Abstract. Osteosarcoma is the second leading cause of cancer-associated mortality worldwide in children and adolescents. ZC3H12D has been shown to negatively regulate Toll-like receptor signaling and serves as a possible tumor suppressor gene. MicroRNAs (miRNAs/miRs) are known to play an important role in the proliferation of human osteosarcoma cells. However, whether miRNAs can affect tumor development by regulating the expression of ZC3H12D has not yet been investigated. The aim of the present study was to investigate the role of miR-128-3p in regulating ZC3H12D expression, as well as its function in tumor cell proliferation, apoptosis, and metastasis. Reverse transcription-quantitative PCR, western blotting and dual luciferase reporter assays were performed to analyze the regulation of ZC3H12D expression by miR-128-3p. MTT, colony formation and flow cytometry assays were also used to analyze the effect of miR-128-3p on cell proliferation and apoptosis. A wound healing assay was performed to investigate the cell migration ability. The results demonstrated that miR-128-3p directly targeted ZC3H12D and downregulated its expression, thereby promoting cell proliferation and migration. miR-128-3p overexpression also improved resistance to cisplatin in MG-63 and 143B cell lines, supporting the hypothesis that miR-128-3p may function as an oncogene in osteosarcoma cells. The potential clinical significance of miR-128-3p as a biomarker and therapeutic target provides rationale for further investigation into the miR-128-3p-mediated molecular pathway and how it is associated with osteosarcoma development.

Introduction

As the most commonly occurring primary malignant bone tumor, osteosarcoma accounts for >10% of all solid tumors worldwide (1-3). Osteosarcoma is the second leading cause of cancer-associated mortality worldwide and primarily affects young individuals, including children and adolescents (4,5). Owing to advancements in surgical technology and combined therapeutic strategies, the 5-year overall survival rate of osteosarcoma has increased to 60-70% (6). However, the survival rate of patients in the advanced clinical stage and pulmonary metastases remains poor (7). Therefore, it is necessary to explore novel drugs or therapeutic strategies against this disease.

ZC3H12D, also known as TFL and p34, is a member of the CCCH-type zinc finger-containing protein family that also includes ZC3H12A, ZC3H12B and ZC3H12C (8-10). ZC3H12D, which is enriched in the lymphoid and inflamed tissues, such as lymph node, lung, and spleen (11), plays an important role in inflammation. ZC3H12D has been shown to negatively regulate Toll-like receptor signaling and suppresses both the initial inflammation storm and chronic inflammation by targeting the mRNA of cytokines as well as NF-κB and c-fos (12). Huang et al (11), for the first time, identified ZC3H12D as a tumor suppressor in transformed follicular lymphoma. Subsequently, other researchers have also demonstrated that overexpression of ZC3H12D inhibited cell proliferation in lung cancer (13). ZC3H12D also inhibits DNA synthesis and promotes apoptosis in lung cancer (14). The present study investigated the expression of ZC3H12D in osteosarcoma cells and analyzed its effects on the malignant behaviors of these cells.

miR-128-3p, also known as TFL and p34, is a member of the CCCH-type zinc finger-containing protein family that also includes ZC3H12A, ZC3H12B and ZC3H12C (8-10). ZC3H12D, which is enriched in the lymphoid and inflamed tissues, such as lymph node, lung, and spleen (11), plays an important role in inflammation. ZC3H12D has been shown to negatively regulate Toll-like receptor signaling and suppresses both the initial inflammation storm and chronic inflammation by targeting the mRNA of cytokines as well as NF-κB and c-fos (12). Huang et al (11), for the first time, identified ZC3H12D as a tumor suppressor in transformed follicular lymphoma. Subsequently, other researchers have also demonstrated that overexpression of ZC3H12D inhibited cell proliferation in lung cancer (13). ZC3H12D also inhibits DNA synthesis and promotes apoptosis in lung cancer (14). The present study investigated the expression of ZC3H12D in osteosarcoma cells and analyzed its effects on the malignant behaviors of these cells.

microRNAs (miRNAs/miRs) are a family of endogenous non-coding single-stranded RNA molecules with a length of 19-22 nucleotides, which act as either oncogenes or tumor suppressors based on the function of their target genes (15). In our study, we aimed to confirm that miR-128-3p targets ZC3H12D and to identified miR-128-3p as an oncogene in osteosarcoma. The present study aimed provide novel therapeutic targets for the treatment of osteosarcoma.
Materials and methods

Cell lines and cell culture. U2OS, 143B, Saos-2, MG-63 and hFOB1.19 cell lines were obtained from The American Type Culture Collection. Negative control cells and transfected cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM; Xiamen Yimo Biological Technology Co., Ltd.) containing 10% fetal bovine serum, 100 U/ml penicillin, 100 U/ml streptomycin and 2 mM l-glutamine (all Gibco; Thermo Fisher Scientific, Inc.) at 37˚C in a 5% CO₂ atmosphere with 21% oxygen.

Predict the miRNAs targeting ZC3H12D. TargetScan (URL: http://www.targetscan.org/vert_72/) was used to screen the miRNAs targeting ZC3H12D and predict the binding site of miR-128-3p at ZC3H12D 3’untranslated region (UTR).

Plasmid constructs. The 3’UTR of the human ZC3H12D gene was amplified using 2x Phanta Master Mix (Vazyme Biotech Co., Ltd.) by PCR from the genomic DNA of MG-63. The thermocycling conditions were as follows: Initial denaturation at 95˚C for 3 min, followed by 30 cycles of amplification (95˚C for 15 sec, 60˚C for 15 sec and 72˚C for 30 sec), and a final extension step at 72˚C for 5 min. The primers used were as following: 3’ UTR wild-type (WT), forward, 5’-AGCTCG CTAGCTTCAGGGAGCACCACCGCCTTCAG-3’ and reverse, 5’-GCAATGCTCCGATGTCAGCTCAAACACGCGTGGATCC-3’; 3’UTR mutant (Mut), forward, 5’-TTTGTAG CAGCCTAATAAGACAGTACAAAAAATAAG-3’ and reverse, 5’-GTCTTATTACGCTGATCAATCAAATAAACACGCTTAC-3’.

The ZC3H12D 3’UTR fragment was the cloned downstream of the firefly luciferase reporter gene in the pmirGLO vector (Promega Corporation). The mutant version of the ZC3H12D 3’UTR fragment was generated by using the QuikChange II Site-Directed Mutagenesis kit (Agilent Technologies, Inc.) according to the manufacturer’s instructions.

Total RNA was isolated from MG-63 cells using Total RNA Extraction Reagent (Vazyme Biotech Co., Ltd.) according to the manufacturer’s instructions. Total RNA was then reversely transcribed to cDNA using the Advantage RT-for-PCR kit (cat. no. 639505; Takara Bio, Inc.) and the following protocol: 42˚C for 30 min and 85˚C for 5 sec. The cDNA of ZC3H12D was amplified using PCR with the following primers: ZC3H12D, forward, 5’-GAGATGCTACGCGCTACCCGTCGGATCC -3’ and reverse, 5’-CTAGATCTCAGAGTGGAGCTGCCCTGCGGAAAGGC TCCGGAATC GTGACTCACGAGGAGTACGACAG , forward, 5’-CGCGTCAGTTGATGACAGTGGAGTGGAGCTGCCCTGCGGAAAGGC TCCGGAATC GTGACTCACGAGGAGTACGACAG , forward, 5’-CGCGTCAGTTGATGACAGTGGAGTGGAGCTGCCCTGCGGAAAGGC TCCGGAATC GTGACTCACGAGGAGTACGACAG , forward, 5’-CGCGTCAGTTGATGACAGTGGAGTGGAGCTGCCCTGCGGAAAGGC TCCGGAATC GTGACTCACGAGGAGTACGACAG . The miRNA and negative control (NC) mimic and inhibitor were obtained from Guangzhou Ribobio Co., Ltd. The sequences were as following: miR-128-3p Mimic: 5’-UCACUGUGACCCGUCCUU-3’, Mimic NC: 5’-UUUACUACAAAAAGUACUG-3’, miR-128-3p inhibitor: 5’-AAAGAGACCGGUUCAUGUGA-3’ and inhibitor NC: 5’-CAGUACUUUGUGUGAGUCAA-3’.

Transfection. The miRNA and negative control (NC) mimic and inhibitor were obtained from Guangzhou Ribobio Co., Ltd. After a 24 h-transfection, the cells were harvested for the subsequent experiments.

Dual-luciferase reporter assay. MG-63 and 143B cells were seeded into a 24-well plate at a density of 4x10⁴/well. When the cells reached 60-80% confluence, they were co-transfected with 50 pmol/well miR-128-3p mimic (or Mimic NC) and 1 µg/well pmirGLO-ZC3H12D 3’UTR WT or Mut plasmid using the Exfect Transfection Reagent (Vazyme Biotech Co., Ltd.) at 37˚C. The cells were lysed using Reporter 5x Lysis Buffer (Promega Corporation) at 48 h post-transfection, and the luciferase activity was measured using the Dual-Glo Luciferase Assay system (Promega) and normalized to Renilla luciferase activity.

Reverse transcription-quantitative (RT-q)PCR assay. Total RNA was isolated from transfected hFOB1.19, Saos-2, U2OS, MG-63 and 143B cells using the RNA Isolater Total RNA Extraction reagent (Vazyme Biotech Co., Ltd.) according to the manufacturer’s instructions. cDNA of ZC3H12D was synthesized using a HiScript II 1st Strand cDNA Synthesis kit (Vazyme Biotech Co., Ltd.) and the following protocol: 50˚C for 30 min and 85˚C for 5 sec. The reverse transcription reaction for miR128-3p was performed using a miRNA 1st Strand cDNA Synthesis kit (Vazyme Biotech Co., Ltd.) and the following protocol: 50˚C for 30 min and 85˚C for 5 sec. qPCR was conducted using an iQ5 Real-Time PCR Detection system (Bio-Rad Laboratories, Inc.) with a ChamQ SYBR qPCR Master Mix kit (Vazyme Biotech Co., Ltd.). The thermocycling conditions were: 94˚C for 3 min, followed by 94˚C for 15 sec, 60˚C for 20 sec and 72˚C for 20 sec, for 40 cycles. Each detection was carried out in triplicate. The primers used in the reverse transcription reaction and qPCR were as follows: miR-128-3p, RT:5’-GTCGATATCCAGTGACCGTTGAGGATTTGCACAGGATTTGCATGCTGCTGTAGTTGACAGTGGAGTGGAGCTGCCCTGCGGAAAGGC TCCGGAATC GTGACTCACGAGGAGTACGACAG , forward, 5’-CGCGTCAGTTGATGACAGTGGAGTGGAGCTGCCCTGCGGAAAGGC TCCGGAATC GTGACTCACGAGGAGTACGACAG , forward, 5’-CGCGTCAGTTGATGACAGTGGAGTGGAGCTGCCCTGCGGAAAGGC TCCGGAATC GTGACTCACGAGGAGTACGACAG , forward, 5’-CGCGTCAGTTGATGACAGTGGAGTGGAGCTGCCCTGCGGAAAGGC TCCGGAATC GTGACTCACGAGGAGTACGACAG .
Western blotting. The transfected hFOB1.19, Saos-2, U2OS, MG-63 and 143B cells were lysed in ice-cold RIPA buffer (Shanghai Yeasen Biotechnology Co., Ltd.). The proteins were quantified using a BCA protein assay kit (E112-01, Vazyme). In total, 20 μg protein/per lane was loaded on 10% denaturing SDS-PAGE gels per lane for electrophoresis and transferred onto PVDF membranes (Roche Diagnostics). After blocking with 5% bovine serum albumin (BSA; Sigma-Aldrich; Merck KGaA) in Tris-HCl buffer at 28˚C for 2 h, the membranes were incubated with the primary antibody Anti- ZC3H12D (1:1,000; cat. no. 24991-1-AP; ProteinTech Group, Inc.) or anti-GAPDH (1:5,000; cat. no. YM3029; Immunoway; http://www.immunoway.com/) prepared in Tris-HCl buffer containing 5% BSA at 4˚C overnight. Next day, the membranes were washed with Tris-HCl buffer, followed by incubation with the corresponding secondary antibody HRP-Goat Anti-Rabbit IgG (H + L) (1:5,000; cat. no. SA00001-2; ProteinTech Group, Inc.) or HRP-goat anti-mouse IgG (H + L) (1:5,000; cat. no. SA00001-1; ProteinTech Group, Inc.) at 28˚C for 1 h. Subsequently, the membranes were washed with Tris-HCl buffer. Protein bands were visualized using the reagent Pierce ECL (Thermo Fisher Scientific, Inc.) and a Chemo XRS + luminometer (Bio-Rad Laboratories). GAPDH was used as the loading control. Protein expression levels were quantified using the Quantity One software (Bio-Rad Laboratories).

Cell proliferation assay. After transfection, 143B and MG-63 cells were seeded into 96-well plates at an initial density of 3x10^4 cells/well. The cells were stained with MTT (Shanghai Yeasen Biotechnology Co., Ltd.) and a Chemo XRS + luminometer (Bio-Rad Laboratories) at each time point for 4 h at 37˚C. The purple formazan was dissolved with dimethyl sulfoxide (Sigma-Aldrich; Merck KGaA) in Tris-HCl buffer at 28˚C for 24 h. The absorbance at 570 nm was measured with SpectraMax Absorbance Reader (Molecular Devices). All experiments were performed in triplicates.

Colony formation assay. After transfection, 143B and MG-63 cells were seeded into 6-well plates at a density of 1,000 cells/well and maintained in DMEM containing 10% FBS at 37˚C for 7-10 days. After most of the colonies had expanded to >50 cells, the cells were washed with PBS, fixed in 4% methanol at 28˚C for 15 min and stained with 0.1% crystal violet (Beijing Solarbio Science & Technology Co., Ltd.) at 28˚C for 15 min. The colonies were photographed and the number of them were counted using ImageJ (1.8.0; National Institutes of Health). At least three independent experiments were carried out for each assay.

Cell cycle assay. After transfection, 143B and MG-63 cells were seeded at a density of 2x10^5 cells per well in 6-well plates. After 24 h the cells were harvested and fixed in 70% ethanol at 4˚C overnight. The fixed cells were incubated with 0.5 ml PBS containing 10 μg/ml RNase and 0.2% Triton X-100 for 30 min at 37˚C, then stained with 20 μg/ml PI for 30 min in the dark at 28˚C. A flow cytometer Finally, the stained cells were analyzed by flow cytometry. A flow cytometer NovoCyte 1300 (ACEA Bioscience) and software NovoExpress 1.4.1 (ACEA Bioscience) were used to determine the cell cycle distribution.

Apoptosis assay. Annexin V-FITC and PI (Vazyme Biotech Co., Ltd.) were used to detect cell apoptosis by flow cytometry. After transfection, 143B and MG-63 cells were seeded into 6-well plates at a density of 4x10^3 per well, followed by incubation with 20 μM cisplatin at 37˚C for 24 h. The cells were then collected, rinsed with PBS, and stained with 200 μg/ml Annexin V-FITC and 30 μg/ml PI in the dark at 28˚C for 10 min. Immediately, the fluorescence was analyzed in 10,000 cells/sample using the flow cytometer NovoCyte (ACEA) and software NovoExpress 1.4.1 (ACEA). The results were expressed as the percentage of cells exhibiting early and late apoptosis relative to total cells analyzed.

Wound healing assay. After transfection, MG-63 and 143B cells were seeded into a 6-well plate (4x10^5/well). Once they reached 100% confluence, the wound was created using a 200-μl pipette tip. The cells were continuously cultured in serum-free medium for 24 h and observed under a light microscope at a magnification of 40x. Images were captured at 0 and 24 h.

Statistical analysis. All statistical analyses were performed using SPSS software 22.0 (IBM Corp.). GraphPad Prism 5 (GraphPad Software, Inc.) was used to construct the graphs. The data are presented as the mean ± standard deviation (SD) of three independent experiments, unless otherwise shown. Tukey's test was used to identify significant differences among multiple groups. Unpaired student's t-tests were used to compare two groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Comparison of expression levels of ZC3H12D in osteoblast and osteosarcoma cells. RT-qPCR and western blotting were used to analyze the expression of ZC3H12D in mRNA and protein levels, respectively. The results showed that both the mRNA and the protein levels of ZC3H12D were decreased in the osteosarcoma cells (U2OS, 143B, Saos-2 and MG-63) compared with those in the osteoblast cell hFOB1.19 (P<0.0001; Fig. 1A and B). The expression level of ZC3H12D was the highest in the MG-63 and 143B cell lines, therefore these were selected for further experiments.

miR128-3p targets ZC3H12D and reduces the expression level of ZC3H12D in osteosarcoma cells. TargetScan software was used to scan possible miRNAs targeting ZC3H12D and miR-128-3p was selected as the potential miRNA (Fig. 1C). Then, a Dual-luciferase reporter assay was performed. As shown in Fig. 1D, transient co-transfection of an miR-128-3p mimic and a pmirGLO-ZC3H12D 3' UTR plasmid resulted in a notable decrease in the luciferase activity compared with the NC group in MG-63 (P=0.0001) and 143B (P<0.0001) cells. Moreover, the regulatory effect of the miR-128-3p mimic or inhibitor on ZC3H12D expression was examined using RT-qPCR and western blotting. As shown in Fig. 1E, the mRNA levels of ZC3H12D in the MG-63 and 143B cells were markedly decreased after transfection with the miR-128-3p mimic, but increased after transfection with the miR-128-3p inhibitor (Fig. 1F), compared with those in the NC group.
The change in ZC3H12D protein levels was consistent with the mRNA levels. In addition, the expression of miR-128-3p was upregulated in the osteosarcoma cells, compared with that in the osteoblast cell hFOB1.19 (Fig. 1G). These findings suggested that miR128-3p directly targets ZC3H12D and downregulates its expression in osteosarcoma cells.

**miR128-3p promotes the proliferation of osteosarcoma cells.**

The present study aimed to determine whether miR-128-3p serves as an oncogenic miRNA by targeting ZC3H12D. miR-128-3p-overexpression was induced in the MG-63 and 143B cells with or without ZC3H12D-overexpression (Fig. 2A-D). An MTT assay was performed to evaluate cell viability. As shown in Fig. 2E, the miR-128-3p mimic significantly promoted cell proliferation (P<0.001) compared with the Mimic NC. The results of the colony formation assay showed that the colony numbers were also significantly increased after overexpression of miR-128-3p (Fig. 2F). The number was increased from 372±24 to 800±21 (P=0.0002, Fig. 2G) for MG-63 cells and from 211±10 to 326±15 (P=0.0005, Fig. 2G) for 143B cells. The effect of miR128-3p on cell cycle progression was analyzed using flow cytometry. As shown in Fig. 2H and I, the miR-128-3p mimic reduced the percentage of cells in the G0/G1 phase and increased the proportion of cells in the S phase in comparison with the Mimic NC. Taken together, these results indicated that miR128-3p could prevent G1 phase arrest in MG-63 and 143B cells and promote proliferation. Moreover, ZC3H12D-overexpression reversed the effects of miR-128-3p (Fig. 2E-I).
miR128-3p enhances the resistance of osteosarcoma cells against cisplatin. After treating the cells with 20 µM cisplatin for 24 h, flow cytometry was performed to evaluate apoptosis. The results revealed that the proportions of the apoptotic cells were 5.29±0.49 and 8.14±0.32% for MG‑63 and 143B cells overexpressing miR‑128‑3p, respectively. The rate of apoptosis in the mimic NC + vector group was higher (10.84±0.21%, P=0.0064 and 16.42±1.58%, P=0.0426, respectively) (Fig. 3A and B). These data indicated that miR‑128‑3p improved the resistance of osteosarcoma cells to cisplatin. As expected,
co-overexpression of ZC3H12D eliminated the effects of
miR128-3p overexpression. The percentage of apoptotic cells
was increased from 5.29±0.49 to 21.84±1.19% (P<0.0001) for
MG-63 cells and from 8.14±0.32 to 29.48±5.50% (P=0.0026)
for 143B cells (Fig. 3A and B). These results suggested that
ZC3H12D increases the sensibility of osteosarcoma cells
against cisplatin.

miR128-3p promotes the migration of osteosarcoma cells.
To examine the effect of miR-128-3p on cell migration, a
wound healing assay was performed. The results revealed
that miR-128-3p improved the migration ability of the MG-63
and 143B cells (Fig. 4). Moreover, ZC3H12D overexpression
reversed the miR-128-3p-induced improvement of migration
ability (Fig. 4).

Discussion

ZC3H12D has identified as a tumor suppressor in trans-
formed follicular lymphoma, lung and advanced endometrial
cancer (11,13,23). In the present study, it was demonstrated that
ZC3H12D expression was notably decreased in osteosarcoma
cell lines. Thus, it was hypothesized that ZC3H12D may also
be a tumor suppressor in osteosarcoma. Next, it was confirmed
that miR-128-3p directly targeted ZC3H12D and downregu-
lated its expression, which suggested that miR-128-3p may act
as an oncogenic miRNA. Finally, the effects of miR-128-3p
and ZC3H12D on cellular functions in osteosarcoma cell lines
were investigated and the results demonstrated that miR-128-3p
indeed promotes cell proliferation and migration, but inhibits
apoptosis. Moreover, ZC3H12D reversed the effects induced
by miR-128-3p. The expression level of ZC3H12D was the
highest in MG-63, while the expression level of miR-128-3p
was the lowest in 143B, and this divergence may be explained
by the fact that the expression of ZC3H12D is regulated by
other molecules or miRNAs.

A miRNA can target numerous genes, and several targets
of miR-128-3p, such as SAM and SH3 domain-containing
protein 1, PTEN and integrin α2, have been reported (24-27).
miR-128-3p directly targets these genes and regulates
their expression, promoting cancer progression in
osteosarcoma (24-27). Thus, miR-128-3p may serve as an
oncogenic miRNA by downregulating not only ZC3H12D
but also other tumor suppressors. However, some studies
have reported that miR-128-3p serves as an antioncogenic miRNA in several types of cancer (27-29). For example, Huang et al (29) demonstrated that miR-128-3p suppresses the proliferation of hepatocellular cancer cells by regulating PIK3R1. miR-128-3p-overexpression sensitizes hepatocellular cancer cells to sorafenib-induced apoptosis through regulating Parkinson disease protein 7 (27). Zhao et al (28) showed that miR-128-3p overexpression suppresses breast cancer progression through targeting LIM domain kinase 1 (28). These divergences may be explained by the fact that the expression level of miR-128-3p in different types of cancer may differ, and that miR-128-3p can targets numerous genes, the expression levels of which may also be different in different cancer types. One of the limitations of present study is that we did not decrease the miR-128-3p level to observe the effect of miR-128-3p on osteosarcoma cell. Additional experiments using an miR-128-3p inhibitor to elucidate the effect of miR-128-3p on cellular function could further support our conclusion. Moreover, the objective of future research would be to find molecules downstream of the miR-128-3p/ZC3H12D pathway. In addition, the role of miR-128-3p/ZC3H12D in osteosarcoma should be studied in vivo in the next work. The present study, has found a long non-coding RNA (lncRNA) CKMT2-AS1 that exhibits antitumor activity through targeting miR-128-3p (unpublished data). We are presently constructing an animal model, which will be used to determine the function of CKMT2-AS1 in vivo, along with the effect of miR-128-3p, with the aim to elucidate the complete lncRNA/miR-128-3p/ZC3H12D axis.

Patients with localized disease have a 65-70% 5-year survival rate (30), while those with metastasis, most commonly in lung parenchyma and distant skeletal sites, exhibit poor survival rates of 19-30% (31,32). The present study reported that miR-128-3p was highly expressed in the osteosarcoma cells, promoting cell migration, and thus it may serve as a potential therapeutic target. Suppression of miR-128-3p would inhibit the migration of osteosarcoma cells, and may increase the survival rate. Currently, the main treatment for osteosarcoma is surgery and cycles of neoadjuvant chemotherapycombinedly using three to four kinds of cytotoxic agents, such as cisplatin, doxorubicin, methotrexate and ifosfamide (30). The survival time of patients with recurrent disease with drug resistance is ~1 year (33). The present study revealed that miR-128-3p improved the resistance of osteosarcoma cells to cisplatin, and thus miR-128-3p may be a target for advancing the efficiency of chemotherapy drugs.

In conclusion, the present study demonstrated that ZC3H12D serves as a tumor suppressor in osteosarcoma cells for the first time, to the best of our knowledge. Moreover, it was also reported that the miR-128-3p/ZC3H12D axis could further support our conclusion. Moreover, the objective of future research would be to find molecules downstream of the miR-128-3p/ZC3H12D pathway. In addition, the role of miR-128-3p/ZC3H12D in osteosarcoma should be studied in vivo in the next work. The present study, has found a long non-coding RNA (lncRNA) CKMT2-AS1 that exhibits antitumor activity through targeting miR-128-3p (unpublished data). We are presently constructing an animal model, which will be used to determine the function of CKMT2-AS1 in vivo, along with the effect of miR-128-3p, with the aim to elucidate the complete lncRNA/miR-128-3p/ZC3H12D axis.

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Availability of data and materials

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

Authors’ contributions

FL and XC designed the experiments. FL, XC, MZ and YW performed the statistical analysis, interpreted the data and wrote the manuscript. MZ, YW, ZW, ML, BS and CL performed the experiments. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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