LncRNA THUMPD3-AS1 promotes invasion and EMT in gastric cancer by regulating the miR-1297/BCAT1 pathway

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Research Article

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Abstract

Objective Long noncoding RNAs (lncRNAs) are significant regulators in gastric cancer GC; however, studies of their mechanisms of action are needed to determine their clinical value. In this study, we investigated the effects and mechanism of action of THUMPD3-AS1 in GC.

Methods Candidate lncRNAs and mRNAs were getted from The Cancer Genome Atlas, revealing the differential expression and prognostic significance of THUMPD3-AS1-BCAT1 in GC. qRT-PCR was performed to detect THUMPD3-AS1 levels in GC samples and cell lines. CCK8, scratch wound healing, and Transwell assays as well as experiments in vivo were conducted to evaluate the function of THUMPD3-AS1 in GC. Related genes were analysed to detect interactions between THUMPD3-AS1, BCAT1, and miR-1297.

Results THUMPD3-AS1 levels were significantly elevated in GC and were positively correlated with poor prognosis. Functionally, THUMPD3-AS1 promoted GC cell proliferation, migration, invasion, and epithelial–mesenchymal transition (EMT) and induced tumour growth in vivo. THUMPD3-AS1 regulated BCAT1 by competitively binding to miR-1297; further analyses revealed that both THUMPD3-AS1 and miR-1297 can interact with BCAT1.

Conclusions These findings demonstrate that THUMPD3-AS1 promotes GC cell invasion and EMT via the miR-1297/BCAT1 pathway, suggesting that THUMPD3-AS1 is a novel biomarker and therapeutic target for GC.

Background

Recurrence and metastasis are the main causes of death in patients with gastric cancer GC. Treatment outcomes are not ideal, emphasising the importance of exploring the underlying molecular mechanism and identifying novel biomarkers and anti-cancer molecular targets.

Recent research has revealed a large number of long noncoding (lncRNAs) with aberrant expression in tumour tissues. Many lncRNA molecules are closely related to the occurrence and development of GC. lncRNAs play role in the pathogenesis of cancer, including cell proliferation, migration, and invasion, epithelial–mesenchymal transition (EMT), apoptosis, and immune escape. The lncRNA THUMPD3-AS1 acts as a proto-oncogene in some tumours. However, its role and mechanism of action in GC have not been established. In this study, a bioinformatics approach was used to evaluate the role of THUMPD3-AS1 in the progression of GC via the competitive endogenous RNA (ceRNA) mechanism. Subsequent in vitro and in vivo analyses were performed to confirm this hypothesis. The results provide a basis for elucidating the mechanism underlying the progression of GC and for identifying novel potential target genes.

Materials and Methods
Clinical samples

Fifty patients with GC who were surgically treated at the Fourth Hospital of Hebei Medical University from January 2019 to June 2022 were enrolled, and paired GC and paracancerous tissue samples were obtained. Patients did not receive radiotherapy or chemotherapy and had no comorbid cancers. Patients were between 40 and 75 years of age. During surgery, tumour tissues and paracancerous tissues were collected, placed in liquid nitrogen, and transferred to a -80°C freezer for long-term storage as soon as possible. All patients provided signed informed consent, and the study was reviewed and approved by the Ethics Committee of the Fourth Hospital of Hebei Medical University (ethics number: 2019MEC039).

Cell lines and main reagents

The human GC cell lines AGS, SGC7901, and HGC-27 and normal gastric epithelial cell line GES-1 were purchased from the Cell Resource Centre of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences and were maintained and passaged at the scientific research centre of our hospital. Nude mice were purchased from the Cell Resource Center of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. Primers were designed according to the sequences of THUMPD3-AS1, miR-1297, BCAT1, U6 (internal control), and β-actin (internal control) and were synthesized by Shanghai Genechem Biotechnology Co. (Shanghai, China). Foetal bovine serum, Dulbecco's modified Eagle's medium (DMEM), streptomycin, β-actin antibody, reconstituted basement membranes, and Transwell chambers were purchased from Shanghai Zemai Biological Company (Shanghai, China). CCK-8 reagent, trypsin, penicillin and streptomycin, and phosphate-buffered saline (PBS) were purchased from the Cell Resource Centre of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. Dual-luciferase reporter assay kits and BCA kits were purchased from Beijing Solarbio Company (Beijing, China). TRIzol kits, fluorescence quantitative PCR kits, and reverse transcription kits were purchased from Dalian Takara Biotechnology Co. (Dalian, China). The neural cadherin (N-cadherin) antibody, epithelial cadherin (E-cadherin) antibody, and glyceraldehyde-3-phosphate dehydrogenase antibody were purchased from Sigma (St. Louis, MO, USA).

Culture conditions

When cells formed confluent monolayers, the medium was discarded. Cells were rinsed twice with PBS and digested with 0.25% trypsin. When the intercellular space increased, the trypsin was aspirated and new medium was added to prepare a single-cell suspension. Experiments were performed when cells reached 60–80% confluence.

Animal experiments

The experiment followed the principles of the Animal Care and Use Committee of Hebei Medical University and was approved by the Medical Ethics Committee of the Fourth Hospital of Hebei Medical University. Twenty BALB/c nude mice (4–5 weeks old) were obtained from the Cell Resource Center of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences and housed in a specific pathogen-free environment at 23–25°C. Xenograft tumours were established by subcutaneous injection,
and tumour sizes were determined weekly. Mice were sacrificed after 4 weeks, and tumours were harvested for the determination of size and weight. Xenograft tumours were formalin-fixed for follow-up studies.

**Bioinformatics analysis**

The GC dataset from The Cancer Genome Atlas (TCGA) database (http://cancergenome.nih.gov/) was used. Gene expression data were obtained from TCGA using the R package “TCGAbiolinks”. The R package DESeq2 was used to identify differentially expressed genes based on the original read count data. Genes with a corrected p-value of < 0.05 and fold change of > 2 were considered significantly differentially expressed. A differential expression analysis, functional enrichment analysis, and correlation analysis to determine the prognostic value were performed.

**CCK8 assay**

Cells in each group were retrieved and placed in an incubator for 24, 48, and 72 h separately. The culture was interrupted by discarding the medium and adding 100 µL of medium that contained CCK-8 reagent. The cells were then returned to the incubator for 2 h. The OD value at 450 nm was measured using a microplate reader. Six parallel wells were set up for each group. The experiment was repeated three times.

**Real-time fluorescent quantitative RT-PCR for the detection of THUMPD3-AS1 and miR-1297**

Total RNA was extracted from tumour tissues and cells from each group after transfection. According to the instructions provided with the reverse transcription kit, 2 mg of total RNA was added to establish a 20 mL reaction system, and cDNA was obtained by reverse transcription. A real-time quantitative PCR system was established using the corresponding primers for target genes and GoTaq qPCR Master Mix. The TaqMan Real-Time PCR Kit was used with β-actin and U6 as internal controls. The final relative expression levels were calculated by using the $2^{-\Delta \Delta Ct}$ method. The experiment was repeated three times and average values were obtained. The primer sequences were as follows: THUMPD3-AS1, forward primer: ATTCTGTCCCTGACCGTCT, reverse primer: GTTCTCTTCTGTTTCCACAC; miR-1297, forward primer: TTCAAGTAAATTCAGGTGGTGC, reverse primer: GTCGTATCCAGTGACTTC; BCAT1, forward primer: TGTATCGCTCTGCTGTGAGG, reverse primer: CAGTTCCAATGAATGTAGGACG; internal control 6U forward primer: 5′-CTCGCTTCCAGGCAATGACTGC-3′, reverse primer: 5′-AACGCTTCAGGAAATTGTGGAG-3′, internal control β-actin, forward primer: GGTCATCACCATTGGCAA, reverse primer: GAGTTGAAGGTAGTTTCGTGGA; miR-1297, forward primer: TTCAAGTAAATTCAGGTGGTGC, reverse primer: GTCGTATCCAGTGACTTC; BCAT1, reverse primer: GGTCCACATTGAGGCAAA, reverse primer: GAGTTGAAGGTAGTTTCGTGGA; miR-1297, forward primer: TTCAAGTAAATTCAGGTGGTGC, reverse primer: GTCGTATCCAGTGACTTC.

**Detection of protein expression by western blotting**

The total protein from each group of samples was extracted by the ABC method, followed by SDS-PAGE (initial voltage 80 V, increased to 180 V after proteins entered the separation gel, and continued until the front of the sample reached the bottom of the separation gel). Separated proteins were then transferred to membranes at 150 mA for 2 h. The membranes were blocked by incubating in blocking solution for 1h
at room temperature with gentle rocking. The membranes were placed in hybridisation bags, which were sealed after air bubbles were driven out and were placed on a shaker at 4°C for overnight shaking. Then, the hybridisation bag was cut open, and the membranes were rinsed three times before they were placed in the hybridisation bag containing the secondary antibody and incubated at room temperature for 2h with gentle shaking. Subsequently, the membranes were placed in the colour developing solution for reaction, after which they were rinsed with distilled water and air-dried. Images were obtained, and grey values and relative expression levels were analysed.

**Detection of cell migration ability by scratch test**

Five horizontal lines were drawn on the back of a 6-well plate. After 48 h of transfection, cells from each group were collected and inoculated into the 6-well plate at a density of $5 \times 10^5$ per well. Cells were grown overnight before the wells were scratched with a 200 µL pipette tip perpendicular to the horizontal lines on the back and rinsed gently with PBS. The scratched cells were washed with PBS, and serum-free medium were added. Cells were cultured for 48 h and images were obtained under an inverted microscope. Three duplicate wells were set up for each group. The resulting images were processed using ImageJ (NIH, Bethesda, MD, USA). The number of cells that migrated across the edges of scratches and cell morphology were observed at 24 h, 48 h, and 72 h after the scratch.

**Transwell detection of cell invasion ability**

Transwell invasion chambers were placed in a clean, sterile 24-well plate. After pipetting, the Transwell chambers were placed in a 37°C incubator for incubation and hydration. Cells that reached the logarithmic phase of growth were retrieved in 2 h and washed three times. The cell density was adjusted to $8.0 \times 10^4$ cells/mL after the buffer solution was aspirated. Then, 500 µL of the cell suspension was transferred into each chamber, and the Transwell chambers were placed in a pre-treated 24-well plate. A total of 500 µL of DMEM containing 10% foetal bovine serum was added to each well and the plate was placed in an incubator. After 24 to 48 h of incubation, the Transwell chambers were gently removed and the cells were fixed with paraformaldehyde and stained with crystal violet dye. Cells were counted under a microscope to determine the average number of migrating cells for each membrane and to evaluate the invasive ability of cells.

**Dual luciferase reporter assay**

The potential binding sites of THUMPD3-AS1 were predicted using StarBase v2.0, miRcode, and RNAhybrid, and miR-1297 primers were designed according to the prediction results. The amplified fragment was introduced into a luciferase reporter plasmid to construct the reporter vector THUMPD3-AS1-Wt. The predicted fragment of miR-1297 was modified, and the mutant reporter vector THUMPD3-AS1-Mut was established. The plasmid and miR-1297 mimics were transfected into HGC-27 cells, and luciferase activity was detected in 48 h. The regulatory relationship with BCAT1 was verified by the same method.

**Statistical analysis**
Experimental data are expressed as the mean ± standard deviation of three independent experiments, and SPSS 21.0 was used for statistical analyses. The measurement data conforming to a normal distribution are expressed as the mean ± standard deviation (x ± s). The \( t \)-test was used for comparisons between two groups. One-way ANOVA was used for comparisons among multiple groups. All statistical tests were two-way and results with \( p < 0.05 \) were considered statistically significant.

Results

Differentially expressed IncRNAs in GC

Significantly differentially expressed IncRNAs based on recent GC data were obtained (|logFC| > 1, FDR < 0.05). A total of 1043 differentially expressed IncRNAs and 2841 differentially expressed genes were obtained [Fig. 1(a), Fig. 1(b)]. The differentially expressed IncRNAs and genes can significantly distinguish normal samples from cancer samples [Figure 1(c)]. Based on a corrected p-value of < 0.05 and expression change of > 2-fold, candidate genes were identified. As shown in a heatmap [Fig. 1(c)], the differentially expressed IncRNAs in cancer tissues were significantly up-regulated.

Identification of candidate ceRNA pairs in GC

According to known IncRNAs in databases and miRNA targets of genes, overlap in target miRNA sets for each differentially expressed IncRNA-gene pair was evaluated. The results uncovered top-ranked IncRNAs and interacting genes. THUMPD3-AS1 was highly ranked among IncRNAs [Figure. 2(a)] and \( BRCA1 \), \( RACGAP1 \), and others were top-ranked among mRNAs with significant differences [Figure. 2(b)]. A boxplot displays the number of candidate ceRNAs that were significantly associated with each gene or IncRNA [Fig. 2(c)] and the p-values for all candidate ceRNAs that were significantly related to the gene or IncRNA were obtained [Fig. 2(d)]. For candidate IncRNA-gene pairs with significant correlations determined by the hypergeometric test, Pearson correlation coefficients in cancer samples and normal samples were calculated for all differentially expressed genes and IncRNAs [Fig. 2(e)]. There were 4457 significantly correlated pairs in cancer samples and 11097 significantly correlated pairs in normal samples. Sixty-four IncRNA-gene pairs with significant correlations in cancer samples were identified as candidates by the random perturbation method for subsequent analyses.

Prognosis and functional enrichment analyses of candidate ceRNA pairs

We performed a survival analysis of 2298 candidate IncRNA-gene pairs in GC to examine associations with prognosis. As a result, 460 ceRNA pairs related to survival were obtained. HAGLR had the highest number of connections in the functional protein association network, indicating its central role in protein interactions and signalling pathways [Fig. 3(a)]. The genes in the ceRNA network were subjected to functional enrichment analyses. Candidate ceRNA pairs were mainly enriched in extracellular matrix
tissue, focal adhesions, PI3K-AKT and other signalling pathways, indicating that they are related to stromal tissue remodelling and tumour cell migration [Fig. 3(b)].

Expression and prognostic value of THUMPD3-AS1-BCAT1 in tissues and cell lines

THUMPD3-AS1-BCAT1 is an important pair of candidate ceRNAs. The expression level of the THUMPD3-AS1 pair in the tumour group was significantly higher than that in the normal group [Fig. 4(a)]. A dot plot demonstrated that THUMPD3-AS1 and BCAT1 exhibited a general upwards trend in 50 GC tissues [Fig. 4(b)]. A series of analyses revealed that THUMPD3-AS1-BCAT1, an important pair of ceRNAs, is highly expressed in cancer, with significantly correlated expression. A correlation analysis revealed that the expression levels of BCAT1 and THUMPD3-AS1 were significantly positively correlated, and the distributions of expression levels were both unimodal [Fig. 4(c)]. The relationship between THUMPD3-AS1-BCAT1 and overall survival was analysed based on a multivariate Cox regression analysis. The actual 5-year overall survival and predicted 5-year overall survival were significantly positively correlated, indicating that the model was highly effective [Fig. 4(d)]. The risk rate of the BCAT1 and THUMPD3-AS1 treatment group was 1.4 times that of the control group [Fig. 4(d)]. A survival curve analysis of TCGA data showed that the survival rate in patients with high expression of THUMPD3-AS1 was significantly lower than that in patients with low expression of THUMPD3-AS1, indicating that the expression of the THUMPD3-AS1 pair was significantly related to survival [Fig. 4(d)].

Effects of THUMPD3-AS1 on cell viability and invasion

THUMPD3-AS1 was differentially expressed in five cell lines, including GES-1, AGS, and HGC27, with the weakest expression in GES-1 [Fig. 5(a)]. In an overexpression experiment of THUMPD3-AS1-siRNA, miR1297 was significantly expressed in the transfection group, and the expression of miR1297 was significantly higher in the vehicle control group and negative control group than in the THUMPD3-AS1-siRNA group [Fig. 5(b)]. During the overexpression of THUMPD3-AS1, the vehicle control group and negative control group exhibited a similar trend, with higher expression levels than those in the transfection group. In an analysis of the inhibition of THUMPD3-AS1, the viability of cells in the transfection group was significantly higher than those in the vehicle control group and the negative control group from 0 to 72 h. Compared with viability in the control group, the viability of cells in the overexpression group was higher and the viability of cells in the inhibition group was lower [Fig. 5(c)]. After the overexpression of THUMPD3-AS1, the wound healing rate of cells in the transfection group was significantly higher than those in the control and negative groups. The number of cells that migrated through the membrane and expression levels of related genes were also significantly higher in the transfection group than in control and negative groups. After inhibition, the wound healing rate of cells in the transfection group was significantly lower than those in the control group and the negative group, and the number of cells that migrated through the membrane and expression levels of related genes were also significantly reduced in the transfection group [Fig. 5(d)].
In vivo validation

After transfection with MNK45 (an inhibitor of THUMP3-AS1), the tumour volume and weight were significantly lower than those in the control group [Fig. 6(a)]. In solid tumour specimens, the tumour size in the treatment group was significantly smaller than that in the control group [Fig. 6(b)]. Levels of TIMP3 and E-cadherin in the transfection group were significantly higher than levels of other proteins, and levels of other proteins were all lower in the transfection group than in the control group [Fig. 6(c)].

Relationship between THUMP3-AS1 and miR-1297

As determined by the hypergeometric test, the target miRNA sets of THUMP3-AS1 and BCAT1 had significant overlap [Fig. 7(a)], and miRDB predicted that THUMP3-AS1 had a miR-1297 binding site [Fig. 7(b)]. In 50 clinical samples, miR-1297 levels were significantly lower in tumour tissues than in paracancerous tissues, further verifying that this locus acts as a tumour suppressor in GC tissue; however, the latter was significantly lower than the former in analyses of the overexpression and inhibition of THUMP3-AS1 expression [Fig. 7(c)]. The expression levels of THUMP3-AS1 and miR-1297 in 50 GC tissues were negatively correlated [Fig. 7(d)]. In the THUMP3-AS1 overexpression experiment, miR-1297 levels were significantly higher in the transfection group than in the vehicle control group and the negative control group [Fig. 7(e)]. A dual luciferase reporter assay showed that derivative THUMP3-AS1 affected downstream gene transcription and translation and that luciferase levels decreased at 560 nm. Additionally, THUMP3-AS1 had a binding site for miR-1297 [Fig. 7(f)].

Relationship between BCAT1 mRNA and miR-1297

In terms of the binding relationship between BCAT1 mRNA and miR-1297 [Fig. 8(a)], in 50 GC tissues, BCAT1 mRNA levels were significantly higher in tumour tissues than in paracancerous tissues [Fig. 8(b)]. The expression levels of THUMP3-AS1 and BCAT1 mRNA in tissues were positively correlated [Fig. 8(c)], while the expression level of miR-1297 was negatively correlated with the BCAT1 mRNA expression level in tissues [Fig. 8(d)]. BCAT1 mRNA levels were lowest in the GES-1 cell line, highest in the MKN45 cell line, and significantly higher in the MKN45 cell line than in the NCIN87 cell line [Fig. 8(e)]. When miR-1297 was overexpressed, BCAT1 expression was evaluated at the mRNA and protein levels. BCAT1 mRNA levels in the transfection group were significantly lower than those in the vehicle control group and the negative control group [Fig. 8(f)]. When miR-1297 was inhibited, BCAT1 mRNA levels in the transfection group were significantly higher than those in the vehicle control group and the negative control group [Fig. 8(g)]. Derivative BCAT1 affected the transcription and translation of downstream genes, and luciferase activity decreased at 560 nm, while the mutant had no effect on transcription and translation. These findings indicated that BCAT1 had a binding site for miR-1297 [Fig. 8(h)].

Effect of the THUMP3-AS1/miR-1297 axis on the viability, invasion, and migration of GC cells
When THUMPD3-AS1 was inhibited, BCAT1 mRNA and protein expression levels were significantly higher in the control and negative groups than in the THUMPD3-AS1-siRNA group. Its expression in the THUMPD3-AS1-siRNA + miR-1297 inhibition group was slightly lower than that in the THUMPD3-AS1-siRNA group [Fig. 9(a)]. When THUMPD3-AS1 was overexpressed, BCAT1 mRNA and protein expression levels were significantly lower in the negative control group than in the overexpression group. However, the expression was low in the THUMPD3-AS1 overexpression + miR-1297 mimic group [Fig. 9(b)]. The cell viability, wound healing rate, and number of cells that migrated through the membrane were significantly lower in the THUMPD3-AS1-siRNA group than in the THUMPD3-AS1-siRNA + miR-1297 inhibition group [Fig. 9(c)]. When THUMPD3-AS1 was overexpressed, the cell viability, wound healing rate, and number of cells that migrated through the membrane were significantly higher than those in the mimic group [Fig. 9(d)].

**Discussion**

In gastric cancer (GC), distant metastasis is an important reason for the high recurrence rate and mortality\(^6\). The EMT plays an essential role in the process of GC metastasis\(^7\). LncRNAs regulate the expression levels of cancer-related genes at multiple levels and are involved in the occurrence and development of GC\(^8\). Studies have revealed the aberrant status of many lncRNAs in gastrointestinal cancers compared with levels in healthy tissues\(^9\). In this study, we identified a significant lncRNA THUMPD3-AS1 associated with GC and explored the mechanism by which it regulates the invasion and EMT of GC cells using clinical tissue samples and comprehensive biological analyses\(^10\)\(^11\).

LncRNAs are involved in the occurrence and development of GC\(^10\)\(^11\). Many lncRNAs are associated with cancer, such as GC, via the ceRNA mechanism. For example, LncRZNFX1-AS1 promotes the invasion and spread of GC\(^12\), and LncCYTOR triggers GC progression by targeting miR-103/RAB10\(^13\). Mining and analyses of TCGA data showed that the expression level of THUMPD3-AS1 in GC tissues was significantly higher than that in normal tissues and was positively correlated with invasion and metastasis. We verified these findings by detecting the level of THUMPD3-AS1 in 50 GC tissues, revealing higher levels in cancer tissues than in paracancerous tissues. Functional experiments showed that THUMPD3-AS1 promotes the growth and metastasis of GC in vitro and in vivo. Studies have shown that THUMPD3-AS1 can promote the growth and invasion of colorectal cancer\(^14\), non-small cell lung cancer\(^15\), and liver cancer\(^16\) cells. These results show that THUMPD3-AS1 is generally highly expressed in tumours and has the characteristics of a proto-oncogene. However, another study suggested that the level of lncRNA THUMPD3-AS1 is significantly reduced in GC tissues and cells\(^17\). Further research is still needed to clarify these conflicting results. The present findings suggest that THUMPD3-AS1 may exert its function via the regulation of BCAT1, as it acts as a “sponge” for hsa-miR-1297 in cells.

The exogenous overexpression of miR-1297 in GC can inhibit cell proliferation and induce apoptosis, and the inhibition of miR-1297 can promote cell proliferation and reduce cell apoptosis in vitro\(^18\). miR-1297 can promote the progression of GC by regulating E2F3\(^19\) and can enhance GC cell growth by targeting
CREB1. Other studies have indicated that miR-1297 may act as an anti-tumour molecule in lung cancer, pancreatic cancer, and colorectal cancer. Indeed, miR-1297 exhibited low expression levels in GC tissues. A dual-luciferase reporter assay showed that THUMPD3-AS1 binds to miR-1297, and the tumorigenic effect of THUMPD3-AS1 in GC could be partially reversed by the overexpression of miR-1297.

BCAT1, as a key catalytic enzyme, is involved in the regulation of GC cell growth, and its overexpression is associated with TNM staging, local invasion, lymph node involvement, and metastasis. Studies have shown that miR-1297 is a tumour suppressor gene in GC. In this study, the dual-luciferase reporter assay showed that BCAT1 binds to miR-1297. When miR-1297 was overexpressed, BCAT1 levels in the transfection group were significantly lower than those in the vehicle control group and the negative control group. In contrast, when miR-1297 was inhibited, BCAT1 levels were significantly higher than those in the control groups. It was predicted that BCAT1 can act as a target of miR-1297. Previous reports have shown that BCAT1 overexpression promotes proliferation, invasion, and metastasis in bladder cancer, cervical cancer, hepatocellular carcinoma, and ovarian cancer. The results of this study verify the relationship between expression levels of miR-1297 and BCAT1 in GC and the effects of the correlation on cancer cell migration and invasion.

This study is the first to report the clinical significance and function of THUMPD3-AS1, miR-1297, and BCAT1 in GC. The three loci have binding sites for each other, further suggesting that THUMPD3-AS1 promotes tumour metastasis via the regulation of mRNA. We preliminarily conclude that the highly expressed lncRNA THUMPD3-AS1 regulates the expression of BCAT1 in the tumour by specifically reducing the expression of miR-1297, thereby promoting the invasion and EMT of GC cells. However, our study had some limitations. A number of bioinformatics analyses and cell experiments were performed; however, the number of clinical samples was small and prospective studies were lacking. Future studies will involve the collection of a larger number of clinical samples for more detailed analyses of THUMPD3-AS1, BCAT1, and miR-1297.

Declarations

Acknowledgements

Not applicable.

Ethics approval and consent to participate

Our study was reviewed and approved by the Ethics Committee of the Fourth Hospital of Hebei Medical University (ethics number: 2019MEC039).

Consent for publication

Not applicable.
Availability of data and materials

The data and materials in this study are available from the corresponding author on request.

Competing interests

The authors declare that they have no conflict of interest.

Funding

Not applicable.

Data availability statements

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

ZZ wrote the paper, BW LY made important revisions to the paper, WL. FQ JC collected the literature, TB approved the final version of the paper for publication. BT carried out the high-throughput sequencing experiments and performed the bioinformatics analysis. ZZ and TB confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Patient consent for publication

Not applicable

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References


Figures
Figure 1

Differentially expressed lncRNAs in gastric cancer. Histogram, volcano plots, and heat maps are shown. a: Histogram, b: volcano plots, c: heat maps
Figure 2

Figure 3

Results of a prognostic analysis and enrichment analysis of candidate ceRNA pairs. a: Prognostic value of candidate ceRNA pairs, b: GO and KEGG pathway enrichment analyses of candidate ceRNA pairs.
Figure 4

Expression levels of THUMP3-AS1-BCAT1 in tissues and cell lines and its prognostic value. a: Box plot (TCGA) of the differential expression of THUMP3-AS1 and BCAT1 in gastric cancer tissues, b: scatter plot of the differential expression in tissues, c: correlation analysis of THUMP3-AS1 and BCAT1 expression levels in gastric cancer tissues, d: Prognostic analysis based on TCGA data.
Figure 5

Effect of THUMPD3-AS1 on cell viability and invasion. a: Expression of THUMPD3-AS1 in different cell lines, b: THUMPD3-AS1-siRNA inhibition/overexpression assays, c: comparison of cell viability, d: alterations in migration, invasion, and levels of invasion- and EMT-related genes (MMP-2, MMP-9, TIMP-3, E-cadherin, N-cadherin, and Vimentin) after the inhibition and overexpression of THUMPD3-AS1.

Figure 6

In vivo validation results. a: tumour volume and weight, b: tumour comparison, c: detection of protein levels (western blotting).
Figure 7

Relationship between THUMPD3-AS1 and miR-1297 expression. a: Hypergeometric test results for the identification of target miRNAs of THUMPD3-AS1 and BCAT1, b: online prediction results using miRDB, c: expression levels of miR-1297 in tissues and cell lines, d: expression levels of THUMPD3-AS1 and miR-1297 in tissues were negatively correlated, e: effect of the overexpression and inhibition of THUMPD3-AS1 on miR-1297 levels, f: results of a dual-luciferase reporter assay.
Figure 8

Relationship between BCAT1 mRNA and miR-1297 levels. a: Online prediction using TargetScan and miRDB, b: BCAT1 mRNA expression in tissues, c: THUMPD3-AS1 levels were positively correlated with mRNA expression levels of BCAT1 in tissues, d: miR-1297 levels were negatively correlated with BCAT1 mRNA expression levels in tissues, e: expression levels of BCAT1 mRNA in different cell lines, f: effect of the overexpression of miR-1297 on BCAT1 mRNA and protein levels, g: effect of the inhibition of miR-1297 on BCAT1 mRNA and protein levels, h: Dual-luciferase reporter assay.
Figure 9

Effects of the THUMP3-AS1/miR-1297 axis on the activity, invasion, and migration ability of gastric cancer cells. a: Effect of THUMP3-AS1 inhibition on BCAT1 mRNA and protein levels in cells (control group, negative group, THUMP3-AS1-siRNA group, THUMP3-AS1-siRNA+miR-1297 inhibition group), b: Effect of THUMP3-AS1 overexpression on BCAT1 mRNA and protein levels in cells (negative group, THUMP3-AS1 overexpression group, THUMP3-AS1 overexpression + miR-1297 mimics group), c: Effects of THUMP3-AS1 inhibition on cell viability, migration, and invasion (vehicle control group, negative control group, THUMP3-AS1-siRNA group, THUMP3-AS1-siRNA+miR-1297 inhibition group), d:
Effect of THUMPD3-AS1 overexpression on cell migration and invasion (negative control group, THUMPD3-AS1 overexpression group, THUMPD3-AS1 overexpression+miR-1297 mimics group).