Overexpression of antisense long non-coding RNA ZNF710-AS1-202 promotes cell proliferation and inhibits apoptosis of clear cell renal cell carcinoma via regulation of ZNF70 expression

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Abstract. Antisense long non-coding RNAs (AS lncRNAs) have been increasingly recognized as important regulators of gene expression and have been found to play crucial roles in the development and progression of tumors. The present study explored the roles of AS IncRNA ZNF710-AS1-202 in clear cell renal cell carcinoma (ccRCC). The expression levels of ZNF710-AS1-202 were detected in 46 human ccRCC tissues and 34 healthy adjacent renal tissues. The associations between the levels of ZNF710-AS1-202 expression and the clinicopathological features of the patients were evaluated by the $\chi^2$ test. Gain- and loss-of-function experiments were performed to analyze the role of ZNF710-AS1-202 in ccRCC cell proliferation and survival in vitro. Reverse transcription-quantitative PCR and/or western blotting were employed to detect ZNF710-AS1-202, zinc finger protein 710 (ZNF710) and cyclin B1 expression. The Cell Counting Kit-8 and colony formation assays, as well as flow cytometry, were used to detect cell proliferation or apoptosis. The subcellular localization of ZNF710-AS1-202 was analyzed by RNA fluorescence in situ hybridization. The results revealed that ZNF710-AS1-202 was downregulated in human ccRCC tissues and was associated with the pathological grade, tumor size, local invasion and TNM stage, but not with lymph node metastasis or distant metastasis. However, ZNF710-AS1-202 overexpression promoted the proliferation of RCC cells and inhibited apoptosis. Opposite results were observed when ZNF710-AS1-202 was knocked down by small interfering RNA. Furthermore, ZNF710-AS1-202, which was mainly expressed in the cytoplasm of RCC cells, regulated ZNF710 mRNA and protein expression in opposing manners. In conclusion, the present study revealed that ZNF710-AS1-202 and ZNF710 may serve as promising therapeutic targets for ccRCC.

Introduction

Renal cell carcinoma (RCC), which originates from renal parenchyma urinary tubular epithelial cells, is one of the most common malignant tumors of the urinary system (1-3). Clear cell RCC (ccRCC) accounts for ~80% of all RCC cases (1,3-6). As ccRCC is not sensitive to chemoradiotherapy (7), surgical resection is the main treatment strategy for the disease (1-3,8). However, the rate of recurrence and distant metastasis remains as high as ~30% (3,5,8). Therefore, the identification of novel therapeutic targets is urgently required to prevent the progression of ccRCC.

Increasing evidence suggests that long non-coding RNAs (lncRNAs) are involved in the occurrence and progression of ccRCC (2-4,6). lncRNAs, defined as transcribed RNA molecules >200 nt in length (9-12), are an important class of non-coding RNAs involved in several biological functions (12-17). Antisense lncRNAs (AS lncRNAs), a subclass of lncRNAs, are transcribed from complex genetic loci on the opposite strands of sense protein-coding genes (13-15,18-20). AS lncRNAs may overlap exons and/or introns of their associated sense protein-coding transcripts to regulate epigenetic silencing, transcription and mRNA stability by forming sense-antisense pairs (18-24). The genomic arrangement of AS lncRNA genes also suggests that they may be involved in pathways that allow genes to regulate their own expression (23).

In the present study, RNA-sequencing (seq) was applied to detect changes in the transcriptome of five paired surgically resected ccRCC and para-cancerous (PC) tissues. The five AS lncRNAs with the most significant differences in expression were selected and AS lncRNA ZNF710-AS1-202 was subsequently chosen for further experimentation.
ZNF710-AS1-202 is transcribed from the ZNF710-AS1 gene, which is antisense to zinc finger protein 710 (ZNF710). The ZNF710-AS1 gene has two transcripts, ZNF710-AS1-201 and ZNF710-AS1-202. The ZNF710-AS1-202 transcript is wholly mapped to the fourth intron of the ZNF710-201 transcript. The present study revealed that ZNF710-AS1-202 negatively regulated ZNF710 mRNA expression and positively regulated ZNF710 protein expression. Furthermore, Uniprot analysis revealed that ZNF710 is involved in transcription regulation.

Reverse transcription-quantitative PCR (RT-qPCR) verified that ZNF710-AS1-202 expression was significantly decreased in ccRCC tissues and was associated with the pathological grade, tumor size, local invasion and TNM stage, suggesting that ZNF710-AS1-202 exhibited antitumor effects. However, ZNF710-AS1-202 overexpression significantly increased the proliferation of RCC cells. Opposite results were observed when ZNF710-AS1-202 was knocked down by small interfering (si) RNA. Furthermore, the levels of ZNF710-AS1-202 expression in ccRCC cells was significantly increased compared with in normal cells.

Materials and methods

Patient samples. A total of 34 pairs of ccRCC tissues and corresponding PC tissues, as well an additional 12 ccRCC tissues, were obtained from surgeries performed between May 2016 and December 2018 at the First Affiliated Hospital of Zhengzhou University (Zhengzhou, China). The age of the included patients ranged from 28-72 years old, and the ratio of male to female was 32:14. All samples were immediately frozen in liquid nitrogen and stored at -80˚C until RNA extraction. The postoperative pathology of all tumor specimens in the present study was ccRCC. Tumor specimens whose postoperative pathology was not ccRCC were excluded from the present study.

Databases. Ensembl database (http://asia.ensembl.org/index.html) was used to query the gene sequences of ZNF710-AS1 and ZNF710. The GEPIA website (http://gepia2.cancer-pku.cn/#analysis) was used to analyze TCGA data using its preset program. Moreover, the Uniprot database (https://www.uniprot.org/) was used to search for information on the ZNF710 protein.

Gene expression profile analysis. A total of five paired ccRCC and PC tissues were analyzed by high throughput RNA-seq. A paired Student's t-test was used to compare the AS lncRNA levels between ccRCC and PC tissues.

Cell culture and reagents. The human RCC cell lines (786-O, ACHN and 769-P) and the human renal tubular epithelial cell line (HK-2) were obtained from The Cell Bank of the Type Culture Collection of the Chinese Academy of Sciences. 786-O and 769-P cells were cultured in RPMI-1640 medium (GE Healthcare Life Sciences) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.). ACHN cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM; GE Healthcare Life Sciences) containing 10% FBS. HK-2 cells were cultured in DMEM/F12 medium (GE Healthcare Life Sciences) with 10% FBS. Penicillin (100 U/ml) and streptomycin (100 µg/ml) were used in the culture of all of these cell lines. All cells were maintained at 37˚C in a humidified atmosphere containing 5% CO2.

Cell transfection. pcDNA3.1-ZNF710-AS1-202 and pcDNA3.1-NC vectors were designed and synthesized by Hanbio Biotechnology Co., Ltd. and transfected into 786-O and ACHN cells using Lipofectamine™ 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) adding 2.5 µg plasmids into each well of the 6-well plates or 0.1 µg per well of the 96-well plates according to the manufacturer’s protocol. Small interfering (si)RNAs were designed and synthesized by Guangzhou RiboBio Co., Ltd. Then, 75 pmol siRNAs were added into each well of the 6-well plates and 3 pmol each well of the 96-well plates, according to the manufacturer's protocol of Lipofectamine™ 3000. The following sequences were used: SiZNF710-AS1-202#1, 5'-CCCACATGCACTGATT-3'; SiZNF710-AS1-202#2, 5'-TCCCATACATCTTATTGAA-3'; and Si negative control, 5'-TTTCCGGAACGTTACAGT-3'. Cells were harvested for RNA and protein extraction 48 and 72 h after transfection, respectively.

RNA extraction and strand-specific RT-qPCR. Total RNA from ccRCC tissues and cells was extracted using TRIzol® reagent (Beijing Leagene Biotech Co., Ltd.) according to the manufacturer's protocol. Total RNA was treated with gDNA Remover (Toyobo Life Science) at 37˚C for 15 min and then reverse transcribed into cDNA separately with strand-specific primers (25) using the ReverTra Ace qPCR RT Master Mix (Toyobo Life Science) with the following conditions: 37˚C for 15 min, and 98˚C for 5 min. qPCR was performed using a SYBR Green Mix (Roche Diagnostics) and a QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific, Inc.) in a 20 µl reaction mixture using the following conditions: Initial denaturation at 95˚C for 10 min, followed by 40 cycles of 95˚C for 10 sec, 60˚C for 10 sec and 72˚C for 45 sec, then followed by one cycle of 95˚C for 15 sec, 60˚C for 1 min. The heating rate of all the above steps was 1.6˚C/sec. Then, the temperature was increased to 95˚C at 0.15˚C/sec from 60˚C, remaining at 95˚C for 1 sec. The following primer pairs were used for qPCR: ZNF710-AS1-202 forward, 5'-CTTACTACTGCACCAGTGT-3' and reverse, 5'-CCGCTCTGATGTGTT-3'; ZNF710-AS1-201 forward, 5'-AAAGACTGTTGGAGAGGTT-3' and reverse, 5'-GGCGCCGAAAGATAGG-3'; ZNF710 forward, 5'-TTCCTACTACGCGACAGTGT-3' and reverse, 5'-GTCAGGGTGGCCCTTGGAGTT-3'; β-actin forward, 5'-CTTGCGACCCACGAC-3' and reverse, 5'-GGCCGGAGGACTCCTACAC-3'; and GAPDH forward, 5'-CAGGAGGCTTGGTATGAT-3' and reverse, 5'-GAAAGGCTTGGGCTCATTT-3'. mRNA levels were quantified using the 2-ΔΔct method (26) and normalized to the internal reference genes GAPDH or β-actin.

Western blot analysis. RCC cells were lysed with RIPA buffer (Beyotime Institute of Biotechnology) and protein concentrations were determined with a bicinchoninic acid protein assay kit (Beijing Leagene Biotech Co., Ltd.). Protein samples (25 µg/lane) were separated by SDS-PAGE on 12% gels and transferred onto PVDF membranes. The membranes were
blocked by 10% skim milk for 1 h at 25°C and then were incubated with primary antibodies against ZNF710 (cat. no. bs-4373R; 1:1,000; BIOSS), cyclin B1 (cat. no. ab32053; 1:10,000; Abcam) and β-actin (cat. no. ab8227; 1:5,000; Abcam) overnight at 4°C. Following primary antibody incubation, the membranes were incubated with a horseradish peroxidase-conjugated anti-rabbit secondary antibody (cat. no. ab6721; 1:10,000; Abcam) at 25°C for 1 h. Protein bands were subsequently visualized using an enhanced chemiluminescence reagent kits (Beyotime Institute of Biotechnology) and scanned by an imaging system (Bio-Rad Laboratories, Inc.). The densitometry was quantified using ImageJ software v1.8.0 (National Institutes of Health).

Cell Counting Kit-8 (CCK-8) assay. RCC cells were seeded in 96-well plates at a density of 3,000 cells/well in sextuplicate and cultured in 100 µl complete media with 10% FBS. Cells were subsequently transfected with the plasmid vectors or siRNAs using Lipofectamine™ 3000 according to the manufacturer's protocol. At the indicated time points (0, 24, 48, 72, 96, 120 and 144 h post-transfection), 10 µl CCK-8 reagent (dojindo Molecular Technologies, Inc.) was added to each well and incubated for 90 min according to the manufacturer's instructions. Then, the absorbance at a wavelength of 450 nm was measured using a microplate reader.

Cell cycle analysis. Cells were collected 48 h after transfection and washed twice with PBS. The cells were fixed with ice-cold 75% ethanol and incubated at 4°C overnight. Fixed cells then were washed twice with PBS and incubated with 500 µl propidium iodide (PI) solution with rna-seq was used to detect changes in the transcriptome of cc rcc and PC tissues. The five AS lncRNAs with the most significant

| Table I. Relevance analysis of LncZNF710-AS1-202 expression in patients with ccRCC. |
|------------------|-------|-------|-------|
| Variable         | Patients | Low | High | P-value |
| Cases            | 46     | 23   | 23   | 0.536   |
| Age, years <60   | 30     | 16   | 14   |         |
| Age, years ≥60   | 16     | 7    | 9    |         |
| Sex Male         | 32     | 16   | 16   |         |
| Sex Female       | 14     | 7    | 7    |         |
| Tumor diameter, cm <5 | 12 | 3   | 9    | 0.044  |
| Tumor diameter, cm ≥5 | 34 | 20  | 14   |         |
| T Stage T1       | 22     | 7    | 15   | 0.018   |
| T Stage T2-4     | 24     | 16   | 8    |         |
| N Stage N0       | 27     | 12   | 15   | 0.369   |
| N Stage N1, Nx   | 19     | 11   | 8    |         |
| M Stage M0       | 36     | 17   | 19   | 0.475   |
| M Stage M1       | 10     | 6    | 4    |         |
| TNM Stage I      | 17     | 4    | 13   | 0.006   |
| TNM Stage II-IV  | 29     | 19   | 10   |         |
| Grade I          | 12     | 3    | 9    | 0.044   |
| Grade II-IV      | 34     | 20   | 14   |         |

Low/high by the sample mean. Pearson χ²-test. *P<0.05. ccRCC, clear cell renal cell carcinoma; lncRNA, long non-coding RNA.

Apoptosis assay. The cells were trypsinized without EDTA and washed twice with cold PBS. Subsequently, the cells were stained using the Annexin V-FITC/PI kit (BD Biosciences; Becton, Dickinson and Company) at room temperature for 15 min in the dark according to the manufacturer's protocol. The cells were subsequently analyzed using a flow cytometer (FACScan®; BD Biosciences; Becton, Dickinson and Company) and data were processed using ModFit LT 5.0 (Verity Software House).

RNA fluorescent in situ hybridization (FISH). The Cy3-labeled ZNF710-AS1-202 probes were designed and synthesized by Guangzhou RiboBio Co., Ltd. The probes were ~27 nucleotides in length. ZNF710-AS1-202 was hybridized in situ with the Cy3-labeled ZNF710-AS1-202 probes in RCC cells according to the manufacturer's protocol of Ribo™ Fluorescent In Situ Hybridization kit (RiboBio Co., Ltd.). RCC cells were fixed in 4% paraformaldehyde at room temperature for 10 min. For permeabilization, RCC cells were incubated in 1X PBS with 0.5% Triton X-100 at 4°C for 5 min. For pre-hybridization, RCC cells were incubated in pre-hybridization buffer at 37°C for 30 min. Enhanced antifade mounting medium (Beijing Leagene Biotech Co., Ltd.) was used at room temperature in the dark after hybridization, according to the manufacturer's protocol. Subcellular localization of ZNF710-AS1-202 was detected by laser scanning confocal microscopy (Carl Zeiss AG; ZEN System).

Statistical analysis. Data are expressed as the mean ± standard deviation of three independent experiments. Statistical analyses were performed using GraphPad Prism software (version 8.0; GraphPad Software, Inc.). The groups were analyzed using the Student's t-test, Mann Whitney test, one-way analysis of variance followed by Student-Neuman-Keuls test, the χ² test, or the log-rank test as applicable. P<0.05 was considered to indicate a statistically significant difference.

Results

ZNF710-AS1-202 is downregulated in ccRCC tissues and is associated with clinicopathological features. RNA-seq was used to detect changes in the transcriptome of ccRCC and PC tissues. The five AS IncRNAs with the most significant
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Differences in expression were identified (Fig. 1A) and lncRNA ZNF710-AS1-202 was subsequently selected for further experimentation.

ZNF710-AS1-202 expression was analyzed in a cohort of 34 pairs of ccRCC tissues and corresponding PC tissues as well as an additional 12 ccRCC tissues using RT-qPCR. ZNF710-AS1-202 was significantly downregulated in human ccRCC tissues (n=34) compared with their corresponding PC tissues (n=34), which was consistent with the results of RNA-seq and The Cancer Genome Atlas (TCGA) analysis (Fig. 1A-C).

Furthermore, a total of 46 human ccRCC tissue samples was used for the analysis between clinicopathological data and expression level of ZNF710-AS1-202. As the results show, the levels of ZNF710-AS1-202 were associated with the pathological grade, tumor size, local invasion and TNM stage, but not with lymph node metastasis or distant metastasis (Table I).

These results were validated with TCGA data (27) (Fig. 1D). What's more, TCGA analysis revealed that the overall survival time as well as the disease-free survival time of patients with higher ZNF710-AS1-202 levels tended to be longer than that of patients with lower ZNF710-AS1-202 levels (Fig. 1E and F). However, the expression levels of ZNF710-AS1-202 were
higher in the ccRCC cell lines compared with the HK-2 cells in vitro (Fig. 1G).

**ZNF710-AS1-202 overexpression promotes ccRCC cell proliferation and survival, and inhibits apoptosis.** RT-qPCR analysis revealed that ZNF710-AS1-202 expression was significantly increased after transient transfection with pcDNA3.1-ZNF710-AS1-202 compared with the control cells (Fig. 2A). The CCK-8 assay revealed that the proliferation of ACHN and 786-O cells transfected with pc-ZNF710-AS1-202 was significantly increased compared with the control cells (Fig. 2B and C). Similarly, ZNF710-AS1-202 overexpression
ZnF710-aS1-202 ProMoTeS ccrcc cell ProliFeraTion and reGulaTeS ZnF710 eXPreSSion

significantly enhanced the colony formation ability of RCC cells (Fig. 2D). A significant and reproducible increase in the S phase and a decrease in the G2/M phase were observed following ZnF710-aS1-202 overexpression in RCC cells (Fig. 2E-G) (20). Additionally, western blot analysis revealed a significant increase in cyclin B1 expression in ACHN and 786-O cells overexpressing ZnF710-aS1-202 (Fig. 2H and I). This suggested that ZnF710-aS1-202 overexpression promoted cell proliferation. Moreover, ACHN and 786-O cells transfected with the ZnF710-aS1-202 overexpression plasmid exhibited lower levels of apoptosis compared with the control cells (Fig. 2J).

ZnF710-aS1-202 is mainly expressed in the cytoplasm of RCC cells. ZnF710-aS1-202 transcripts were mainly expressed in cytoplasm of ACHN, 786-O and 769-P cells (Fig. 3).

Depletion of ZnF710-aS1-202 inhibits ccRCC cell proliferation and survival, and promotes apoptosis. After siZnF710-aS1-202 transfection, ZnF710-aS1-202 expression was significantly decreased compared with control cells (Fig. 4A). Furthermore, ZnF710-aS1-202 knockdown significantly decreased the proliferation rate in 786-O and 769-P cells (Fig. 4B and C). The colony formation assay revealed that the colony formation ability of ccRCC cells was impaired after ZnF710-aS1-202 knockdown (Fig. 4D). Furthermore, ZnF710-aS1-202 knockdown increased the number of cells in the G2/M phase and decreased the number of cells in the S phase (Fig. 4E). Western blotting revealed that ZnF710-aS1-202 knockdown downregulated cyclin B1 protein expression in 786-O and 769-P cells (Fig. 4F). Moreover, 786-O and 769-P cells transfected with siZnF710-aS1-202...
exhibited higher levels of apoptosis compared with the control cells (Fig. 5).

Dysregulation of ZNF710-AS1-202 causes significant changes in ZNF710 mRNA and protein expression. ZNF710 is the sense protein-coding gene relative to ZNF710-AS1 (Fig. 6A). ZNF710-AS1-202 downregulated the expression of ZNF710 mRNA and upregulated the expression of ZNF710 protein (Fig. 6B-E and K).

TCGA analysis revealed that there was a significant difference in ZNF710 mRNA expression levels in ccRCC tissues with different pathological grades (Fig. 6F).
Furthermore, the low level of ZNF710 mRNA expression was associated with poor prognosis (Fig. 6G and H).

The expression of ZNF710-AS1-201, which is the other isoform of ZNF710-AS1, was not significantly changed after ZNF710-AS1-202 overexpression in RCC cells (Fig. 6I). Unlike ZNF710-AS1-202, the expression of ZNF710-AS1-201 tended to be upregulated in ccRCC tissues. Furthermore, increased expression of ZNF710-AS1-201 was significantly associated with poor overall survival in patients with ccRCC (Fig. 6J).

Discussion

The results from the present study revealed that ZNF710-AS1-202 promoted the proliferation and inhibited apoptosis of RCC cells in vitro. However, ZNF710-AS1-202 expression was downregulated in ccRCC tissues compared with PC tissues. These contradictory results may be explained if the downregulation of ZNF710-AS1-202 expression is considered as negative feedback regulation in response to the body’s endogenous antitumor activity. This phenomenon also suggests that not all downregulated genes in tumor tissues are tumor suppressor genes. Furthermore, in the ccRCC cell lines investigated in vitro, the cell lines with high ZNF710-AS1-202 expression exhibited faster proliferation rates.

The ZNF710-AS1-202 transcript plays an important role in regulating ZNF710 expression. The effect of ZNF710-AS1-202 on RCC cells may be achieved through the ZNF710 protein (19,23,28). Therefore, further research is required to elucidate the mechanism by which ZNF710-AS1-202 downregulated ZNF701 mRNA expression and upregulated ZNF710 protein expression.

The present study used FISH to reveal that lncRNA ZNF710-AS1-202 is distributed in both the cytoplasm and the nucleus of RCC cells. ZNF710-AS1-202 in the nucleus may bind to the fourth intron of ZNF710-201 pre-mRNA to form complimentary strands, thereby inhibiting ZNF701 pre-mRNA processing such as RNA splicing. In the cytoplasm of RCC cells, lncRNA ZNF710-AS1-202 may recruit polyribosomes to induce the expression of the ZNF710 protein. As ZNF710-AS1-202 is mainly distributed in the cytoplasm, the expression level of ZNF710 protein was upregulated.

A hypothesis for the downregulation of ZNF710-AS1-202 expression in ccRCC tissues observed in the present study is that only the expression of the ZNF710-AS1-202 in the nucleus was downregulated (19). Therefore, further research is required to verify whether the expression of ZNF710 protein was upregulated in ccRCC tissues compared with adjacent normal tissues using techniques such as immunohistochemistry.

While there was no significant difference in the levels of ZNF710 mRNA expression between ccRCC and PC tissues from TCGA database, low levels of ZNF710 mRNA expression were associated with poor prognosis in patients with ccRCC.

To the best of our knowledge, this is the first study to investigate the AS lncRNA ZNF710-AS1-202, the ZNF710 protein and their associations. The Uniprot database revealed that ZNF710 is a transcription factor/cofactor that belongs to the Kruppel C2H2-type zinc-finger protein family and is closely related to gene transcription by binding to DNA (29). RIP technology may be used to identify the gene sequence ZNF710 binds to and to explore the downstream molecules in this pathway (28).

Exogenous ZNF710-AS1-202 was thought to promote the ZNF710-AS1 gene to express ZNF710-AS1-201 rather than ZNF710-AS1-202. However, the RT-qPCR results in the present study revealed that there was no significant change in the expression of ZNF710-AS1-201 after ZNF710-AS1-202 overexpression. Furthermore, TCGA analysis demonstrated that there was no significant difference in the expression of ZNF710-AS1-201 between ccRCC and adjacent healthy tissues. However, ZNF710-AS1-201 expression was closely related to the prognosis of patients with ccRCC. In contrast to ZNF710-AS1-202, the high expression level of ZNF710-AS1-201 in ccRCC tissues is associated with poor prognosis in patients with ccRCC. The first exon of ZNF710-AS1-201 overlaps the last 769 nucleotides of the fifth exon of ZNF710-201 mRNA and the second exon of ZNF710-AS1-201 overlaps 399 nucleotides of the fourth intron of ZNF710-201 pre-mRNA. Therefore, ZNF710-AS1-201 may also be relevant to the expression of ZNF710 (28).
Figure 6. Dysregulation of ZNF710-AS1-202 causes significant changes in ZNF710 expression at both the mRNA and protein levels. (A) Schematic of the lncRNA-ZNF710-AS1 (green) and ZNF710-201 transcript (yellow) from ENSEMBL. The levels of ZNF710 expression in RCC cells was detected by (B) reverse transcription-qPCR or (C) western blotting following ZNF710-AS1-202 overexpression. The levels of ZNF710 expression in RCC cells was detected by (D) reverse transcription-qPCR or (E) western blotting ZNF710-AS1-202 suppression. (F) The levels of the ZNF710-201 transcript in different pathological grade ccRCC tissue samples obtained from TCGA (from GEPIA). (G) Overall survival and (H) disease free survival of patients with ccRCC in TCGA according to ZNF710-201 expression (from GEPIA). (I) The expression levels of ZNF710-AS1-201 in RCC cells following ZNF710-AS1-202 overexpression. (J) Overall survival of patients with ccRCC in TCGA according to ZNF710-AS1-201 transcript expression (from GEPIA). (K) Model of ZNF710-AS1-202-dependent regulation of ZNF710 mRNA and protein. In the nucleus of RCC cells, ZNF710-AS1-202 tethered to the fourth intron of ZNF710 pre-mRNA and formed complimentary strands that inhibited ZNF710 mRNA splicing. In the cytoplasm, ZNF710-AS1-202 induced the expression of ZNF710 protein. **P<0.01 and ***P<0.001 vs. corresponding pc-NC or SiNC. ZNF710, zinc finger protein 710; TCGA, The Cancer Genome Atlas; ccRCC, clear cell renal cell carcinoma; GEPIA, Gene Expression Profiling Interactive Analysis; Inc, long noncoding; OD, optical density; ns, not significant; siNC, small interfering negative control.
In conclusion, the results of the present study suggested that ZNF710-AS1-202 and ZNF710 may be used as promising therapeutic targets for ccRCC. However, one drawback of the present study was that the in vitro results were not validated by in vivo experiments. Additionally, rescue experiments may be helpful to clarify the regulatory effects of ZNF710-AS1-201 and ZNF710-AS1-202 on ZNF710. Furthermore, the function of the ZNF710 protein in ccRCC cells was not investigated and the mechanisms by which ZNF710-AS1-202 regulated the ZNF710 mRNA and protein levels were not explored. Future studies are required to investigate the mechanism of the ZNF710-AS1-ZNF710 axis to identify specific markers and targets for the diagnosis and treatment of ccRCC.

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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

GL and MX performed the experiments and analyzed the data, and were major contributors in writing the manuscript. ZH performed high throughout RNA-sequencing. PL performed the western blot assay. HL, ZZ, YD, ZJ and JY conceived and performed high throughout RNA-sequencing. Pl performed GL and MX performed the experiments and analyzed the data.

Competing interests

The authors declare that they have no competing interests.

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