LncRNA-URHC Functions as a ceRNA to Regulate Danjb9 Expression by Competitively Binding to miR-5007-3p in Hepatocellular Carcinoma

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Abstract

Background: Hepatocellular carcinoma (HCC) is often diagnosed at a late stage, when the prognosis is poor. The regulation of long non-coding RNAs (lncRNAs) plays a crucial role in HCC. However, the precise regulatory mechanisms of lncRNA signaling in HCC remain largely unknown. We study aim to investigate the underlying mechanisms of lncRNA (upregulated in hepatocellular carcinoma) URHC in HCC.

Methods: RT-qPCR, fluorescence in situ hybridization (FISH) staining, EdU, colony formation, and tumor xenografts experiments were used to identify localized and biological effects of URHC on HCC cells *in vitro* and *in vivo*. The bioinformatics analysis, Dual-luciferase reporter assay, and rescue experiments revealed the potential mechanism of URHC.

Results: URHC silencing may inhibit the HCC cells proliferation *in vitro* and *in vivo*. We found that URHC was mainly localized in the cytoplasm. The expression of miR-5007-3p was negatively regulated by URHC. And miR-5007-3p could reverse the effect of URHC in HCC cells. The expression of DNAJB9 was negatively regulated by miR-5007-3p but positively regulated by URHC. These suggesting of lncRNA-URHC positively regulated the level of DNAJB9 by sponging miR-5007-3p.

Conclusion: Together, our study elucidated the role of URHC as a miRNA sponge in HCC, and shed new light on lncRNA-directed diagnostics and therapeutics in HCC.

Background

Hepatocellular carcinoma (HCC) is the most common type of liver tumor worldwide. It has become the second leading cause of cancer-related death in China [1, 2]. Tumor resection is currently the best treatment choice among the several therapeutic methods available. However, HCC is often diagnosed at a late stage, when the prognosis is poor, and many patients die within 1 year after the detection of HCC [3]. Therefore, enhancing our understanding of the molecular mechanisms in HCC is essential for the development of effective treatment strategies. Recent experimental studies provide strong evidence that lncRNAs play an important role in cancer progression, and the abnormal expression of lncRNAs has been found in the HCC [4-7]. For example, lncRNA HOST2 can activate the JAK2-STAT3 signaling pathway promote epithelial-mesenchymal transition, proliferation, invasion and migration of HCC cells ⁷. A lncRNA PVT1/miR-150/HIG2 axis regulates the proliferation, invasion, and balance of iron metabolism in HCC [8]. lncRNAs have been shown to function as miRNA sponges, which interact with miRNAs and play crucial roles in human carcinogenesis by modulating the expression of miRNA target genes [9-12]. These may contribute to the development of new effective therapeutic strategies to improve the prognosis of HCC patients.

lncRNAs are a class of conserved non-protein-coding RNAs with more than 200 nucleotides that are widely distributed in the human genome and reside in the nucleus or cytoplasm [13-15]. Many aberrant expression of lncRNAs have been found to be associated with human diseases, in particular, cancers [16, 17]. They can regulate gene expression in cis or in trans by diverse mechanisms [18-20], and have been

demonstrated to cause dysregulation of lncRNAs and impact on cellular functions such as cell proliferation, apoptosis, migration, invasion, tumorigenicity, and metastasis, by interacting with DNA, RNA, and proteins [21, 22]. miRNAs are defined as small ncRNAs with approximately 19–25 nucleotides that modulate gene expression via binding to the 3'-untranslated region (3'-UTR) of their target mRNAs, leading to translation inhibition or degradation [23, 24]. By negatively regulating the expression of target genes, miRNAs play a significant role at the posttranscriptional level and participate in a large number of biological processes, including cell proliferation, cell cycle, apoptosis, and differentiation [25–27]. Recent studies have revealed that lncRNAs can function as a competing endogenous RNAs (ceRNAs) to regulate the expression pattern and biological characteristics of miRNAs [28, 29].

In the present study, we investigated the biological effects of URHC overexpression on cell proliferation in HCC. Bioinformatics prediction and experimental analysis confirmed that URHC directly targeted miR-5007-3p to regulate its expression. Furthermore, mechanistic analysis revealed that URHC positively regulated DNAJB9 by sponging miR-5007-3p, indicating that it plays an oncogenic role in HCC pathogenesis. Together, these results suggest that the molecular mechanisms of URHC-miR-5007-3p-DNAJB9 axis are likely to be enriched in HCC.

Methods

Cell culture and transfection

The human HCC cell line Hep3B and 293T cells were purchased from ATCC. The normal human liver cell line MIHA was purchased from YaJi Biological (Shanghai, China). HCC cells Bel-7402 and Bel-7404 were obtained from Beina Biological (Beijing, China) in 2017. The SMMC-7721 was bought from Fenghui Biotechnologies Inc (Hunan, China). MIHA, SMMC-7721, Bel-7402, and Bel-7404 cells were cultured in RPMI 1640 (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS, Invitrogen). Hep3B and 293T were cultured in Dulbecco's modified Eagle medium (DMEM, Invitrogen) containing 10% FBS. All cells were incubated in a humidified atmosphere of 5% CO₂ at 37°C. Dimethyl sulfoxide was purchased from Sigma-Aldrich (St. Louis, MO).

Scrambled siRNA of URHC (siRNA-Con) and URHC siRNAs were purchased from GenePharma (Shanghai, China). miR-5007-3p inhibitor, inhibitor negative control (NC inhibitor), miR-5007-3p mimic and NC mimic were also purchased from GenePharma (Shanghai, China). Full-length URHC cDNA was subcloned into GV230 lentiviruses (Genechem, Shanghai, China) and infected into Bel-7404 and Hep3B cells to generate URHC-overexpressing cells. Lipofectamine 2000 Reagents (Invitrogen Co, USA) were used for cell transfections.

Human tissue samples

We obtained 26 pairs of primary HCC and adjacent non-tumor tissues from patients undergoing surgery at Xijing Hospital, the Fourth Military Medical University; all diagnoses were based on a biopsy. These

tissues were immediately frozen in liquid nitrogen after surgical resection. All patients provided written informed consent, and these studies were approved by the Ethics Review Committees of Xijing Hospital.

Reverse transcription and quantitative real-time PCR.

Total RNA was prepared from the indicated cells using TRIzol reagent (Invitrogen) based on the manufacturer's instructions. One microgram of total RNA was converted to cDNA using PrimeScript® RT Master Mix Perfect Real-Time (Takara, Inc). SYBR Premix EX Taq II (TaKaRa) was used based on the manufacturer's instructions under the following conditions: 95°C for 2 min, 95°C for 15 s, and 60°C for 60 s for 40 cycles. miR-5007-3p expression levels were quantified using MicroRNA First-Strand Synthesis and miRNA Quantitation Kits including U6 control primer (Takara) based on the manufacturer's instructions. The results were analyzed based on the $2^{-\Delta\Delta Cq}$ formula. The primers used for RT-qPCR were as follows: b-actin: sense 5'-CTGGAACGGTGAAGGTGACA-3', and antisense 5'-CGGCCACATTGTGAACTTTG-3'; URHC: sense 5'-TGTTTATGTGAGAGGAGAAAGGAAG-3'; and antisense 5'-CACTAGAGGTCTGCAAATAAAGTGA-3'; DNAJB9: sense 5'-TAGGCACACACCACCACATC-3'; and antisense 5'-CTTTGGGAGGCCAAGGTAGG-3'; and miR-5007-3p 5'-CCATATGAACCAAACCTCTAATA-3'. The internal control genes were b-actin for URHC, DNAJB9, and U6 snRNA for miR-5007-3p.

Fluorescence in situ hybridization

SMMC-7721 and Hep3B cells were plated to achieve 70% confluency for staining. Frozen sections of HCC tissues were treated with 4% DNase/RNase-free paraformaldehyde. After fixation, the sections were treated with Proteinase K (20 mg/ml) for 5 min, and then washed three times with phosphate-buffered saline (PBS). The cells were incubated with a prehybridization solution for 1 h at 37°C. Then, the prehybridization solution was removed, and the cells were covered with a URHC probe (8 ng/μl) hybridization solution overnight in a 37°C incubator. The slices were washed three times using Wash Buffer Solution. The cells were extensively washed three times using Wash Buffer Solution, counterstained with DAPI, and mounted with an anti-fade reagent (Invitrogen). The URHC probe for fluorescence in situ hybridization (FISH) was: 5'-GFP-AGTACATACTCACTACACTAGAGGTCTGCA-GFP-3' (Servicebio, China). The mir-5007-3p probe for FISH was: 5'-Cy3-ATTAGAGTTTGGTTCATATGAT-Cy3-3' (Servicebio).

CCK-8 and colony formation assays

The CCK-8 assay was used to assess HCC cell proliferation. HCC cells were seeded in a 96-well plate at a density of 2×10^3 cells per well. Cell proliferation was measured at 24, 48, 72, and 96 h. CCK-8 solution (10mL) was added to each well, and incubated for 1 h at 37°C, in a 5% CO₂ incubator. After incubation, the optical density (OD) 450 value was read using an Epoch Spectrophotometer (USA). Experiments were repeated at least three times.

The colony formation assay was performed to measure the capacity of cell proliferation. SMMC-7721 and Hep3B cells (300 cells/well) were seeded in a six-well plate and cultured for 10 days. Colonies were

then fixed with methanol for 15 min and stained for 10 min with 0.5% crystal violet. Then, colonies were imaged with a Phone and analyzed by ImageJ software. The assays were repeated at least three times.

5-Ethynyl-2'-deoxyuridine (EdU) assay

Cell proliferation was also determined by the 5-ethynyl-2'-deoxyuridine assay using an Apollo®488 EdU Kit (RIBOBIO, Guangzhou, China). The EdU assay was performed based on the manufacturer's instructions. The cells were then visualized under a fluorescence microscope (20 × 10). To assess cell proliferation, the ratio of EdU-stained cells (with red fluorescence) to Hoechst-stained cells (with blue fluorescence) was calculated. Experiments were repeated at least three times.

Luciferase reporter assay

The luciferase reporter assay was performed in 293T cells. The 293T cells were seeded in a six-well plate and cotransfected with luciferase reporter constructs encoding the wild-type 3'-UTR region of DNAJB9 or URHC (DNAJB9/URHC-WT-3'-UTR) or a mutated 3'-UTR region of DNAJB9 or URHC 3'-UTR region (DNAJB9/URHC-MUT-3'-UTR) (RIBOBIO, Guangzhou, P.R. China) and miR-5007-3p mimic or mi-NC using Lipofectamine 2000 (Invitrogen). After 48 h of incubation, the cells were washed with PBS and lysed with Passive Lysis Buffer (Promega). Firefly and Renilla luciferase activities were measured using the Dual-Luciferase Reporter Assay Kit (Promega) based on the manufacturer's protocol.

Western blot assay

Cells were lysed using RAPI buffer (Beytime, China). Total proteins were quantified using the BCA Protein Assay Kit (Beytime, China). Proteins were separated by 12% SDS-polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride (PVDF) membranes (Milipore, Inc). The membranes were washed three times for 10 mins with Tris-buffered saline-0.5% Tween 20 (TBS-T) and blocked with 5% nonfat milk-TBS-T at room temperature for 1 h. Subsequently, they were incubated with primary antibodies (anti-DNAJB9 and anti-GAPDH obtained from Abcam and Cell Signaling Technology, respectively) at 4°C overnight. Following, incubation with corresponding secondary antibodies, the signal were detected with ECL Western Blotting Detection Reagents (Milipore, Inc). GAPDH was used as an endogenous reference.

Immunohistochemical (IHC) assay

DNAJB9 and Ki67 were detected in xenograft tumor specimens of nude mice, which were fixed with paraformaldehyde, embedded with paraffin and cut into 4 mm sections. The subsequent steps accomplished with the biotin-streptavidin peroxidase method (SPLink Detection Kit, ZSGB-Bio, Beijing, China) were followed based on the manufacturer's instructions. Briefly, paraffin-embedded samples were deparaffinized, dehydrated in a graded series of ethanol and blocked with endogenous peroxidase for 10 min, and incubated in goat serum for 30 min. The slides were incubated with the corresponding primary antibodies at 4°C overnight. Then, the slides were washed with PBS, incubated with biotinylated goat anti-rabbit IgG, and then incubated with horseradish peroxidase (HRP) conjugated streptomycin.

Diaminobenzidine (ZSGB-Bio) was added to the slides for chromogenic reaction. The slides were observed with an optical microscope (Olympus, Tokyo, Japan).

In vivo proliferation assay

Six week-old male BALB/c nude mice were obtained from Vital River Laboratory Animal Technology Co. (Beijing, China). Animal studies were approved by the Institutional Animal Care and Use Committee of Fourth Military Medical University. The mice were housed in pathogen-free conditions. To evaluate the *in vivo* tumorigenic effects, mice were randomly divided into the following two groups with five mice in each group: the control group and the LncRNA URHC group. siRNA-URHC and URHC-NC cells (1×10^7) were inoculated subcutaneously in the right flank of nude mice. Tumors were measured every 7 days, and tumor volumes were calculated. Five weeks after HCC cell inoculation, the mice were sacrificed, and the tumors were collected.

Statistical analysis

All experimental data were reported as means $\pm$ experimental standard deviations (SD). Student's t-test was used to determine the significance of the results (significance: * $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$).

Results

URHC promotes cell proliferation of HCC in vitro and in vivo

Previous data indicate that URHC expression is upregulated in HCC cell lines and tissues [30]. 7404 and Hep3B cells exhibited lower URHC expression than the 7721 and 7402 HCC cell lines (Figure 1A), so we selected 7404 and Hep3B cells to construct stable cell lines to overexpression URHC, and the transfection efficiency was detected by RT-qPCR (Figure 1B). The previous results of CCK-8 and EdU assays demonstrated that the downregulated expression of URHC attenuated the proliferation of 7721 cells [30], whereas the overexpression of URHC enhanced 7404, and Hep3B cells proliferation by CCK-8 and colony formation assays (Figure 1C and D). To further determine the anti-tumorigenesis potential of URHC *in vivo*, 7721 cells transfected with siRNA-NC and URHC-siRNA were inoculated into male nude mice. Mice were randomly divided into two groups with five mice in each group, but each group only included three tumors. Mice injected with siRNA-URHC 7721 cells showed a reduction in tumor volume and weight at the end of the experiment compared with the control groups (Figure 1E, F, and G). Moreover, IHC staining revealed that Ki67 (proliferation marker) expression was decreased in the siRNA-URHC xenograft tumor tissues (Figure 1H). These results suggest that URHC expression may play an important role in the cell proliferation of HCC in vitro and in vivo.

URHC is predominantly localized in the cell cytoplasm

Since many lncRNA are not restricted to either the nuclear or cytoplasmic compartment [21], but cellular location is known to dictate the function of lncRNAs. However, the subcellular location of URHC is

unclear. A FISH assay was performed to identify the subcellular localization of URHC in 7721 cells, Hep3B cells, HCC tissues, and adjacent tissues. Moreover, cellular fractionation was performed to identify the subcellular localization of URHC in 7721 and Hep3B cells. The results confirmed that URHC was mainly localized in the cell cytoplasm (Figure 2A, B, and C).

URHC can function as a ceRNA to sponge miR-5007-3p

Increasing evidence has revealed that lncRNAs contain sequences that are complementary to miRNAs and that they can directly or indirectly regulate the expression and activity of miRNAs. The potential targets of URHC were predicted by the bioinformatics database (StarBase). We observed that several potential miRNAs had putative binding sites for URHC, such as miR-5007-3p, miR-4698, miR-559, and miR-3942-3p (Table 1). Compared with the other three potential miRNAs, the expression level of miR-5007-3p was the lowest in the URHC-high expressing of HCC cells and highest in the URHC-low expressing HCC cells (Figure 3A and Figure S1). Next, we measured the expression of miR-5007-3p in HCC tissues and normal tissues. As shown in the RT-qPCR results in Figure 3B, the expression of miR-5007-3p was significantly downregulated in HCC tissues compared with that in the adjacent tissues. To confirm our prediction that miR-5007-3p targets to URHC, we first measured the expression levels of miR-5007-3p in 7404, Hep3B, 7721 and 7402 cells that were transfected with over-URHC or siRNA-URHC, respectively, by RT-qPCR. The results showed that the expression of miR-5007-3p was decreased in the over-URHC group compared with the over-control group, whereas the expression of miR-5007-3p was increased in the siRNA-URHC group compared with the siRNA-control group (Figure 3C). Meanwhile, RT-qPCR analysis of resected tumor tissues demonstrated same results (Figure 3D). Additionally, we determined the location of URHC and miRNA-5007-3p in HCC tissues, normal tissues, and cell lines. The FISH results showed that URHC and miRNA-5007-3p were colocalized in the cell cytoplasm (Figure 3E). These results suggest that URHC may negatively regulate the expression of miR-5007-3p. Moreover, we confirmed the direct relationship between URHC and miR-5007-3p using a dual-luciferase reporter assay. Using the bioinformatic database, we predicted the potential miRNA binding sites in URHC, and the alignment of miR-5007-3p with the 3'-UTR of URHC was shown (Figure 3F). 293T cells were cotransfected with miRNAs and URHC (URHC-NC, URHC mutant). As shown in Figure 3G, miR-5007-3p mimic transfection significantly decreased the luciferase activities of URHC compared with NC transfection, whereas there were no effects on the luciferase activities by miR-5007-3p mutant transfection. These results indicate that URHC directly targets miR-5007-3p to regulate its expression.

miR-5007-3p reverses the promoting effect of URHC on cell growth

We next determined whether URHC exerted its function through miR-5007-3p in 7721 and Hep3B cells. In EdU and colony formation assays, the proliferation of 7721 cells in the siRNA-URHC + miR-5007-3p inhibitor group was remarkably increased compared with that in the siRNA-URHC and siRNA-URHC significant + miR-5007-3p inhibitor NC groups, whereas, HCC cell proliferation was evidently decreased in the over-URHC + miR-5007-3p mimic group compared with the over-URHC and over-URHC + miR-5007-3p

mimic NC groups (Figure 4A and B). These results suggest that URHC function was reversed by transfection with miR-5007-3p inhibitor or mimic in 7721 and Hep3B cells.

URHC regulates the expression of DNAJB9 by targeting miR-5007-3p in HCC cells

It is well known that miRNAs play biological roles by targeting the 3'-UTR of target genes. The bioinformatics databases (miRDB, miRTarBase and miWalk) predicted that potential downstream genes of miR-5007-3p include *BCOR*, *AJAP1*, *DNAJB9*, *RAI1*, and others (Figure S2A and B). Among the targets of miR-5007-3p, we focused on *DNAJB9*, because the expression level of *DNAJB9* was the highest in the HCC cells that highly expressed URHC (Figure S2C). We first measured the expression of *DNAJB9* in HCC tissues, and its expression was found to be significantly increased (Figure 5A). In 7721 and 7402 cell lines, the mRNA level of *DNAJB9* was significantly decreased in cells transfected with siRNA-URHC compared with control cells, whereas the expression of *DNAJB9* in over-URHC cells showed the opposite result (Figure 5B). Meanwhile, RT-qPCR and Western blot analysis of resected tumor tissues demonstrated *DNAJB9* was decreased in the siRNA-URHC group compared with the siRNA-control group (Figure 5C and D). Furthermore, the URHC and *DNAJB9* expression showed a significant positive correlation in HCC patients by Spearman's correlation analysis, whereas miR-5007-3p and *DNAJB9* expression showed a significant negative correlation (Figure 5E). Using the bioinformatic database, we predicted the potential miRNA binding sites in *DNAJB9*, and the alignment of miR-5007-3p with the 3'-UTR of *DNAJB9* was shown (Figure 5F). We then confirmed the direct interaction between miR-5007-3p and the 3'-UTR of *DNAJB9* using a dual-luciferase reporter assay. 293T cells were cotransfected with miRNAs and *DNAJB9* (*DNAJB9* NC, *DNAJB9* mutant). As shown in Figure 5G, miR-5007-3p transfection significantly decreased the luciferase activities of *DNAJB9*, compared with NC transfection, whereas there were no effects on the luciferase activities by miR-5007-3p mutant transfection. Furthermore, we determined whether miR-5007-3p was involved in the URHC regulation *DNAJB9* expression. We assessed the effects of URHC and miR-5007-3p on the mRNA and protein levels of *DNAJB9* by RT-qPCR and western blot, and found that *DNAJB9* expression levels were apparently affected by URHC and miR-5007-3p. As shown in Figure 5H and I, *DNAJB9* expression was significantly decreased after transfecting with siRNA-URHC and miR-5007-3p inhibitor NC. The inhibitory effect of siRNA-URHC was notably reversed by cotransfection with siRNA-URHC and miR-5007-3p inhibitor in 7721 cells. Otherwise, the expression of *DNAJB9* was significantly increased in the over-URHC and miR-5007-3p mimic NC groups. The above results suggested that URHC functioned as a ceRNA by sponging miR-5007-3p and indirectly regulated *DNAJB9* expression.

Discussion

In this study, we added the function of URHC in vivo and in vitro. URHC is predominantly in the cell cytoplasm, and indicate that URHC can function as a ceRNAs to regulate the expression pattern and biological characteristics of miRNAs. Our study showed that URHC high-expression in HCC tissues and cell lines, while miR-5007-3p is low-expression. And miR-5007-3p can reverse the promoting effect of URHC on cell growth. However, URHC promotes cell proliferation of HCC and inhibitor miR-5007-3p is by

regulation downstream DNAJB9. The study highlights the role of URHC in HCC and highlights URHC as a potential diagnostic and therapeutic target.

lncRNAs upregulated in hepatocellular carcinoma (URHC) is our research group screened for a novel lncRNA, it is located on the forward strand of human chromosome 2: 173958088–173958307, and its transcript length is 219bp. The previous result show that it was highly expressed in HCC cell lines, clinical HCC tissues, poor survival in HCC patients, and URHC-high expression promoted tumor growth and inhibited apoptosis via ERK/MAPK inactivation by targeting ZAK [30]. However, the precise regulatory mechanisms of URHC signaling in HCC are still largely unknown. In this study, we investigated the expression pattern of URHC in HCC. The results confirmed that URHC was preferentially localized in the cell cytoplasm, suggesting that it could function as a ceRNA to regulate the expression pattern and biological characteristics of miRNAs.

A study has shown that miR-5007-3p is expressed in the lymphatic metastasis tissue from gastric cancer patients via RT-qPCR. In addition, the results revealed that expressed of miR-5007-3p was upregulated in patients with positive lymphatic metastasis of primary gastric tumors [33]. In addition, another experiment by RT-qPCR indicated that the relative expression level of miR-5007-3p was significantly upregulation in the Diabetic kidney disease group compared to type 2 diabetes group [34]. However, these researches did not examine miR-5007-3p specific cellular functions, and miR-5007-3p expression in HCC is unclear. Whether the important roles of URHC in HCC are associated with the dysregulation of miR-5007-3p also remains unknown. In our study, we found that the expression level of miR-5007-3p was the lowest in the URHC-high expressing HCC cells and highest in the URHC-low expressing HCC cells, an inverse correlation between URHC and miR-5007-3p expression. And miR-5007-3p could reverse the promoting effect of URHC on cell proliferation.

DNAJ homolog subfamily B member 9 (DNAJB9), also known as MDJ-1 and ERDJ4, is a 223-amino acid protein that is a member of the DNAJ protein family [35]. The research shown that it is expressed in most cells and displays cytoplasmic immunoreactivity, and DNAJB9 is a unique ER luminal co-chaperone, may involve a pair of chaperones from the cytosol and the ER [36, 37]. Moreover, the study reported that DNAJB9 is involved in ER stress and in the unfolded protein response (UPR), and can inhibit IRE1 activation and degrade SREBP1c, thereby reducing insulin resistance and tumorigenesis in mTORC1/2 constitutively active mice [38]. Another ability of DNAJB9 is to promote liver hypertrophy and suppress food intake. Meanwhile, DNAJB9 influences many cellular processes by regulating the ATPase activity of 70 kD heat shock proteins [35, 39]. However, the cellular function of this DNAJB9 remains largely unknown. Our results showed that URHC bound directly to miR-5007-3p, thereby controlling miR-5007-3p availability for its target gene *DNAJB9*. Furthermore, we confirmed that *DNAJB9* was highly associated with URHC.

Conclusions

In summary, URHC promoted HCC cell proliferation *in vitro* and *in vivo*, suggesting that URHC exhibited oncogenic properties in HCC progression. URHC promoted proliferation via sponging miR-5007-3p from *DNAJB9* both *in vitro* and *in vivo*. This URHC/miR-5007-3p/*DNAJB9* regulatory network may shed light on tumorigenesis in HCC and may contribute to the development of novel diagnostic and therapeutic methods for HCC.

Declarations

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Authors' contributions

Kunwei Niu, Wenjie Song, and Kaishan Tao performed the majority of the experiments, analyzed the data, and wrote and edited the manuscript. Shibin Qu, Jimin Dai, Ye Nie, and Hong Zhang performed these experiments. Xuan Zhang and Jianlin Wang performed the statistical analyses. All authors reviewed the manuscript.

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Availability of data and materials

All the data and materials are available.

Ethics approval and consent to participate

All patients provided written informed consent, and these studies were approved by the Ethics Review Committees of Xijing Hospital, Fourth Military Medical University [grant no: KY20183235-1]. Animal studies were approved by the Institutional Animal Care and Use Committee of Fourth Military Medical University [grant no: KY20191025-1], and in accordance with the Guide for the Care and Use of Laboratory Animals by the US National Institutes of Health.

Consent for publication

All authors have given their consent for the manuscript to be published.

Competing interests

The authors declare that they have no competing interests.

Author details

References

1. Xiao S, Yang M, Yang H, et al. miR-330-5p targets SPRY2 to promote hepatocellular carcinoma progression via MAPK/ERK signaling. *Oncogenesis*. 2018; 7(11): 90.
2. Li H, Zhang M, Linghu E, et al. Epigenetic silencing of TMEM176A activates ERK signaling in human hepatocellular carcinoma. *Clin Epigenetics*. 2018; 10(1):137.
3. Li S, Yao J, Xie M, et al. Exosomal miRNAs in hepatocellular carcinoma development and clinical responses. *J Hematol Oncol*. 2018; 11(1): 54.
4. Zhao J, Greene CM, Gray SG, Lawless MW. Long noncoding RNAs in liver cancer: what we know in 2014. *Expert Opin Ther Targets*. 2014; 18(10): 1207-18.
5. DiStefano JK. Long noncoding RNAs in the initiation, progression, and metastasis of hepatocellular carcinoma. *Noncoding RNA Res*. 2017; 2(3-4): 129-136.
6. He JH, Han ZP, Liu JM, et al. Overexpression of Long Non-Coding RNA MEG3 Inhibits Proliferation of Hepatocellular Carcinoma Huh7 Cells via Negative Modulation of miRNA-664. *J Cell Biochem*. 2017; 118(11): 3713-3721.
7. Xiao JN, Yan TH, Yu RM, et al. Long non-coding RNA UCA1 regulates the expression of Snail2 by miR-203 to promote hepatocellular carcinoma progression. *J Cancer Res Clin Oncol*. 2017; 143(6): 981-990.
8. Wu Y, Yuan T, Wang WW, et al. Long Noncoding RNA HOST2 Promotes Epithelial-Mesenchymal Transition, Proliferation, Invasion and Migration of Hepatocellular Carcinoma Cells by Activating the JAK2-STAT3 Signaling Pathway. *Cell Physiol Biochem*. 2018; 51(1): 301-314.
9. Xu Y, Luo X, He W, et al. Long Non-Coding RNA PVT1/miR-150/ HIG2 Axis Regulates the Proliferation, Invasion and the Balance of Iron Metabolism of Hepatocellular Carcinoma. *Cell Physiol Biochem*. 2018;49 (4): 1403-1419.
10. Wang J, Liu X, Wu H, et al. CREB up-regulates long non-coding RNA, HULC expression through interaction with microRNA-372 in liver cancer. *Nucleic Acids Res*. 2010; 38(16): 5366-83.
11. Chang YN, Zhang K, Hu ZM, et al. Hypoxia-regulated lncRNAs in cancer. *Gene*. 2016; 575(1): 1-8.
12. Li T, Meng XL, Yang WQ. Long Noncoding RNA PVT1 Acts as a "Sponge" to Inhibit microRNA-152 in Gastric Cancer Cells. *Dig Dis Sci*. 2017; 62(11): 3021-3028.
13. Guo XB, Hua Z, Li C, et al. Biological significance of long non-coding RNA FTX expression in human colorectal cancer. *Int J Clin Exp Med*. 2015; 8(9): 15591-600.
14. Hombach S, Kretz M. Non-coding RNAs: Classification, Biology and Functioning. *Adv Exp Med Biol*. 2016; 937: 3-17.
15. Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. *Cell*. 2009; 136(4): 629-41.

16. Fang Y, Fullwood MJ. Roles, Functions, and Mechanisms of Long Non-coding RNAs in Cancer. *Genomics Proteomics Bioinformatics*. 2016; 14(1): 42-54.
17. Bhan A, Soleimani M, Mandal SS. Long Noncoding RNA and Cancer: A New Paradigm. *Cancer Res*. 2017; 77(15): 3965-3981.
18. Quan M, Chen J, Zhang D. Exploring the secrets of long noncoding RNAs. *Int J Mol Sci*. 2015; 16(3): 5467-96.
19. Dimitrova N, Zamudio JR, Jong RM, *et al*. LincRNA-p21 activates p21 in cis to promote Polycomb target gene expression and to enforce the G1/S checkpoint. *Mol Cell*. 2014; 54 (5): 777-90.
20. Zhu L, Liu Y, Chen Q, *et al*. Long-Noncoding RNA Colorectal Neoplasia Differentially Expressed Gene as a Potential Target to Upregulate the Expression of IRX5 by miR-136-5P to Promote Oncogenic Properties in Hepatocellular Carcinoma. *Cell Physiol Biochem*. 2018; 50(6): 2229-2248.
21. Yang G, Lu X, Yuan L. LncRNA: a link between RNA and cancer. *Biochim Biophys Acta*. 2014;1839(11): 1097-109.
22. Marchese FP, Raimondi I, Huarte M. The multidimensional mechanisms of long noncoding RNA function. *Genome Biol*. 2017;18(1): 206.
23. Wentzel EA, Montgomery CL, Hwang HW, *et al*. Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. *Cell*. 2009; 137(6): 1005-17.
24. Hatzia Apostolou M, Polytaichou C, Aggelidou E, *et al*. An HNF4 α -miRNA inflammatory feedback circuit regulates hepatocellular oncogenesis. *Cell*. 2011;147(6): 1233-47.
25. Zheng B, Liang L, Wang C, *et al*. MicroRNA-148a suppresses tumor cell invasion and metastasis by downregulating ROCK1 in gastric cancer. *Clin Cancer Res*. 2011;17(24): 7574-83.
26. Mori M, Triboulet R, Mohseni M, *et al*. Hippo signaling regulates microprocessor and links cell-density-dependent miRNA biogenesis to cancer. *Cell*. 2014; 156(5): 893-906.
27. Zhang JG, Shi Y, Hong DF, *et al*. Corrigendum: MiR-148b suppresses cell proliferation and invasion in hepatocellular carcinoma by targeting WNT1/ β -catenin pathway. *Sci Rep*. 2017; 7: 46886.
28. Liu XH, Sun M, Nie FQ, *et al*. Lnc RNA HOTAIR functions as a competing endogenous RNA to regulate HER2 expression by sponging miR-331-3p in gastric cancer. *Mol Cancer*. 2014; 13: 92.
29. Qu J, Li M, Zhong W, Hu C. Competing endogenous RNA in cancer: a new pattern of gene expression regulation. *Int J Clin Exp Med*. 2015; 8(10): 17110-6.
30. Xu WH, Zhang JB, Dang Z, *et al*. Long non-coding RNA URHC regulates cell proliferation and apoptosis via ZAK through the ERK/MAPK signaling pathway in hepatocellular carcinoma. *Int J Biol Sci*. 2014; 10(7): 664-76.
31. Lennox KA, Behlke MA. Cellular localization of long non-coding RNAs affects silencing by RNAi more than by antisense oligonucleotides. *Nucleic Acids Res*. 2016; 44(2): 863-77.
32. Cabili MN, Dunagin MC, McClanahan PD, *et al*. Localization and abundance analysis of human lncRNAs at single-cell and single-molecule resolution. *Genome Biol*. 2015; 16(1): 20.

33. Yang B, Jing C, Wang J, *et al.* Identification of microRNAs associated with lymphangiogenesis in human gastric cancer. *Clin Transl Oncol.* 2014; 16(4): 374-9.
34. Zhao Y, Shen A, Guo F, *et al.* Urinary Exosomal MiRNA-4534 as a Novel Diagnostic Biomarker for Diabetic Kidney Disease. *Front Endocrinol (Lausanne).* 2020;11: 590.
35. Lee HJ, Kim JM, Kim KH, *et al.* Genotoxic stress/p53-induced DNAJB9 inhibits the pro-apoptotic function of p53. *Cell Death Differ.* 2015; 22(1): 86-95.
36. Kurisu J, Honma A, Miyajima H, *et al.* MDG1/ERdj4, an ER-resident DnaJ family member, suppresses cell death induced by ER stress. *Genes Cells.* 2003; 8(2): 189-202.
37. Huang Y, Arora K, Mun KS, *et al.* Targeting DNAJB9, a novel ER luminal co-chaperone, to rescue Δ F508-CFTR. *Sci Rep.* 2019; 9(1): 9808.
38. Sun F, Liao Y, Qu X, *et al.* Hepatic DNAJB9 Drives Anabolic Biasing to Reduce Steatosis and Obesity. *Cell Rep.* 2020; 30(6):1835-1847.
39. Andeen NK, Yang HY, Dai DF, *et al.* DnaJ Homolog Subfamily B Member 9 Is a Putative Autoantigen in Fibrillary GN. *J Am Soc Nephrol.* 2018; 29(1): 231-239.

Tables

Table 1. Starbase online predicted the miRNAs that target URHC

Name	Accession	Query start	Query start	Subject start	Subject end	Score	Evalue
hsa-miR-5007-3p	MIMAT0021036	74	91	4	21	72	1.5
hsa-miR-4698	MIMAT0019793	59	73	2	16	66	4.7
hsa-miR-559	MIMAT0003223	66	85	1	20	64	6.9
hsa-miR-3942-3p	MIMAT0019230	164	181	4	21	63	8.3

Figures

Figure 1

URHC promotes cell proliferation of HCC in vitro and in vivo. A Relative expression of URHC was detected by qRT-PCR in HCC cell lines. B Increased expression of URHC was detected using RT-qPCR. C 7404 and Hep3B cells were stably transfected with over-URHC or over-control, and cell viability was measured using the CCK-8 assay at 24, 48, 72, and 96 h. D Colony formation assay in 7404 and Hep3B cells stably transfected with over-URHC or over-control. E Xenograft tumors from the nude mice were shown. Each group contained five nude mice. F Tumor growth curves were analyzed. G Tumor weights were analyzed. H Immunohistochemistry analysis of Ki-67 was obtained from tumors. Magnification × 400.

Figure 2

The subcellular location of URHC in HCC cell lines and tissues. A Detection of URHC by fluorescence in situ hybridization (FISH) in HCC cell lines 7721 and Hep3B. URHC: endogenous URHC; DAPI: DNA stained with DAPI; Merge: merged images of DAPI and URHC immunofluorescence. Magnification $\times 400$. B FISH was performed to analyze URHC were performed in HCC tissues and adjacent tissues. C Cellular localization of URHC in 7721 and Hep3B cells were shown. GAPDH and U6 served as a cytoplasmic and nuclear localization marker, respectively. $*P < 0.05$.

Figure 3

URHC was a target of miR-5007-3p. A Relative expression of miR-5007-3p in HCC cell lines and normal hepatic cells was detected by qRT-PCR. B Relative expression of miR-5007-3p in HCC tissues and adjacent tissues was detected by qRT-PCR. C RT-qPCR analysis of URHC-regulated miR-5007-3p expression in 7721, 7402, 7404, and Hep3B cells. D The expression level of miR-5007-3p in tumors was detected. E FISH was performed to analyze the colocalization of URHC and miR-5007-3p in HCC cell lines and tissues. F The predicted miR-5007-3p binding sites in URHC-WT and the designed mutant sequence (URHC-MUT) were indicated. G Luciferase reported assay of 293T cells cotransfected with URHC-WT or

URHC-MUT and miR-5007-3p NC or miR-5007-3p. The bars represent means $\pm$ SD of three independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001 compared with the control group.

Figure 4

miR-5007-3p reversed the promoting effect of URHC on the growth of HCC cells. A and B EdU and colony formation assays identified the phenomenon of URHC regulated each other to compete for the binding of miR-5007-3p in 7721 and Hep3B cell lines.

Figure 5

URHC regulated the expression of DNAJB9, an endogenous miR-5007-3p target. A Relative expression of DNAJB9 in HCC tissues and adjacent tissues was detected by qRT-PCR. B RT-qPCR analysis of URHC-regulated DNAJB9 expression in 7721, 7402, 7404, and Hep3B cells. C and D The mRNA and protein levels of DNAJB9 in tumors were assessed. E The positive correlation between URHC and miR-5007-3p expression and the negative correlation between miR-5007-3p and DNAJB9 expression in HCC tissues were shown in scatter diagrams. F The predicted binding sites of miR-5007-3p in the DNAJB9 sequence were shown. G Luciferase reported assay of 293T cell cotransfected with DNAJB9-WT or DNAJB9-MUT and miR-5007-3p NC or miR-5007-3p. H and I The levels of DNAJB9 in HCC cell transfected with miR-5007-3p inhibitor/mimic or siRNA-URHC/over-URHC were analyzed by RT-qPCR and western blot. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Supplementary Files

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