Research Article
Fluorescent Mitoxantrone Hydrochloride Nanoparticles Inhibit the Malignant Behavior of Giant Cell Tumor of Bone via miR-125b/PTH1R Axis

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Objective. To explore the therapeutic effects and mechanism of fluorescent mitoxantrone hydrochloride nanoparticles on giant cell tumor of bone. Methods. The adsorption capacity of nanoparticles to hydroxyapatite (HA), cell adsorption capacity, encapsulation rate, particle size, and potential of the nanoparticles were determined by HPLC and Zetasizer Nano ZS nanomicelle potentiometer. MTT assay was used to determine the toxicity of nanoparticles to cells. The fluorescent intensity of the nanoparticles and their location in the cells were observed under a fluorescence microscope. RT-qPCR and Western blotting were then used to measure the expression levels of miRNA, mRNA, and proteins in cells. Transwell and Annexin V-FITC/PI staining tests were used to study the cell invasion and apoptotic rate, respectively. The dual-luciferase reporter gene experiment was then carried out to verify the binding relationship between miR-125b and its predicted target. Results. ALN-FOL-MTO-NLC nanoparticles showed a stronger adsorption capacity for HA and stronger toxicity to GCTB28 cells. Compared to normal tissues, the expression level of miR-125b in giant bone tumor tissue and cells was significantly downregulated, and the expression level of miR-125b was upregulated to some extent after treatment. Overexpression of miR-125b or treatment of ALN-FOL-MTO-NLC nanoparticles can inhibit the malignant behavior of GCTB28 cells, whereas the inhibition of the expression of miR-125b can promote the malignant behavior of GCTB28 cells. The result showed that parathyroid hormone receptor 1 (PTH1R) was a downstream target gene for miR-125b. Rescue experiment showed that the treatment of GCTB28 with ALN-FOL-MTO-NLC nanoparticles while inhibiting miR-125b expression can reduce the inhibitory effect of miR-125b on the malignant behavior of GCTB28 cells, whereas upregulating the expression levels of miR-125b and PTH1R in GCTB28 cells had no significant effect on the malignant behavior of GCTB28 cells. Conclusion. ALN-FOL-MTO-NLC nanoparticles have a certain inhibitory effect on the malignant behavior of giant cell tumor of bone through the miR-125b/PTH1R molecular axis.

1. Introduction

Giant cell tumor of bone (GCT) is a bone tumor with high invasiveness and osteolytic nature [1] with potential malignancy [2]. GCT is mainly composed of three kinds of cells, including bone cell-like multinucleated giant cells, spindle-like stromal cells, and monocytes [3]. At present, the preferred treatment for GCT is local treatment with assisted surgery, but this treatment has a higher postoperative recurrence rate [4]; therefore, it is important to find a new way for GCT treatment. Considering the structural characteristics of human tissues, nanoparticles have good advantages in size, can interact with the biological components of the human body, and regulate various biological behaviors of the human body [5]. Nanomaterial-based drug delivery systems are smaller in size, giving them quantum size effects, interfacial effects, and macrochain tunneling effects. Therefore, the nanodrug showed strong permeability, greater solubility, better adsorption, and so on in biology. Due to the small size and large surface area of nanoparticles, nanodrugs are easy to pass...
through blood vessels in vivo and are not easy to cause damage to the inner wall of blood vessels. In addition, the nanoparticles have the advantages of high surface activity, many active centers, and good catalytic efficiency, so nanodrugs can be prepared into sustained-release drugs, change the half-life of drugs in vivo, and extend the action time of drugs. Based on the above advantages, the drug delivery system based on nanomaterials can have better targeting, so that the nanodrug-carrying system can pass through the biological barrier and reach the lesion area [6]. Not surprisingly, a large number of studies have shown that drug delivery systems based on nanomaterials can be used to treat a variety of orthopedic diseases that are difficult to treat with conventional clinical therapies, such as arthritis, osteoporosis, bone cancer, and related bone diseases [7–11]. Although nanoparticles have certain advantages in the treatment of bone diseases, there are still several problems, such as the low drug loading of bone-targeted macromolecular carriers and its easy excretion through the kidney.

Mitoxantrone hydrochloride (Mitoxantro, MTO) is usually used as an anticancer drug in cancer treatment. Shi et al. took MTO as a model drug and developed a new kind of nanodrug-carrying system, and the results of in vivo and in vitro experiments confirmed that the nanodrug-carrying system has a good long-circulation effect and an increased ability to target bone tissue [12]. However, the above research only discussed the theoretical advantages of the new nanodrug-loading system and does not study whether the nanodrug-loading system can improve the therapeutic effect on bone tumors.

MicroRNA (miRNA, miR) is a group of endogenous noncoding RNAs with a length of 20 to 25 nucleotides. It can negatively regulate the expression of mRNA by inhibiting the translation of mRNA or reducing the stability of mRNA [13]. Studies have shown that miRNA can play an important role in many biological processes. Parathyroid hormone-related protein (PThRP) plays a pivotal role in the development of bones [14], and its expression in various cancer tissues and cells is also significantly upregulated [15]. PTH1R as a PThRP receptor is also positively expressed in various cancers and cells [16]. In the previous research, PTH1R was found to be the target gene of miR-125b through the prediction of biological gene database. Therefore, in this article, we will discuss the treatment effects of GCT with the nanodrug-loaded particles at the cellular level and verify the mechanism of action of miR-125b/PTH1R in GCT.

2. Materials and Methods

2.1. Experimental Materials

2.1.1. Tissues and Cells. Bone giant cell tumor tissues and corresponding paracancerous tissues were collected from patients who were diagnosed and operated in our hospital from October 2010 to October 2019. This study was approved by the ethics committee of our hospital. Both patients and their families knew the purpose and significance of this study and signed the informed consent. MG-63 cells and hFOB1.9 cells were purchased from Tongpai (Shanghai) Biotechnology Co., Ltd. GCTB28 cells were purchased from China Center for Type Culture Collection.

2.1.2. Experimental Reagents. Phospholipid, glyceryl trilaurate, cholesterol, mitoxantrone hydrochloride, sodium lauryl sulfate, methanol, methylene chloride, FOL-S100, ALN-S100, hydroxyphosphorus lime, coumarin-6, dimethyl sulfoxide, and polyoxyethylene castor oil were all purchased from Shanghai Houcheng Fine Chemical Co., Ltd. RPMI 1640 medium, Annexin V-FITC/PI kit, and QuikChange site-directed mutation kit were purchased from Shanghai Kemin Biotechnology Co., Ltd. The MTT test kit was purchased from Shanghai Yiyian Biotechnology Co., Ltd. The RT kit (reverse transcription kit) was purchased from Shanghai Zeye Biotechnology Co., Ltd. RIPA lysate was purchased from Shanghai Yuanye Biotechnology Co., Ltd., and the dual-luciferase reporter gene kit was purchased from Beijing Baierdi Biotechnology Co., Ltd.

2.2. Preparation of Mitoxantrone Hydrochloride Nanoparticles. 250 mg of a phospholipid, 25 mg of glyceryl trilaurate, 25 mg of cholesterol, 10 mg of mitoxantrone hydrochloride, and 10 mg of sodium lauryl sulfate were weighed and added to a 250 mL eggplant-shaped bottle, and 10 mL of methanol-dichloromethane (volume ratio 1:1) mixture was then added. The solution was then distilled at 37°C under reduced pressure and vacuum dried overnight. After that, 10 mL of 2% polyoxyethylene castor oil aqueous solution was added and hydrated at 37°C for 30 min. It was then immersed in an ice bath (200 W) for 300 times and squeezed through a 0.22 μm filter to obtain toxantrone hydrochloride nanoparticles (MTO-NLCs).

To obtain the ALN-FOL-MTO-NLCs, 20 mg of folic acid-polyoxyethylene monostearate (FOL-S100) and 100 mg of alenic acid-polyoxyethylene monostearate (ALN-S100) were added to the eggplant-shaped flask before distillation under reduced pressure. The subsequent steps were the same as above, and the double-ligand modified nanoparticles (ALN-FOL-MTO-NLCs) were then obtained.

2.3. Measurement of Nanoparticle Encapsulation Rate, Particle Size, and Potential. 0.1 mL of nanoparticle was added with 0.4 mL of distilled water for dilution, which was then centrifuged at 8000 runs/min for 5 min. The encapsulation rate was then calculated based on the drug content in supernatant and nanoparticle determined by HPLC. 0.1 mL of nanoparticle preparation was added in 3.9 mL of distilled water for dilution. Particle size and potential of nanoparticles were then determined with the Zetasizer Nano ZS nanomicro cell potentiometer.

2.4. Identification of HA Adsorption Capacity of Nanoparticles. The nanoparticles were diluted with distilled water to make the drug content reach 0.1 mg/mL. Two grams of hydroxyapatite (hydroxylapatite, HA) was then weighed and added into 50 mL of the diluted nanoparticle preparation and stirred at room temperature for 60 min, which was then sampled every 15 minutes. After filtering the sample through
a 0.45 μm filter membrane, the drug content in the unab-
sorbed nanoparticles and the diluted nanoparticle prepara-
tion in the filtrate were determined by HPLC, and the
adsorption ratio of the nanoparticles was then calculated.

2.5. Cellular Adsorption Capacity of Nanoparticles. Instead of
mitoxantrone hydrochloride and sodium lauryl sulfate,
coumarin-6 (C6) was used to prepare the C6-coated nano-
particles according to the method in Section 2.2. GCTB28
cells in the logarithmic growth phase were seeded in 96-
well plates at a density of 1 × 10^5 per well and incubated at
37°C for 24 h. After discarding the culture solution, the nano-
particle preparation diluted with RPMI 1640 medium with-
out folic acid was added, the cells were then incubated at
different temperatures for 4 h and washed 3 times with PBS
after the incubation. After lyzing the cells, the BCA kit and
HPLC were then used to determine the concentration of pro-
tein and C6 in the sample. The uptake index was calculated
according to the formula: uptake (%) = intracellular C6
concentration/intracellular protein concentration × 100.

2.6. Determination of Cytotoxicity of Nanoparticles. GCTB28
cells in the logarithmic growth phase were seeded in 96-well
plates at a density of 5 × 10^5 per well. MTO-NLCs and ALN-
FOL-MTO-NLCs were diluted with folic acid-free RPMI
1640 medium and added to each well, and the cells were then
incubated for 48 h. After that, each well was rinsed 3 times
with 37°C HBSS, and 20 μL of MTT solution was then added
to each well. After incubation for another 4 h, 150 μL of
DMSO was then added. A microplate reader was then used
to measure the absorbance of each well at 570 nm after the
solution was thoroughly mixed.

2.7. Cell Transfection. Cells in the logarithmic growth phase
were taken and prepared into a cell suspension, seeded in
96-well plates at a density of 1 × 10^5 per well, and cultured at
37°C for 24 h. The Liposome 2000 kit instructions were
then strictly followed for cell transfection, and the transfection
efficiency was determined with the RT-qPCR experiment.

2.8. RT-qPCR. The total RNA in the cells was extracted with
the TRIzol kit, and then reverse transcription was performed
using the reverse transcription kit according to the reaction
system shown in Table 1. The expression levels of miRNA
and mRNA in cells were quantitatively measured based on
the reaction system shown in Table 2. The U6 and GAPDH
were used as internal references for miRNA and mRNA,
respectively. The setting for PCR was as follows: 40 total
cycles, 95°C for 10 min, 95°C for 30 s, 58°C for 30 s, 72°C for
10 s, and 72°C for 10 min.

2.9. MTT Experiment. Cells in the logarithmic growth phase
of each group were pipetted into single cells in a serum-free
medium for suspension culture. Cells were then seeded in
96-well plates at a density of 5 × 10^3 cells per well. After dif-
ferent treatments, 20 μL of MTT solution dissolved in PBS
was added. After incubating at 37°C for 4 h, the culture
medium in each well was discarded, and 150 μL of DMSO
was added and thoroughly mixed. The absorbance of each
well at 570 nm was then measured with a microplate reader.

2.10. Transwell Experiment. Cells in the logarithmic growth
phase were seeded on the upper layer of the Transwell cham-
er coated with Matrigel gel, and the conventional medium
was added to the lower layer of the Transwell chamber. After
24 h, the cells were fixed with a mixture of acetic acid and
formaldehyde for 15 min, which was then rinsed three times
with the PBS buffer. The cells that did not pass through the
chamber were then gently wiped out with a cotton swab.
Cells that passed through the chamber were then stained with
crystal violet and washed three times with PBS buffer. To
determine the cell invasiveness, the stained cells were then
put under a microscope, and a number of field views were
randomly selected to take pictures.

2.11. Western Blotting. Cells were lysed with RIPA lysate, and
the proteins in the cells were separated using 10% SDS-
PAGE, which was then transferred to PVDF membranes.
The PVDF membranes were then blocked and subsequently
incubated with primary antibody (PTH1R, 1 : 1000) at 4°C
overnight. After washing with PBS, the PVDF membranes
were incubated with secondary antibody (1 : 5000) for 1 h at
room temperature. Using GAPDH as an internal parameter,
ImageJ was then used to determine the gray value of the
band.

2.12. Annexin V-FITC/PI Double-Staining Experiment. After
treating the cells of each group according to the experimental
design, the cell culture was centrifuged at 3000 runs/min for
5 min, and the supernatant was discarded. The pellet was
then rinsed with PBS for 3 times. The collected cells were
then seeded in a 96-well plate at a density of 1 × 10^5 per well
and incubated for 12 h. The Annexin V-FITC/PI double-
staining experiment was then carried out according to the
instructions of Annexin V-FITC/PI Apoptosis Detection
Kit. The apoptotic rates of each group of cells were then mea-
sured with flow cytometry.
2.13. Dual-Luciferase Reporter Gene Experiment. The PTH1R 3′-UTR fragment was amplified and cloned into a vector to construct wild-type PTH1R (PTH1R-wt). And the mutant PTH1R (PTH1R-mut) was constructed using the QuikChange site-directed mutation kit. The liposome transfection change site-directed mutation kit was then used to transfect miR-NC or miR-125b mimics into GCTB28 cells. After incubation for 48 h, the luciferase activity was determined using the dual-luciferase reporter gene system.

2.14. Localization of Drugs in Cells via Immunofluorescence Microscopy. GCTB28 cells in the logarithmic growth phase were inoculated in a special laser confocal Petri dish. After 24 h of incubation, ALN-FOL-MTO-NLC nanoparticles were added and further incubated for another 1 h. After that, the medium was removed, and the cells were rinsed with PBS twice. An immunofluorescence microscope was then used to detect the fluorescence intensity and localization of ALN-FOL-MTO-NLCs in the cells.

2.15. Statistical Analysis. GraphPad Prism 8.0 was used for drawing and statistical analysis. The t-test was used for the comparison between two groups, and the single-factor analysis of variance was used for the comparison between multiple groups. \( P < 0.05 \) was used to indicate that the difference is statistically significant.

3. Results

3.1. Preparation and Identification of Mitoxantrone Hydrochloride Nanoparticles. Microscopic examination results showed that MTO-NLCs and ALN-FOL-MTO-NLCs were spherical and regular in shape. The test results showed that the encapsulation rate, particle size, and potential of MTO-NLCs were 99.37 ± 0.26%, 50.62 ± 3.34 nm, and −3.42 ± 1.13 mV, respectively; the encapsulation rate, particle size, and potential of ALN-FOL-MTO-NLCs were 99.87 ± 0.17%, 46.18 ± 2.53 nm, and −17.11 ± 2.24 mV, respectively. The HA adsorption test showed that compared with MTO-NLCs, the adsorption capacity of ALN-FOL-MTO-NLCs on HA was significantly enhanced, and with the increase of the modification of ALN-S100 and FOL-S100, the adsorption capacity of nanoparticles on HA also increased (\( P < 0.001 \)), as shown in Figure 1(a). The results of cell uptake experiments showed that compared with C6-NLCs, the uptake of ALC-FOL-C6-NLCs by GCTB28 cells was higher (\( P < 0.01 \)), as shown in Figure 1(b).

3.2. The Effects of Mitoxantrone Hydrochloride Nanoparticles on GCTB28 Cells. The cytotoxicity test results showed that ALN-FOL-MTO-NLCs in GCTB28 cells showed stronger cytotoxicity (\( P < 0.05 \)) than MTO-NLCs, as shown in Figure 2(a). After the treatment of GCTB28 cells with ALN-FOR-MTO-NLC nanoparticles, the red fluorescence of the drug can be observed and mainly distributed in the cytoplasm, as shown in Figure 2(b). Based on the above results, we believe that ALN-FOL-MTO-NLCs have a better inhibitory effect on the malignant behavior of GCTB28 cells.

The results of RT-qPCR experiments showed that the expression of miR-125b in MG-63 cells and GCTB28 cells was significantly downregulated (\( P < 0.05 \)), consistent with the results of Fei et al. [17]. And the expression level of miR-125b in hFOB1.19 cells was 4.82 times than that of GCTB28 cells, as shown in Figure 2(c). The expression of miR-125b in normal tissues was 5.16 times higher than that of cancer tissues, as shown in Figure 2(d). After GCTB28 cells were treated with nanoparticles, the expression levels of miR-125b in the MTO-NLCs group and ALN-FOL-MTO-NLCs group were 3.67 and 4.89 times higher than that of the control group, respectively, as shown in Figure 2(e), which is consistent with our predictions; therefore, ALN-FOL-MTO-NLCs were selected for the subsequent experiments.

**Figure 1**: Preparation and identification of nanoparticles. (a) The binding ability of MTO-loaded NLCs to HA. (b) Cell uptake of C6-NLCs and ALN-FOL-C6-NLCs (\( x \pm s, n = 3 \)). \(* * * P < 0.001\) compared with the 0 \( \mu \text{mol/L ALN} + 0 \mu \text{mol/L FOL}\) group; \( ^{\#} P < 0.01\) compared with the C6-NLCs group.
Figure 2: Continued.
After transfection with miR-NC, miR-125b mimics, and miR-125b inhibitor, RT-qPCR was used to detect the transfection efficiency, and the results are shown in Figure 2(d). Furthermore, the MTT test showed that the proliferation capabilities of GCTB28 cells transfected with miR-NC liposomes were not significantly different from that of the control group, whereas the proliferation capabilities of GCTB28 cells after transfection with miR-125b mimics and ALN-FOL-MTO-NLCs was significantly reduced compared with the control group ($P < 0.05$), and the proliferation capabilities of GCTB28 transfected with miR-125b inhibitor liposomes was significantly increased compared with the control group ($P < 0.05$), as shown in Figure 2(g). Annexin V/PI double-staining results showed that the apoptotic rates of GCTB28 cells transfected with miR-NC liposomes were not significantly different from that of the control group. GCTB28 cells transfected with miR-125b mimics and ALN-FOL-MTO-NLCs had higher apoptotic rates compared with the control group ($P < 0.05$), and the apoptotic rates of GCTB28 cells transfected with miR-125b inhibitor liposome were significantly reduced compared with the control group ($P < 0.05$) as shown in Figure 2(h) and Figure S1. The Transwell experiments showed that the number of migratory GCTB28 cells transfected with miR-NC liposomes was not significantly different from that of the control group, whereas GCTB28 cells transfected with miR-125b mimics and ALN-FOL-MTO-NLCs had a significantly lower migration rate compared with the control group ($P < 0.05$). Furthermore, the number of migratory GCTB28 cells transfected with miR-125b inhibitor liposomes was significantly higher than that of the control group ($P < 0.05$), as shown in Figure 2(i) and Figure S2.

3.3. The Targeting Relationship between miR-125b and PTH1R. The bioinformatic analysis showed that parathyroid hormone receptor 1 (PTH1R) is the target gene of miR-125b, and the binding sequence is shown in Figure 3(a). The results of the dual-luciferase reporter gene experiment showed that overexpression of miR-125b induced a decrease in the luciferase activity of PTH1R-wt ($P < 0.05$); however,
it had no significant effect on the luciferase activity of PTH1R-mut, as shown in Figure 3(b). Thus, the above dual-luciferase reporter gene experiment verified the targeting relationship between PTH1R and miR-125b. Western blotting experiments and RT-PCR results also showed that overexpression of miR-125b significantly inhibited the expression of PTH1R protein and mRNA (P < 0.05), as shown in Figures 3(c) and 3(d).

3.4. ALN-FOL-MTO-NLCs Inhibit the Malignant Behavior of GCTB28 Cells through the miR-125b/PTH1R Molecular Axis. RT-qPCR results showed that the expression of PTH1R mRNA in GCTB28 cells was significantly increased after transfection with pc-PTH1R (P < 0.05), and the expression of PTH1R mRNA in GCTB28 cells was significantly decreased after transfection with si-PTH1R (P < 0.05), as shown in Figure 4(a). MTT results showed that compared with the control group, the proliferation activities of GCTB28 cells in the si-PTH1R group and ALN-FOL-MTO-NLCs group were significantly reduced (P < 0.05), whereas the number of invasive GCTB28 cells in the miR-inhibitor + ALN-FOL-MTO-NLCs group and miR-mimics + pc-PTH1R group did not change significantly from the control group, as shown in Figure 4(d) and Figure S4.

4. Discussion

As one of the most common primary bone tumors, GCT is frequently occurred in young people and often happens around the knee joint. Although the malignant degree of GCT is low, it still has strong invasiveness and osteolysis, and the recurrence rate after surgical treatment is extremely high, which seriously affects the quality of patients’ life. Therefore, finding new treatment strategies is essential to improve the quality of life for patients and reduce their recurrence rates after surgery. Studies have shown that nanoparticles have unparalleled advantages in the treatment of bone tumors. In this study, based on the research of Shi et al., MTO-NLC and ALN-FOL-MTO-NLC nanoparticles were prepared. Careful examination of the prepared nanoparticles revealed that both nanoparticles were spherical and regular...
in shape, and the rate of drug coverage, particle size, and potential was consistent with the results of Shi et al. Furthermore, compared with MTO-NLCs, ALN-FOL-MTO-NLCs have a certain degree of improvement in terms of HA adsorption capacity, cellular uptake capacity, and toxicity to cancer cells. This improvement is partially attributed to ALN. As a

![Figure 4: Reply experiment. (a) The relative expression level of PTH1R mRNA. (b) GCTB28 cell proliferation activity. (c) GCTB28 cell apoptosis rate. (d) GCTB28 cell invasion number. (x ± s; n = 3). **P < 0.01 compared with the pc-NC group; #P < 0.05 compared with the si-NC group; △△△P < 0.001 compared with the control group.](image-url)
bone metabolism regulator, ALN has a strong affinity with HA. Therefore, the modifications of nanoparticles with FOL not only ensured the steric hindrance of PEG but also increased the ingestion of nanoparticles by target cells. Immunofluorescence microscopy results showed that the ALN-FOR-MTO-NLC nanoparticles in GCTB28 cells were red fluorescent and mainly distributed in the cytoplasm, which is also consistent with the results of Vibet et al. [18].

Specifically, various studies have confirmed the expression changes of miR-125b in a variety of cancer tissues and cells. For example, miR-125b as a tumor suppressor gene was downregulated in breast cancer tissues and cells [19], and miR-125b expression in gastric cancer tissues and cells was also significantly downregulated [20]. In this study, it was found that the expression level of miR-125b in GCT tissues and cells was also significantly downregulated. The expression of miR-125b in GCT cells was upregulated to varying degrees after treatment with MTO-NLCs and ALN-FOL-MTO-NLCs, and the overexpression of miR-125b produced certain effects on proliferation, invasion, and apoptosis of GCT cells. Therefore, we think miR-125b played a role in the occurrence and development of GCT, which may be related to the role of ALN-FOL-MTO-NLC nanoparticles.

In this study, inhibiting the expression of PTH1R in GCTB28 cells reduced the proliferation and invasion of GCTB28 cells and induced its apoptosis. Moreover, bioinformatics analysis showed that PTH1R and miR-125b have partially complementary sequences, and their targeting relationship was further verified via the dual-luciferase reporter gene experiment, Western blotting, and RT-qPCR.

At the end of the study, through a series of rescue experiments, it was confirmed that ALN-FOL-MTO-NLCs inhibited the malignant behavior of GCTB28 cells by upregulating the expression level of miR-125b which then inhibited the expression of PTH1R. This result provides a theoretical basis for clarifying the mechanism of ALN-FOL-MTO-NLCs in the treatment of GCT, and ALN-FOL-MTO-NLCs combined with surgery may become a new strategy for the treatment of GCT.

Data Availability
All the data is available with the handwritten notebook documented in our lab.

Conflicts of Interest
The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Supplementary Materials
Figure S1: GCTB28 cell apoptosis rate. Figure S2: GCTB28 cell invasion number. Figure S3: GCTB28 cell apoptosis rate. Figure S4: GCTB28 cell invasion number (x ± s, n = 3); *P < 0.05, **P < 0.01, and ***P < 0.001, compared with the control group. (Supplementary Materials)

References


