28].

This study demonstrated that miR-107, miR-609 and Let-7 expression was downregulated by the drug combination, that ACBP downregulated Let-7 expression, and that OXA downregulated miR-609 expression, which showed that the changes in Lin28 gene expression were opposite those in the

expression of these three microRNAs, that Lin28 can target these microRNAs for knockdown, and that these microRNAs may also in turn regulate Lin28 expression. Regarding the role of Let-7 in tumors, Let-7 has been confirmed to have a role in inhibiting cancer cell proliferation [29]. However, ACBP downregulated its expression, and the specific mechanism needs to be further studied. The role of miR-107 in gastric cancer is still controversial, and the role of miR-609 in gastric cancer has not been studied [30]. The combination treatment downregulated the expression of miR-609 and miR-107; thus, we hypothesized that both of these miRNAs may play a carcinogenic role in gastric cancer and that inhibition of their expression by ACBP may be one of the mechanisms inhibiting the proliferation of gastric cancer cells. We will conduct in-depth research based on this hypothesis.

SGC7901 cells have a moderately differentiated status, and our results showed that OXA and ACBP downregulated Lin28 gene expression in SGC7901 cells, while OXA, ACBP and the combination upregulated Lin28 protein expression. Lin28 is highly expressed in multiple tumor cells, and its high expression indicates poor patient prognosis [14]. ACBP downregulated Lin28 gene expression in SGC7901 cells, consistent with the above results. ACBP only slightly upregulated Lin28 protein expression, but OXA obviously upregulated it, and the specific mechanism needs further investigation. In addition, OXA, ACBP and the combination can upregulate the expression of the tumor suppressor gene Let-7 and thus play a role in inhibiting cancer cell proliferation [31].

Let-7 expression levels were inversely correlated with Lin28 gene expression levels, suggesting that Let-7 is targeted by Lin28 for regulation. In addition, OXA upregulated miR-107 expression, but ACBP downregulated its expression. From the analysis of our results, it is hypothesized that miR-107 may play a tumor-promoting role in gastric cancer. There is still much debate about the role of miR-107 in gastric cancer [32].

The experimental results in highly differentiated NCI-N87 gastric cancer cells showed that both OXA and ACBP upregulated Lin28 gene expression, while the combination treatment downregulated Lin28 gene expression. Western blot analysis showed that OXA upregulated Lin28 protein expression, while ACBP and the combination downregulated Lin28 protein expression to inhibit tumor cell proliferation. However, the results of this experiment show that the effect of ACBP is different at the protein level compared with the mRNA level, and further research on the relevant signaling process is needed. GES-1 is a tumorigenic [12], immortalized human gastric mucosal epithelial cell line, and Lin28 is highly expressed in growing and developing GES-1 cells; it exhibits high expression in multiple tumor cell lines [33] and comparatively low expression in human gastric mucosal cells. The results of this study indicated that OXA, ACBP and the combination downregulated Lin28 gene expression, but the Lin28 protein expression level was not measured in GES-1 cells due to its low expression in these cells. Whether ACBP is able to downregulate Lin28 gene expression in GES-1 cells to prevent carcinogenesis needs further study. Both OXA and the combination of ACBP and OXA downregulated Let-7 expression, while ACBP alone upregulated Let-7 expression. Therefore, the role of Let-7 as a tumor suppressor gene was studied. However, ACBP upregulates Let-7 expression and enhances its anticancer effect in humans, thus preventing the occurrence of cancer. Moreover, the results showed that OXA, ACBP and the combination treatment

downregulated miR-609 expression. It is hypothesized that miR-609 may play a tumor-promoting role in gastric cancer and that inhibiting its expression can prevent tumorigenesis and inhibit tumor progression and metastasis.

Furthermore, morphological analysis showed that ACBP, OXA and the combination treatment significantly inhibited proliferation and induced apoptosis and necrosis in MKN-45, SGC7901, and NCI-N87 cells, but the ACBP had a weak effect on GES-1 cells, while OXA had a strong inhibitory effect on GES-1 cell proliferation, indicating that the ACBP has no obvious toxic effects on human gastric mucosal epithelial cells relative to OXA.

Therefore, it is speculated that ACBP exerts a strong inhibitory effect on proliferation in gastric cancer cell lines with different differentiation statuses and a stronger inhibitory effect on the proliferation of poorly differentiated gastric cancer cells. At the protein level, ACBP showed an inhibitory effect on the expression of the putative stem cell marker gene Lin28 but exhibited a different effect at the mRNA level, possibly because the modification levels changed after ACBP acted on cells.

Studies show that the Lin 28/miR-107 pathway was found in knocking down MKN-45 and MKN-28 gastric cancer cell lines with siRNA technology, and Lin28/ miR-107 signaling oxaliplatin sensitivity by regulating the expression of cyclin, p-glycoprotein and topoisomerase, and Lin cell-sensitive chemotherapeutic drugs may be a molecular mechanism that causes chemotherapeutic resistance to gastric cancer. However, the Lin28 and miRNA107 signaling pathway are still less frequently studied.

In this study, results showed that decreased gene expression are observed in the ACBP, OXA groups and mix groups compared with the NS group ($P < 0.05$), but there was no statistical difference between the ACBP and OXA groups ($P > 0.05$). The Lin28 gene expression was decreased in the MIX group, comparison between ACBP and OXA groups ($P < 0.05$). It is seen that short-term intraperitoneal injection of ACBP can inhibit the increase of tumor stem cells and also improve the tumor suppressor sensitivity of oxaliplatin. The expression results of miRNA107 showed that the ACBP and oxaliplatin groups were increased compared with the saline group, with significant statistical significance ($P < 0.01$), while statistical differences were showed in the ACBP and oxaliplatin groups ($P < 0.05$), the MIX group shows statistical differences between the ACBP and oxaliplatin groups ($P < 0.01$). This results shows that the anticancer effect of ACBP may be related to the Lin28/miR-107 signaling pathway, for the first time that the attenuated susceptibility mechanism of ACBP is related to Lin28/miR-107 signaling, and that ACBP is involved in the regulation of other genes during the inhibition of gastric cancer cell growth, which deserves further investigation.

The ACBP, OXA, and MIX groups were mild compared to the NS groups, showing a statistical difference in tumor mass ($P < 0.05$), and no statistical difference between the ACBP, OXA, and MIX groups ($P > 0.05$). Tumor mass in the MIX and NS ($P < 0.05$) shows statistical difference ($P < 0.05$). In the quality of life: the nude mice in the OXA group showed a significant weight loss, as compared with the ACBP and MIX groups ($P < 0.05$). The weight of nude mice was not significant between the ACBP and MIX groups ($P > 0.05$). In the ACBP and MIX groups, no significant weight was increased in nude mice than in the NS

group. Eating was significantly increased in the MIX group compared with the OXA group of tumor-bearing nude mice ($P < 0.05$). It was found that nude mice in the ACBP and MIX groups more active than the NS and OXA groups. It can be seen that ACBP can improve the quality of life of nude mice and also reduce the toxic side effects of OXA. In addition, under the combination treatment, the quality of life and anticancer effect of nude mice have been improved.

In conclusion, the analysis showed application of ACBP inhibited gastric cancer MKN-45 tumor growth and improve the quality of life in tumor-bearing nude mice. The application of ACBP in combination with OXA can not only significantly inhibit the growth of tumor in the nude mice, but also reduce the toxic effect of OXA and improve the quality of life of the tumor-bearing organism. Inhibiting tumor growth with ACBP alone, improved quality of life in tumor-bearing nude mice; and short-term interruption of ACBP combined with OXA significantly increased sensitivity, improved quality of life of tumor-bearing nude mice, and reduced toxic side effects of chemotherapies may also be associated with ACBP inhibition of tumor dry cell proliferation through Lin28/miR-107 signaling. At the same time, the first ACBP inhibited tumor growth in Lin28/miR-107 signaling pathway, explains the regulatory relationship between ACBP-tumor stem cell marker gene-miR107, and undoubtedly helps to understand the expression regulation network of tumor cells, provide some reference for studying the anticancer mechanism of ACBP, and provide some theoretical support for whether it can become a new anti-cancer substance in the future.

Conclusions

Our data demonstrates that ACBP alone and in combination with oxaliplatin influenced the expression of tumor stem cell marker gene Lin28 and regulated the expression of microRNAs specifically regulated by Lin28. ACBP alone has a certain effect in inhibiting tumor growth. The combination of ACBP with the chemotherapy drug oxaliplatin has significant anticancer sensitization on oxaliplatin, and also significantly improves the quality of life of tumor-bearing nude mice and reduces the toxic side effects of chemotherapeutic drugs on nude mice. The anti-cancer effect and attenuated sensitization of ACBP may be related to the Lin28/miR-107 signaling pathway, acting by inhibiting the proliferation of cancerous stem cells. Therefore, It is the first proposed that the inhibitory effect of ACBP on gastric cancer MKN-45 cell lines may play a role through the ACBP-tumor stem cell marker gene-miR107 signaling pathway. Therefore, more detailed studies of the regulatory processes of posttranslationally modified proteins are needed to further confirm the antitumor mechanism of ACBP. The completion of this project provides a scientific basis for exploring the antitumor mechanism of ACBP alone and combined with chemotherapeutic drugs.

Abbreviations

ACBP = Anticancer Bioactive Peptides

OXA = oxaliplatin

MIX=ACBP+OXA = Bioactive Peptide Combined with oxaliplatin

Declarations

Ethical Approval and Consent to Participate

The study on animals were approved by the Ethics Committee for Animal Experiments of Inner Mongolia Medical College, China (number:YKD2016152), all procedures reporting in this study on the animals were carried in accordance with the ARRIVE guidelines, and all methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not Applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Authors Contributions

XLS and XL conceived and designed research. LHK and JQP conducted experiments. WYH contributed new reagents or analytical tools. LHK and JQP analyzed data. XL wrote the manuscript. All authors read and approved the manuscript and all data were generated in-house and that no paper mill was used.

Funding

This work was supported financially by the National Natural Science Foundation of China (22168028, 81660468, 81960560), and Inner Mongolia Natural Science Fund surface project (2021MS02005), and the Inner Mongolia Autonomous Region Health Science and Technology Plan Project (202201274).

Acknowledgement

Not Applicable.

References

1. Feng RM, Zong YN, Cao SM, Xu RH: Current cancer situation in China: good or bad news from the 2018 Global Cancer Statistics? Cancer communications (London, England) 2019, 39(1):22.
2. Li ZJ, Cho CH: Development of peptides as potential drugs for cancer therapy. Current pharmaceutical design 2010, 16(10):1180-1189.

3. Sookraj KA, Bowne WB, Adler V, Sarafraz-Yazdi E, Michl J, Pincus MR: The anti-cancer peptide, PNC-27, induces tumor cell lysis as the intact peptide. Cancer Chemotherapy & Pharmacology 2010, 66(2):325.
4. Choi S, Choi J: Effects of the teach-back method among cancer patients: a systematic review of the literature. Supportive Care in Cancer 2021:1-10.
5. Hou JF, Yan MR, Yan XH: EFFECT OF ANTI-CANCER BIOACTIVE PEPTIDE ON LEUKEMIA MICE. Acta Academiae Medicinae Neimongol 2004.
6. Zhao YY, Peng SD, Su XL: [Effects of anti-cancer bioactive peptide on cell cycle in human nasopharyngeal carcinoma strain CNE]. Zhonghua er bi yan hou tou jing wai ke za zhi = Chinese journal of otorhinolaryngology head and neck surgery 2006, 41(8):607-611.
7. Yan MR, Lan SX: Effect of Anti-gastric Cancer Bilogical Peptide on Lactic Dehydrogenase Isoenzyme. Journal of Qilu Oncology 2002.
8. Ashrafizadeh M, Rafiei H, Mohammadinejad R, Farkhondeh T, Samarghandian S: Anti-tumor activity of resveratrol against gastric cancer: a review of recent advances with an emphasis on molecular pathways. Cancer Cell International 2021, 21(1).
9. Moss EG, Lee RC, Ambros V: The Cold Shock Domain Protein LIN-28 Controls Developmental Timing in C. elegans and Is Regulated by the lin-4 RNA. Cell 1997, 88(5):637-646.
10. Thornton JE, Gregory RI: How does Lin28 let-7 control development and disease? Trends in cell biology 2012, 22(9):474-482.
11. Mayr F, Heinemann U: Mechanisms of Lin28-mediated miRNA and mRNA regulation--a structural and functional perspective. International journal of molecular sciences 2013, 14(8):16532-16553.
12. Shinoda G, Shyh-Chang N, Soysa TY, Zhu H, Seligson MT, Shah SP, Abo-Sido N, Yabuuchi A, Hagan JP, Gregory RI et al: Fetal deficiency of lin28 programs life-long aberrations in growth and glucose metabolism. Stem cells (Dayton, Ohio) 2013, 31(8):1563-1573.
13. Viswanathan SR, Powers JT, Einhorn W, Hoshida Y, Ng TL, Toffanin S, O'Sullivan M, Lu J, Phillips LA, Lockhart VL et al: Lin28 promotes transformation and is associated with advanced human malignancies. Nature genetics 2009, 41(7):843-848.
14. Song H, Xu W, Song J, Liang Y, Fu W, Zhu XC, Li C, Peng JS, Zheng JN: Overexpression of Lin28 inhibits the proliferation, migration and cell cycle progression and induces apoptosis of BGC-823 gastric cancer cells. Oncology reports 2015, 33(2):997-1003.
15. Hsu KF, Shen MR, Huang YF, Cheng YM, Lin SH, Chow NH, Cheng SW, Chou CY, Ho CL: Overexpression of the RNA-binding proteins Lin28B and IGF2BP3 (IMP3) is associated with chemoresistance and poor disease outcome in ovarian cancer. British journal of cancer 2015, 113(3):414-424.
16. Ma W, Ma J, Xu J, Qiao C, Branscum A, Cardenas A, Baron AT, Schwartz P, Maihle NJ, Huang Y: Lin28 regulates BMP4 and functions with Oct4 to affect ovarian tumor microenvironment. Cell cycle (Georgetown, Tex) 2013, 12(1):88-97.

17. Wang Q, Zhou J, Guo J, Teng R, Shen J, Huang Y, Xie S, Wei Q, Zhao W, Chen W et al: Lin28 promotes Her2 expression and Lin28/Her2 predicts poorer survival in gastric cancer. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine 2014, 35(11):11513-11521.
18. Xu WP, Yi M, Li QQ, Zhou WP, Cong WM, Yang Y, Ning BF, Yin C, Huang ZW, Wang J et al: Perturbation of MicroRNA-370/Lin-28 homolog A/nuclear factor kappa B regulatory circuit contributes to the development of hepatocellular carcinoma. Hepatology (Baltimore, Md) 2013, 58(6):1977-1991.
19. Yang H, Cho ME, Li TW, Peng H, Ko KS, Mato JM, Lu SC: MicroRNAs regulate methionine adenosyltransferase 1A expression in hepatocellular carcinoma. The Journal of clinical investigation 2013, 123(1):285-298.
20. Wei W, Zeng H, Zheng R, Zhang S, An L, Chen R, Wang S, Sun K, Matsuda T, Bray F et al: Cancer registration in China and its role in cancer prevention and control. The Lancet Oncology 2020, 21(7):e342-e349.
21. Yu L, Yang L, An W, Su X: Anticancer bioactive peptide-3 inhibits human gastric cancer growth by suppressing gastric cancer stem cells. Journal of cellular biochemistry 2014, 115(4):697-711.
22. Kumar MS, Lu J, Mercer KL, Golub TR, Jacks T: Impaired microRNA processing enhances cellular transformation and tumorigenesis. Nature genetics 2007, 39(5):673-677.
23. Walter J, Monthoux C, Fortes C, Grossmann J, Roschitzki B, Meili T, Riond B, Hofmann-Lehmann R, Naegeli H, Bleul U: The bovine cumulus proteome is influenced by maturation condition and maturational competence of the oocyte. Scientific reports 2020, 10(1):9880.
24. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R et al: Induced pluripotent stem cell lines derived from human somatic cells. Science (New York, NY) 2007, 318(5858):1917-1920.
25. Gaytan F, Sangiao-Alvarellos S, Manfredi-Lozano M, García-Galiano D, Ruiz-Pino F, Romero-Ruiz A, León S, Morales C, Cordido F, Pinilla L et al: Distinct expression patterns predict differential roles of the miRNA-binding proteins, Lin28 and Lin28b, in the mouse testis: studies during postnatal development and in a model of hypogonadotropic hypogonadism. Endocrinology 2013, 154(3):1321-1336.
26. Triboulet R, Pirouz M, Gregory RI: A Single Let-7 MicroRNA Bypasses LIN28-Mediated Repression. Cell reports 2015, 13(2):260-266.
27. O'Day E, Le MTN, Imai S, Tan SM, Kirchner R, Arthanari H, Hofmann O, Wagner G, Lieberman J: An RNA-binding Protein, Lin28, Recognizes and Remodels G-quartets in the MicroRNAs (miRNAs) and mRNAs It Regulates. The Journal of biological chemistry 2015, 290(29):17909-17922.
28. Avalle L, Incarnato D, Savino A, Gai M, Marino F, Pensa S, Barbieri I, Stadler MB, Provero P, Oliviero S et al: MicroRNAs-143 and -145 induce epithelial to mesenchymal transition and modulate the expression of junction proteins. Cell death and differentiation 2017, 24(10):1750-1760.
29. Shen H, Yang Y, Zhao L, Yuan J, Niu Y: Lin28A and androgen receptor expression in ER-/Her2+ breast cancer. Breast cancer research and treatment 2016, 156(1):135-147.

30. Zhang M, Wang X, Li W, Cui Y: miR-107 and miR-25 simultaneously target LATS2 and regulate proliferation and invasion of gastric adenocarcinoma (GAC) cells. Biochemical and biophysical research communications 2015, 460(3):806-812.
31. Spence T, Perotti C, Sin-Chan P, Picard D, Wu W, Singh A, Anderson C, Blough MD, Cairncross JG, Lafay-Cousin L et al: A novel C19MC amplified cell line links Lin28/let-7 to mTOR signaling in embryonal tumor with multilayered rosettes. Neuro-oncology 2014, 16(1):62-71.
32. Li X, Zhang Y, Shi Y, Dong G, Liang J, Han Y, Wang X, Zhao Q, Ding J, Wu K et al: MicroRNA-107, an oncogene microRNA that regulates tumour invasion and metastasis by targeting DICER1 in gastric cancer. Journal of cellular and molecular medicine 2011, 15(9):1887-1895.
33. Venugopal N, Yeh J, Kodeboyina SK, Lee TJ, Sharma S, Patel N, Sharma A: Differences in the early stage gene expression profiles of lung adenocarcinoma and lung squamous cell carcinoma. Oncology letters 2019, 18(6):6572-6582.

Figures

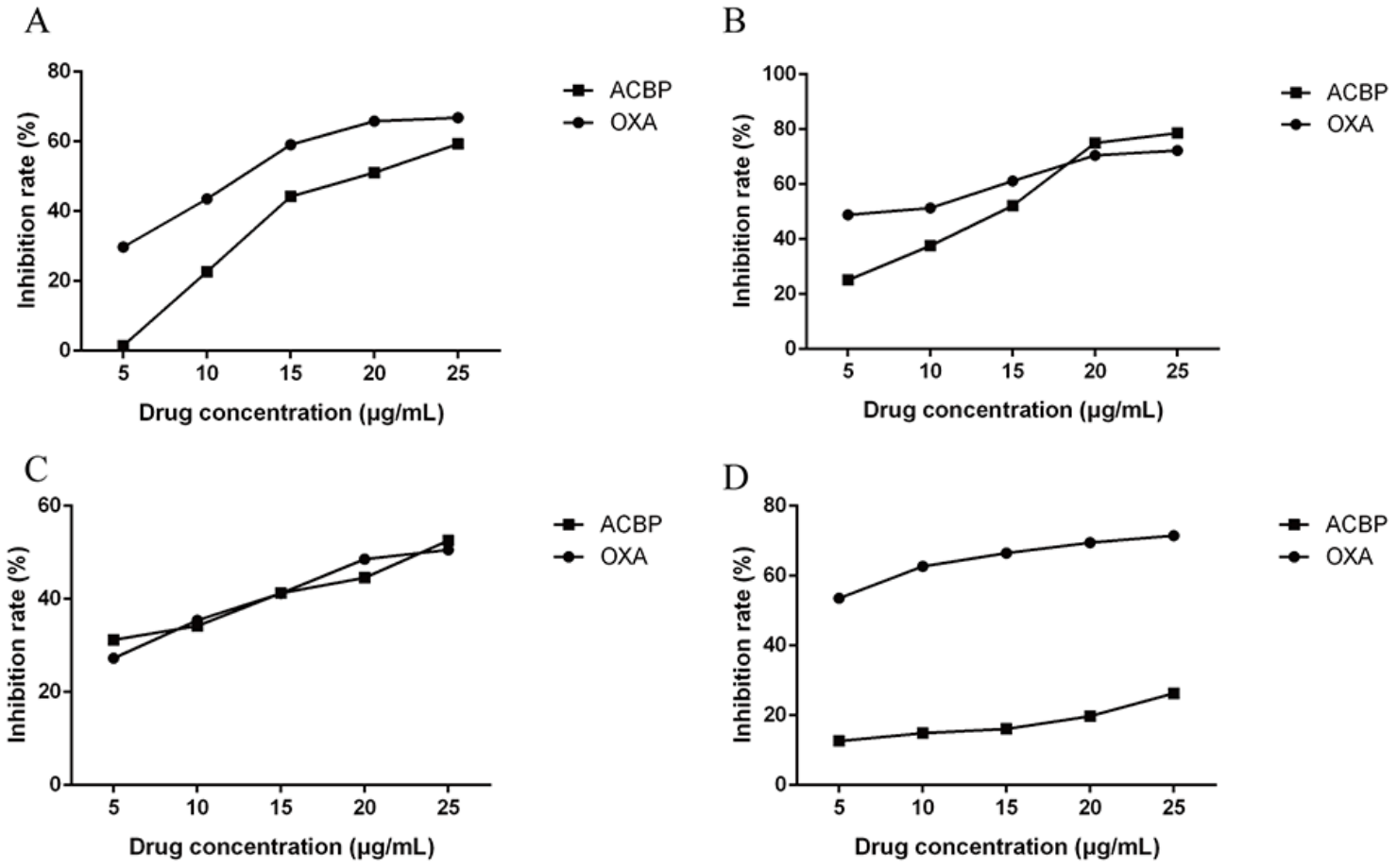

Figure 1

The inhibitory effects of ACBP and OXA at gradient concentrations in four kinds of cells (A: MKN-45 cells; B: SGC7901 cells; C: NCI-N87 cells; D: GES-1 cells).

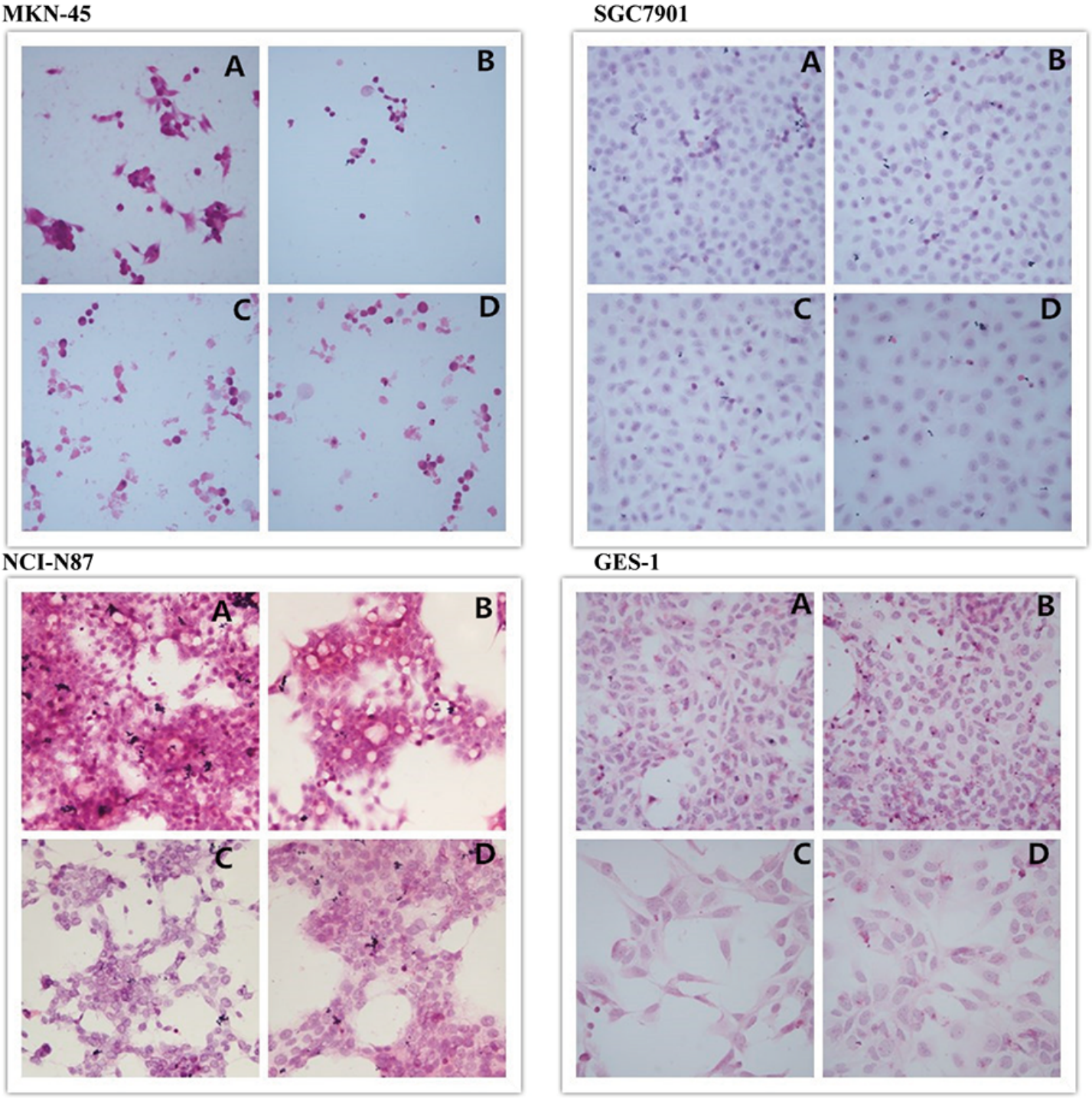

Figure 2

MKN-45 cells: the results of H&E staining in different treatment groups of MKN45 cells SGC7901 cells: the results of H&E staining in different treatment groups of SGC7901 cells NCI-N87 cells: the results of H&E staining in different treatment groups of NCI-N87 cells GES-1 cells: the results of H&E staining in

different treatment groups of GES-1 cells (A: control group, B: ACBP group, C: OXA group, D: combination group; 400× magnification).

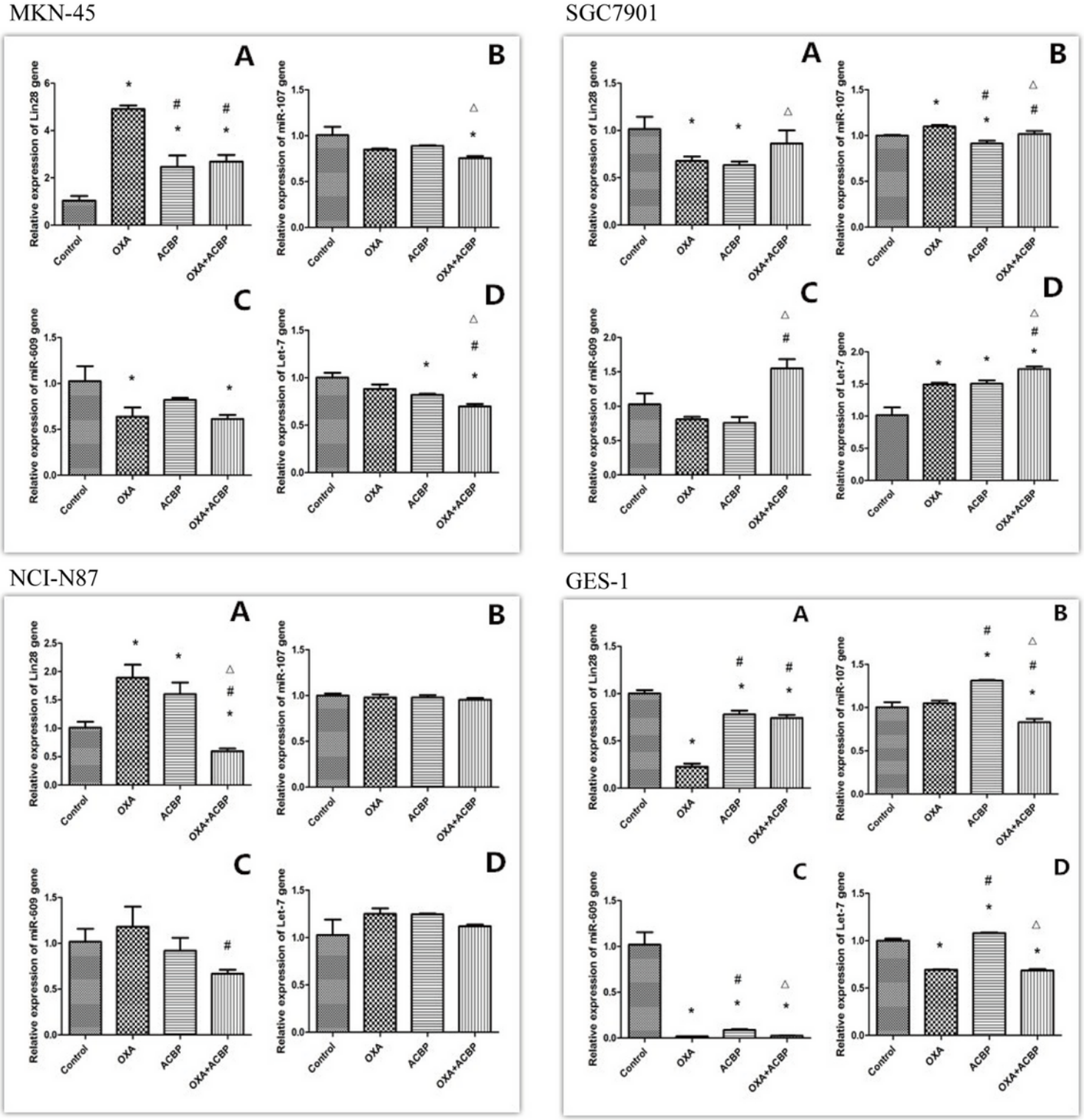

Figure 3

MKN-45 cells: the expression of Lin28, miR-107, miR-609 and Let-7 in different treatment groups of MKN45 cells. SGC7901 cells: the expression of Lin28, miR-107, miR-609 and Let-7 in different treatment

groups of SGC7901 cells. NCI-N87 cells: the expression of Lin28, miR-107, miR-609 and Let-7 in different treatment groups of NCI-N87 cells. GES-1 cells: the expression of Lin28, miR-107, miR-609 and Let-7 in different treatment groups of GES-1 cells (A: Lin28; B: miR-107; C: miR-609; D: Let-7; *: compared with the control group, $P < 0.05$; #: compared with the OXA group, $P < 0.05$; △: compared with the ACBP group, $P < 0.05$).

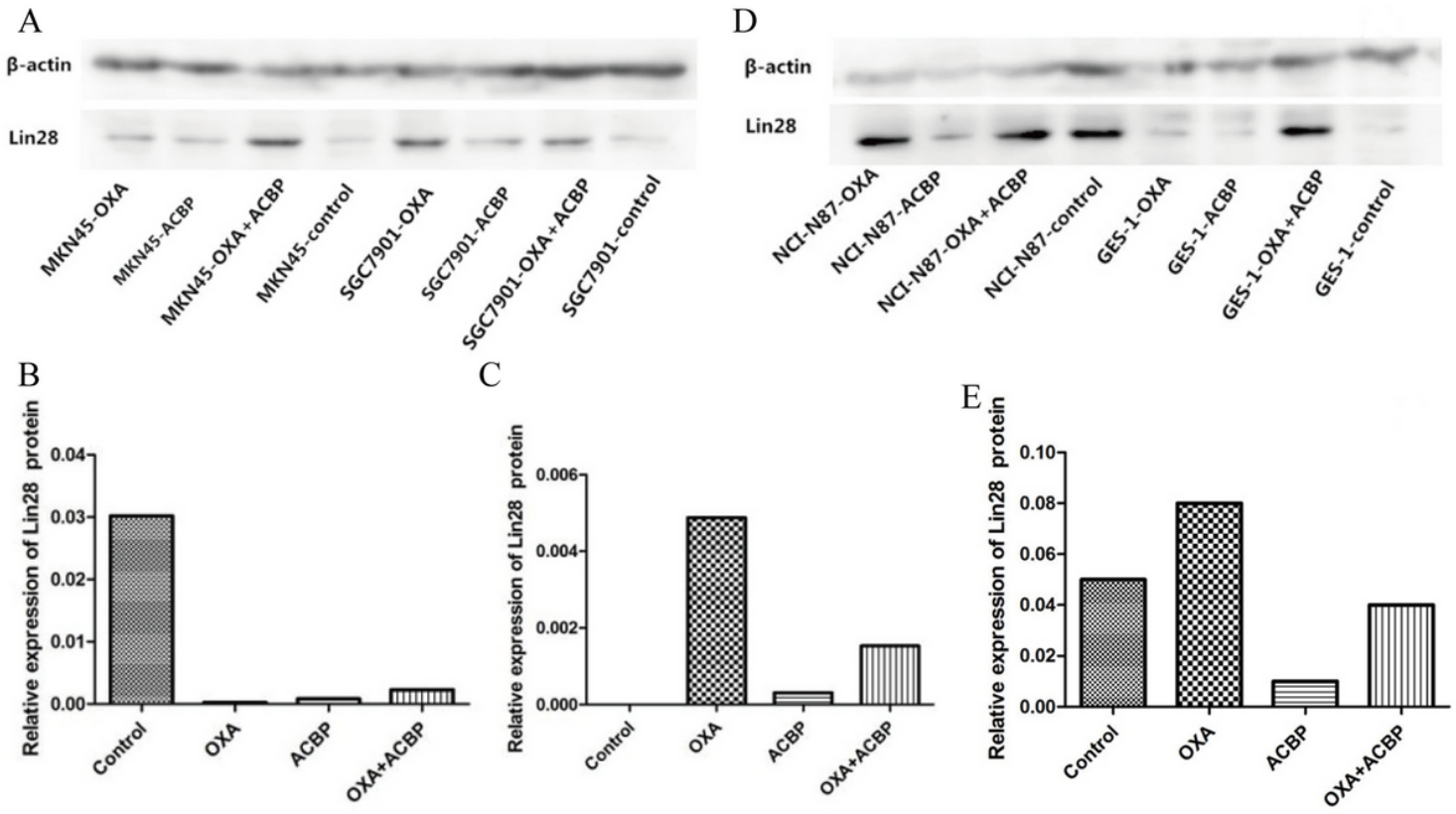

Figure 4

The electrophoresis figure and the expression levels of Lin28 protein in each treatment group of SGC7901 and MKN45 cells (A: electrophoresis figure; B: MKN45 cells; C: SGC7901 cells) and NCI-N87 cells (D: electrophoresis figure; E: NCI-N87 cells).

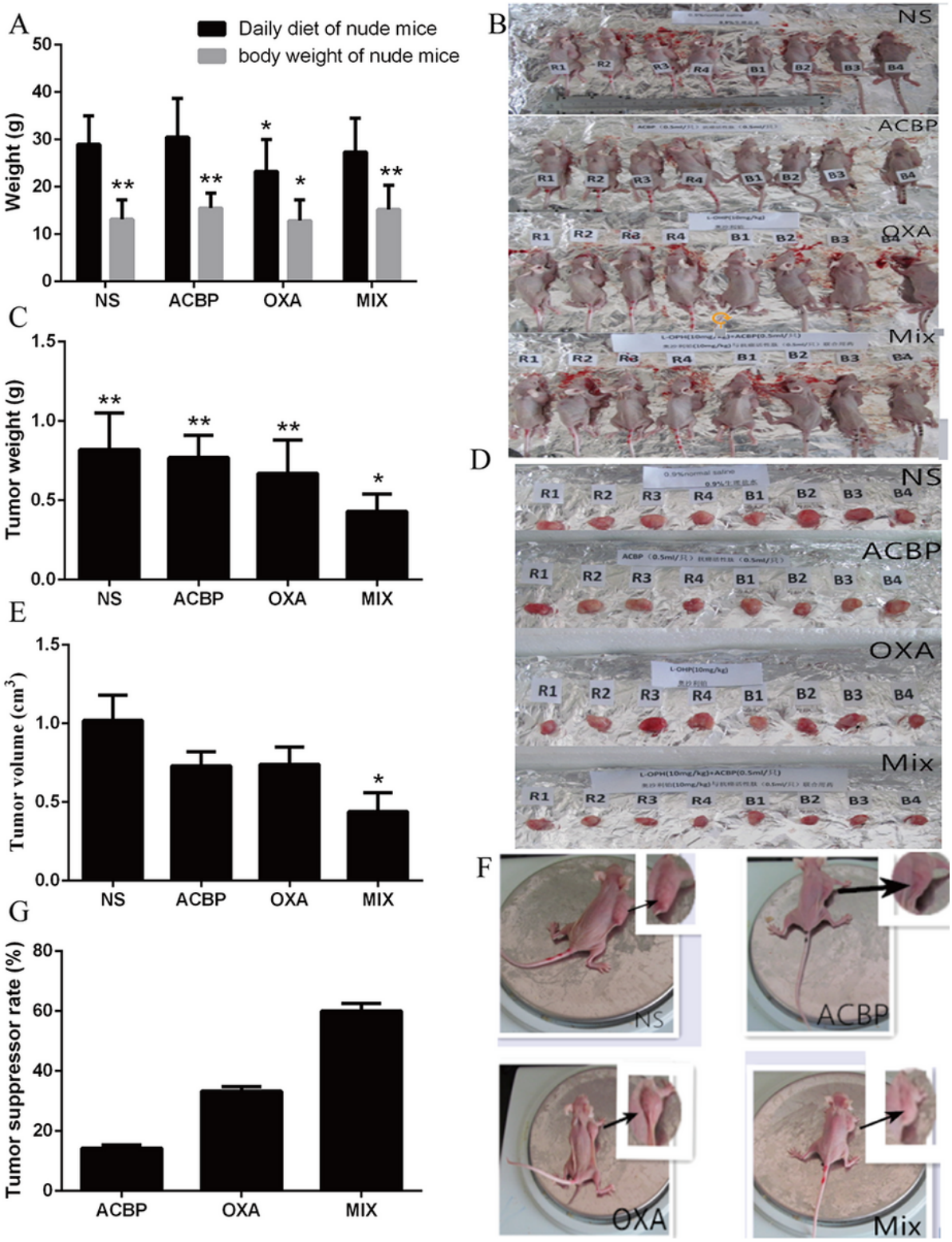

Figure 5

MIX treatment suppresses gastric tumor growth and improves quality of life in the xenograft tumor model. The in vivo tumor growth experiment was established by subcutaneous injection of $1x10^7$ MKN-45 GC cells. A, Daily diet of nude mice and body weight of nude mice, (B) tumor being nude mice were sacrificed, (C) tumor weight, (D) tumor morphology, (E) tumor volume and (F) tumor being nude mice were calculated before the end of the experiment.

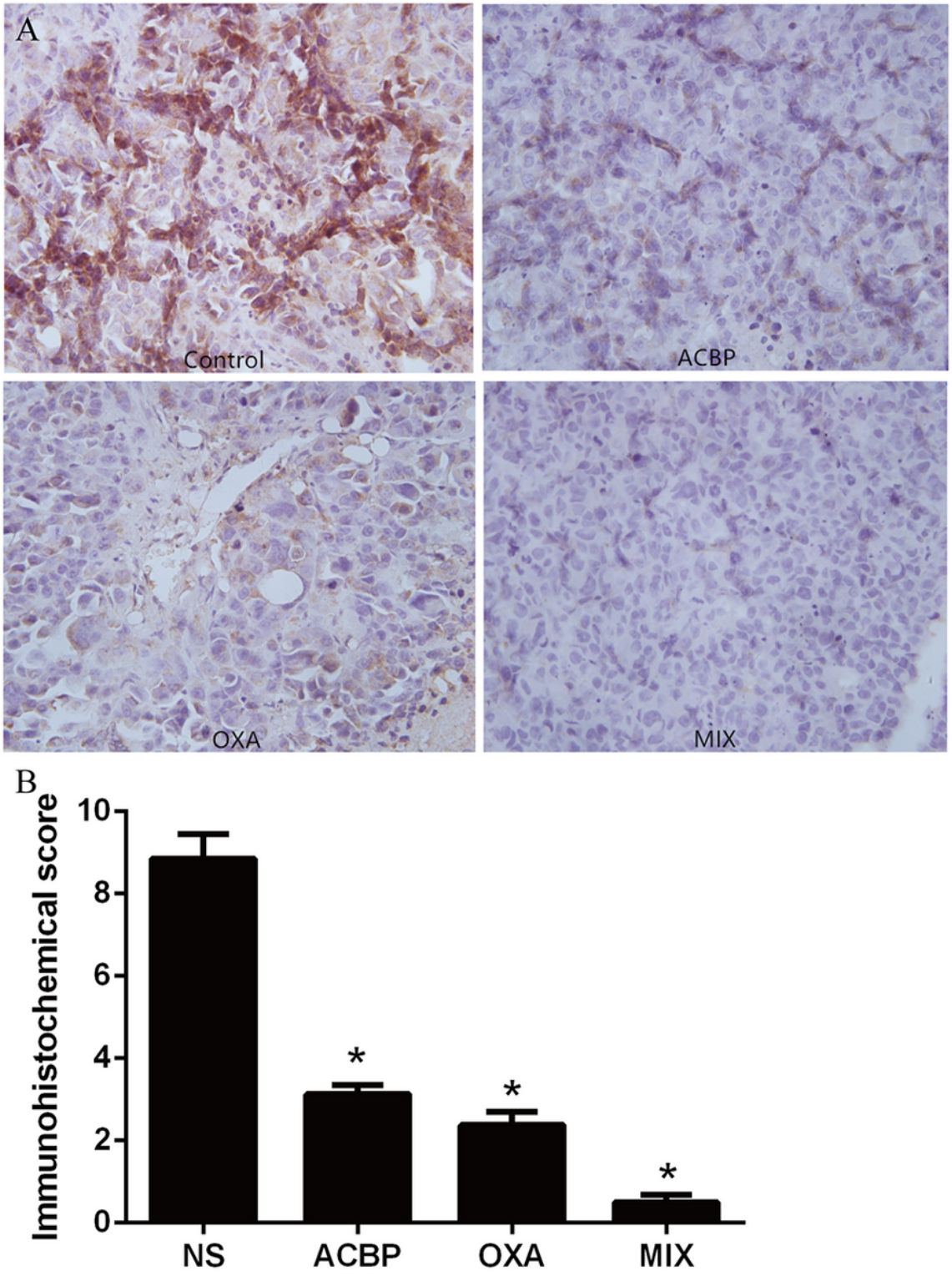

Figure 6

A, Immunohistochemistry staining (x 400) of the tumor of tumor-bearing nude mice treated with Control, ACBP, OXA and MIX. B, Immunohistochemical score were calculated (*: Mix group and OXA group and ACBP group compared with control group, P 0.05).

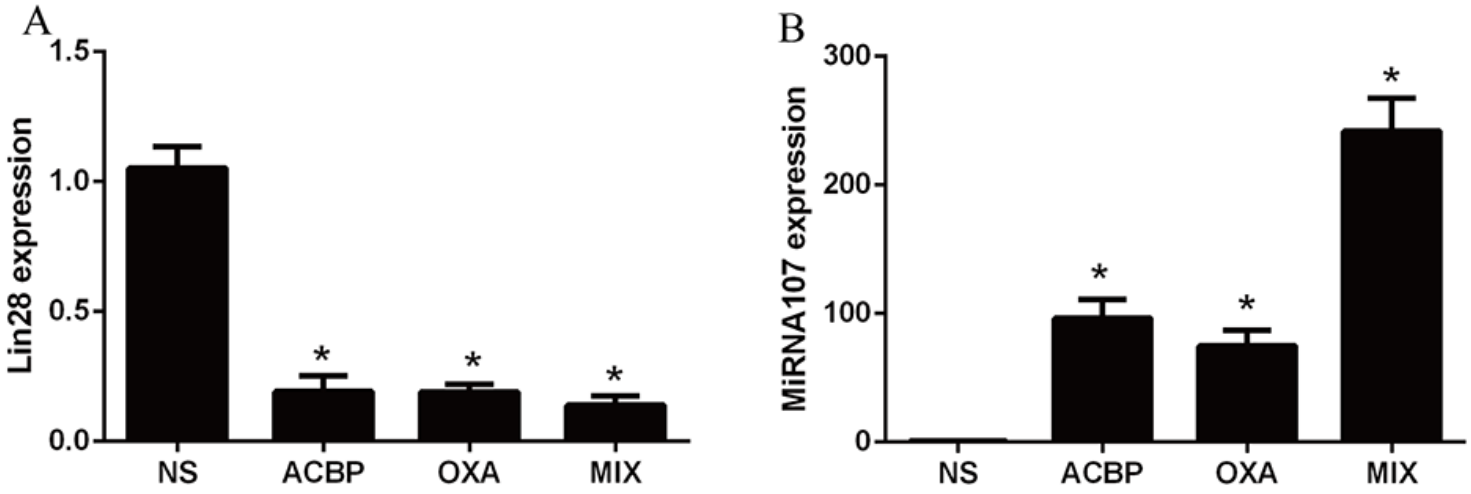

Figure 7

A,The expression of Lin28 in each group was detected by quantitative PCR. B, Comparison of miR-107 gene expression in each group. (*: Mix group and OXA group and ACBP group compared with control group, P 0.05; #: compared with MIX group, P 0.05; ##: compared with MIX group, P 0.01).