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Original Article

LncRNA THUMPD3-AS1/microRNA-4465/KPNA2 axis impacts human hepatocellular carcinoma cell phenotypes

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ABSTRACT

Objective: Hepatocellular carcinoma (HCC) is a lethal malignancy in the world. LncRNA THUMPD3-AS1 is implicated in tumorigenesis and progression in various tumors. Therefore, this study was applied to investigate the action of THUMPD3-AS1 in HCC by regulating microRNA (miR)-4465 and KPNA2.
Methods: The clinical specimens of HCC were collected to determine THUMPD3-AS1, KPNA2, miR-4465, E-cadherin, Vimentin, N-cadherin, ZEB1 and SNAIL levels. HCC cells were screened and transfected with sh-THUMPD3-AS1 or miR-4465 mimic to explore their roles in HCC cell phenotype and epithelial-mesenchymal transition (EMT)-related factors. The involvement of miR-4465 in THUMPD3-AS1-mediated HCC was proved. The relationship of THUMPD3-AS1, KPNA2 and miR-4465 was verified.
Results: Overexpressed THUMPD3-AS1 and KPNA2 and reduced miR-4465 were present in HCC clinical tissues. THUMPD3-AS1 bound to miR-4465 to target KPNA2. Silencing of THUMPD3-AS1 or restoration of miR-4465 repressed HCC cell phenotypes and EMT *in vitro*. Inhibition of miR-4465 mitigated the role of silenced THUMPD3-AS1 in HCC.
Conclusion: This study stresses that THUMPD3-AS1 induces EMT in HCC cells and ultimately promotes HCC cell growth and migration by competitively inhibiting miR-4465 expression and thus upregulating KPNA2.

1. Introduction

Hepatocellular carcinoma (HCC) is a familiar primary liver neoplasm and the 2nd chief cause of cancer death around the world [1], and this disease has high morbidity and mortality [2]. The onset of HCC is more frequent in both Western and Asian countries over the past 10 years [3]. The infection of hepatitis B virus (HBV) is the crucial risk factor related to a rapid increase of incidence of HCC [4]. Besides liver resection, liver transplantation is regarded as the most effective therapeutic method to possibly eliminate HCC lesions [3]. HCC has a poor prognosis because of its underlying systemic manifestations [5]. Therefore, improving HCC prognosis is a main focus.

Recently, growing evidence has shown that long non-coding RNA (lncRNA) exerts functions in cancer occurrence and development because of its epigenetic modification abnormalities, transcription abnormalities, or chromosomal abnormalities [6,7]. THUMPD3-AS1 is known as an oncogenic lncRNA in several cancers, and it has been revealed to be elevated in various cancer tissues, including ovarian cancer and lung cancer [8,9]. These results unveil that THUMPD3-AS1 is commonly upregulated in tumors with proto-oncogene characteristics, while its specific role in HCC are still not comprehensively clear. Thus, exploring this aspect will further help to find out the gene targets, and supply different strategies and ways for clinical diagnosis and treatment. The microRNAs (miRNAs) show post-transcriptional gene regulatory functions, which is able to degrade mRNA or suppress mRNA translation via binding to the target gene mRNA's 3′UTR [10]. miR-26 has been suggested to be linked with tumor progression, and miR-4465 is one member of miR-26 family [11]. miR-4465 has been revealed to be downregulated in ovarian cancer, and the modulation network involving SDHAP1, miR-4465, as well as EIF4G2 might be a potential therapy target for the paclitaxel-resistant ovarian

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cancer [12]. It has been reported that LINC00240 sponges miR-4465 to advance HCC cell progression via the HGF/c-MET signaling pathway [13]. Karyopherin subunit alpha 2 (KPNA2) belongs to the nuclear transporter family, which has been gradually related to the nuclear transport pathway for multiple tumor-associated proteins [14]. KPNA2 multilevel dysregulation in HCC along with its links with the fatty acid metabolism pathway and immune infiltration shows its multiple roles in HCC [15]. Thus, this study was applied to unveil the role of THUMPD3-AS1 in promoting the phenotype of HCC cells by regulating miR-4465 and KPNA2.

2. Materials and methods

2.1. Ethics approval

The study was ratified by the Institutional Review Board of our hospital and followed the principles of the *Declaration of Helsinki*. All patients signed informed consent.

2.2. Clinical sample collection

The cancer tissues together with normal tissues of 120 patients with HCC in our Hospital were collected. The patients including 65 males and 55 females were aged 29–69 years, with a median age of 50 years. Each patient was followed up and the duration of follow-up and survival status were recorded. A follow-up cut-off time of September 2022 was utilized to define 3-year survival (36 months) as the observation endpoint. According to the gene expression levels, they were divided into high and low expression groups. All tissue specimens were confirmed as primary HCC. No preoperative or intraoperative chemo-radiotherapy or immunotherapy was performed in patients, and the HCC tissues were taken from the non-necrotic area in the center of the lesion, and the normal tissues were taken 3 cm from the edge of the cancer tissues. After sectioning, the tissues were kept in liquid nitrogen. The patients were excluded: (1) not meeting the diagnostic criteria of HCC in the clinical guidelines; (2) absence of imaging data or unclear pathological diagnosis; (3) patients with more severe cardiopulmonary diseases and unable to tolerate surgery; (4) those suffering from mental disorders.

2.3. Cell culture and transfection

The immortalized liver cell line (MIHA) and HCC cell lines Huh-7, HLE, Hep3B, MHCC97-H were all available from Xuanya Biotechnology Co., Ltd. (Shanghai, China). All the cells were cultivated in DMEM (Gibco, Grand Island, NY, USA) or RPMI-1640 (Gibco) containing 10 % fetal bovine serum (FBS), 100 μg/mL streptomycin, as well as 100 U/mL penicillin [16]. Cells were transfected with THUMPD3-AS1 silence NC, THUMPD3-AS1 silence sequence, miR-4465 mimic NC, miR-4465 mimic, THUMPD3-AS1 silence sequence + miR-4465 inhibitor NC, or THUMPD3-AS1 silence sequence + miR-4465 inhibitor. The cells in the 3rd passage were detached with trypsin and seeded into a 24-well plate. The initial concentration of the cells was 2×10^6 cells/well. The cells were grown into a single layer by culture. The transfection experiment was conducted with Lipofectamine2000 (Invitrogen, Carlsbad, California, USA). Changes in cell morphology of different transfection groups were observed under inverted phase contrast microscope.

2.4. RT-qPCR

THUMPD3-AS1, miR-4465 and KPNA2 expression in the cells and tissues was detected. Total RNA was extracted from tissues or cell lines using Trizol reagent (ThermoFisher Scientific, Waltham, MA, USA) and then reverse transcription was implemented using complementary DNA transcription kit (ThermoFisher Scientific). The loading control of miR-4465 was U6, and that of THUMPD3-AS1, KPNA2, E-cadherin, Vimentin, N-cadherin, ZEB1 and SNAIL was GAPDH. Gene expression was tested using standard SYBR-GreenRT-PCR kit (Takara, Otsu, Shiga, Japan) and analyzed by $2^{-\Delta\Delta Ct}$ method [17,18]. The primers were listed in Supplementary Table 1.

2.5. Western blot analysis

Total protein in cells and tissues was extracted with radioimmunoprecipitation assay kit (Solarbio, Beijing, China), centrifuged and quantified by bicinchoninic acid method. The proteins were separated by SDS-PAGE, and electro-blotted onto the membrane. Then the membrane was blocked with 5 % bovine serum albumin (BSA) in TBST solution overnight, and cultivatedwith the primary antibody KPNA2 (ab289858, 1: 500, Abcam, USA) overnight, and secondary antibody (7074, 1: 2000, Cell Signaling Technologies) for 1 h. Finally, the membrane was exposed and developed in enhanced chemiluminescence solution, and imaging was implemented on LAS4000C gel imager (Image Quant, GE Healthcare, Piscataway, N.J., USA) [19].

2.6. Luciferase activity assay

The binding sites of THUMPD3-AS1, miR-4465 and KPNA2 were processed using starBase prediction website, and the targeting relationships were further verified. According to the binding sequence of THUMPD3-AS1 to miR-4465 and that of KPNA2 3′untranslated regions (UTR) to miR-4465, the synthetic target sequence and mutation sequence were designed severally. The luciferase reporter plasmids wild type (WT) along with mutant type (MUT) were co-transfected into cells with miR-4465 mimic or mimic-NC. The luciferase activity was measured using a dual luciferase assay system (Promega, Madison, WI, USA) and Glomax20/20luminometer fluorescence detector (Promega) [20].

2.7. RNA pull down assay

Cells were transfected with miR-4465-WT and miR-4465-MUT labelled with 50 nM biotin (Gene Create, Wuhan, China). After 48-h transfection, the cells were cultured with specific cell lysis solution (Ambion, Austin, Texas, USA) for 10 min. The residual lysate was cultivated with M − 280 streptavidin magnetic beads (S3762, Sigma, St. Louis, MO, USA) pre-coated with RNase-free and yeast tRNA (TRNABAK-RO, Sigma, USA) at 4 °C overnight. The antagonistic miR-4465 probe was utilized as a NC. The total RNA was extracted via Trizol, and THUMPD3-AS1 expression was estimated via RT-qPCR [21].

2.8. Cell proliferation assay

The above transfected cells were detached with trypsin, centrifuged, re-suspended in a complete culture medium and counted. After continuous culture at 37 °C with 5 % CO_2 for 0–3 days (0h, 24h, 36h, 72h), 10 μL of cell counting kit (CCK)-8 solution was added to each well and incubated for 4 h. After the color of CCK-8 solution altered from light red to yellow, the optical density $(OD)_{450\ nm}$ value was measured with an microplate reader (Omega Bio-tek Inc, Norcross, GA, USA). Finally, the cell growth curve was drawn [22].

2.9. Colony formation assay

Agar (1.2 %) was heated, dissolved, and set aside in a 46 °C water bath. The prepared cells were counted, and suspended in 40°C-preheated medium. Next, the above cell suspension (1×10^5 cells) was added in 24-well culture plate (325 μL/well), followed by an addition of 50 μL of the samples to be examined and full mixture. A total of 125 μL of pre-warmed 1.2 % agar was pipetted, gently and quickly mixed with the above cell suspension and the sample mixture in 24-well plate, avoiding air bubbles. After natural solidification, the mixture was incubated at 37 °C in 5 % CO_2 incubator, and 8–10 d later, an inverted microscope was utilized for observation.

2.10. Flow cytometry

Apoptosis analysis was performed by staining with fluorescein isothiocyanate AnnexinV Apoptosis Detection Kit I (556547, BD Biosciences) and the early (AnnexinV positive and PI negative) and late (AnnexinV positive and PI negative) apoptotic cells were evaluated via flow cytometry [23].

2.11. Transwell assay

Before the experiment, the cells were starved and detached. The original culture medium was replaced with serum-free DMEM, and the cells were triturated into cell suspension. Approximately 1.5×10^5 cells were placed in the upper Transwell chamber and supplemented with serum-free medium to 200 μL. The low chamber was filled with 700 μL complete culture medium containing 20 % FBS. Post-24 h, the cells were fixed with anhydrous ethanol for 15 min, dyed with crystal violet solution for 15 min and observed microscopically. Cell invasion assay: the cells were treated based on the steps of migration assay, and a chamber covered with matrigel was used [24].

2.12. Immunofluorescence staining

Cells were fixed with 4 % paraformaldehyde for 15 min at room temperature and then treated with 3 % BSA at 37 °C for 30 min to block non-specific staining. Next, the cells were incubated overnight with primary anti-mouse E-cadherin (ab231303, Abcam, Cambridge, UK), rinsed in phosphate-buffered saline for three times, each for 3 min, and then incubated for 2 h at room temperature with secondary Alexa Fluor 647: labelled goat anti-mouse IgG (ab150115, Abcam). All antibodies were diluted according to the instructions [25]. Cell nuclei were counter-stained with 4′,6-diamino-2-phenylindole. Immunofluorescence labelled sections were observed with a fluorescence microscope (Olympus Corporation, Japan). The data were quantified with ImageJ software [26].

2.13. Statistical analysis

SPSS 22.0 statistical software (IBM Corp. Armonk, NY, USA) was utilized to process the data. The measurement data were depicted as mean ± standard deviation. Paired *t*-test was applied in the comparison of cancer tissues to normal tissues, and independent samples *t*-test for other two-group comparison. One-way analysis of variance (ANOVA) was used for comparison among multiple groups, followed by Tukey's post hoc test. The data were compared among groups at different time points by repeated measurement ANOVA, along with Bonferroni post-test. Pearson test was utilized to analyze the relation of indices in clinical samples. Predictors were kept if they were significant at $P < 0.05$.

3. Results

3.1. Overexpressed THUMPD3-AS1 and reduced miR-4465 are detected in HCC tissues and cells

As reported, THUMPD3-AS1 is overexpressed in HCC [27] and miR-4465 can repress hepatocarcinogenesis [13], but the exact mechanism is unclear. RT-qPCR determined that THUMPD3-AS1 was elevated while miR-4465 was reduced in HCC clinical tissues (Fig. 1A and B). The 3-year survival analysis unraveled that low expression of THUMPD3-AS1 displayed longer survival, whereas high expression of miR-4465 displayed longer survival (Fig. 1C–D). Meanwhile, THUMPD3-AS1 expression was negatively linked with miR-4465 expression in clinical HCC tissue samples (Fig. 1E). RT-

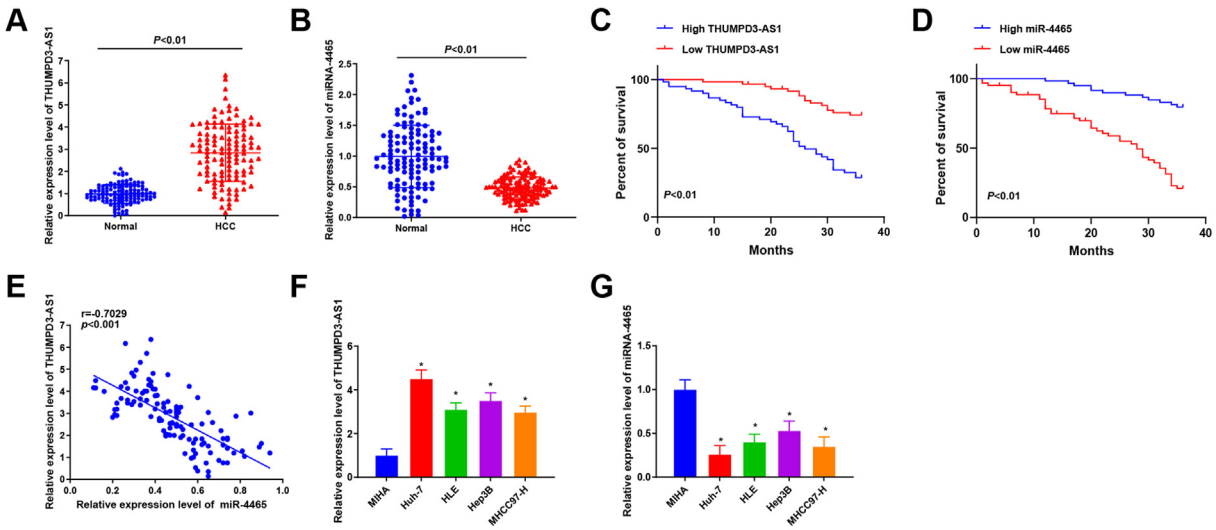

Fig. 1. Up-regulated THUMPD3-AS1 and down-regulated miR-4465 exhibit in HCC tissues and cells. A. THUMPD3-AS1 expression in HCC and normal tissues detected via RT-qPCR; B. miR-4465 expression in HCC and normal tissues detected via RT-qPCR; C. Correlation analysis of THUMPD3-AS1 expression and HCC prognosis; D. Correlation analysis of miR-4465 expression and HCC prognosis; E The correlation of THUMPD3-AS1 and miR-4465 expression in clinical samples analyzed via Pearson correlation analysis; F-G. THUMPD3-AS1 expression in MIHA cell lines and HCC cell lines Huh-7, HLE, Hep3B, and MHCC97-H detected via RT-qPCR; E. miR-4465 expression in MIHA cell lines and HCC cell lines Huh-7, HLE, Hep3B, and MHCC97-H detected via RT-qPCR; Figure A, B, C, n = 120. Figure D, E, * vs the MIHA cell lines, $P < 0.05$.

qPCR also presented that in *in vitro* cell lines, elevated THUMPD3-AS1 and decreased miR-4465 were detected in HCC cell lines Huh-7, HLE, Hep3B, and MHCC97-H versus MIHA cells, and most significantly expressed in Huh-7 cells, so this cell line was selected for the subsequent study (Fig. 1F and G). The above results manifested that overexpressed THUMPD3-AS1 and decreased miR-4465 exhibited in HCC, and THUMPD3-AS1 and miR-4465 expression was negatively correlated.

3.2. THUMPD3-AS1 binds to miR-4465

To further unravel the regulatory mechanism between THUMPD3-AS1 and miR-4465, the binding sites of THUMPD3-AS1 and miR-4465 were forecasted via the website (Starbase). The finding implied the existence of binding site between the two. To verify that, we further mutated the binding site and implemented the luciferase activity and RNA-pull down assays. The outcomes reflected that in Huh-7 cells, the luciferase activity was decreased after miR-4465 mimic + THUMPD3-AS1-WT treatment (Fig. 2A and B). The miR-4465 enrichment was elevated in the cells treated with Bio-THUMPD3-AS1-WT (Fig. 2C). Next, RT-qPCR determined THUMPD3-AS1 and miR-4465 expression, and finally observed that in Huh-7 cells transfected with sh-THUMPD3-AS1, THUMPD3-AS1 expression was reduced while miR-4465 level was elevated (Fig. 2D and E). All of results implied that THUMPD3-AS1 could bind to miR-4465 to regulate miR-4465 expression.

3.3. THUMPD3-AS1 regulates KPNA2 expression through miR-4465

To further unveil the downstream mechanism of miR-4465, the Starbase website was applied to forecast the targeting site between miR-4465 with KPNA2. Then, we further mutated the binding site and the luciferase activity assay confirmed that the luciferase activity was diminished in the cells after co-transfection with miR-4465 mimic and WT-KPNA2 (Fig. 3A and B), suggesting that miR-4465 had targeting sites with KPNA2.

To investigate whether THUMPD3-AS1 regulated KPNA2 by miR-4465, miR-4465 and KPNA2 expression was evaluated by RT-qPCR and Western blot. It was noticed that miR-4465 mimic transfection suppressed KPNA2 and elevated miR-4465 expression, whilst sh-THUMPD3-AS1 transfection reduced KPNA2 expression in Huh-7 cells (Fig. 3C–E).

Also, RT-qPCR manifested that KPNA2 was elevated in HCC clinical tissues (Fig. 3F). The 3-year survival analysis unearthed that low expression of KPNA2 was correlated with longer survival (Fig. 3G). Pearson test found that miR-4465 expression was negatively linked with KPNA2 expression in clinical HCC tissue samples (Fig. 3H). The above results reflected that THUMPD3-AS1 modulated KPNA2 through miR-4465.

3.4. THUMPD3-AS1 advances the growth of HCC cells by restricting miR-4465

In this research, the functional assays including CCK-8 assay, flow cytometry, as well as Transwell assay were implemented to reveal that for Huh-7 cells after transfected with sh-THUMPD3-AS1 or miR-4465 mimic, the proliferation, invasion and migration abilities were diminished, and cell apoptosis was promoted. On the other hand, based on the transfection with sh-THUMPD3-AS1, follow-up transfection with miR-4465-inhibitor could enhance the proliferation, migration and invasion activities, and suppress apoptosis (Fig. 4A–E). To conclude, THUMPD3-AS1 enhanced the growth of HCC cells by inhibiting miR-4465.

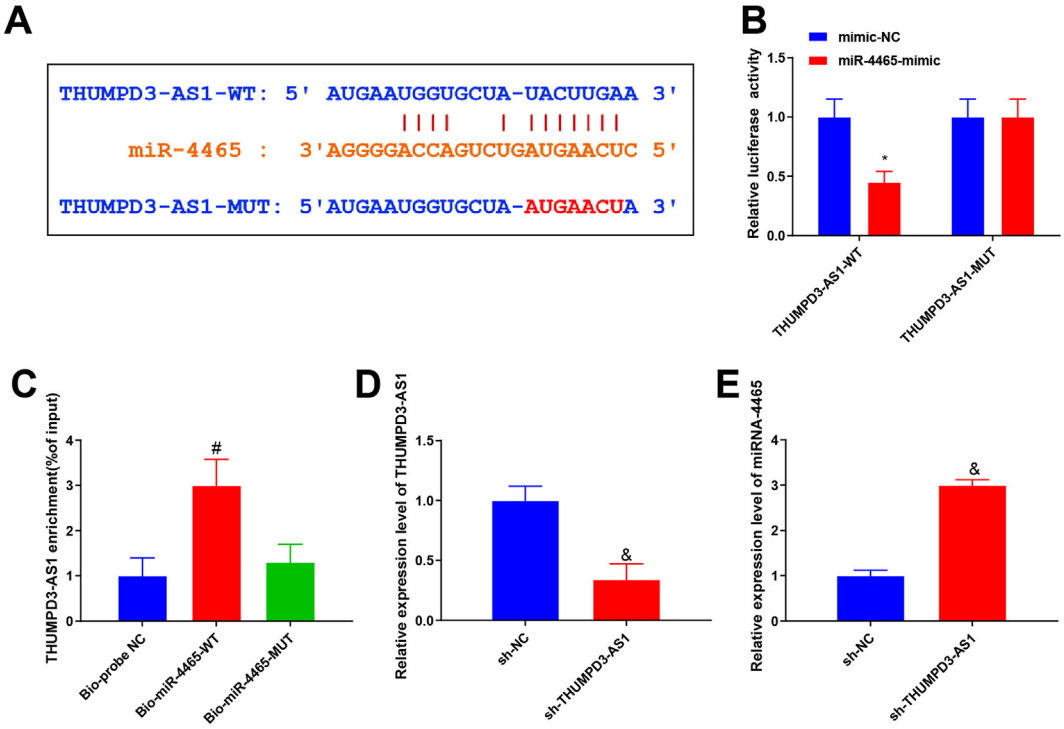

Fig. 2. THUMPD3-AS1 binds to miR-4465. A. The binding site and mutant site of miR-4465 to THUMPD3-AS1; B. The interaction of miR-4465 and THUMPD3-AS1 verified via the luciferase activity assay; C. The relation of THUMPD3-AS1 with miR-4465 in Huh-7 cells detected via RNA pull down assay; D. The changes of THUMPD3-AS1 and miR-4465 expression after silenced THUMPD3-AS1 in Huh-7 cells detected via RT-qPCR; Cell experiments were repeated at least three times, * vs the mimic NC group, $P < 0.05$; # vs the Bio-probe-NC, $P < 0.05$; & vs the sh-NC group, $P < 0.05$.

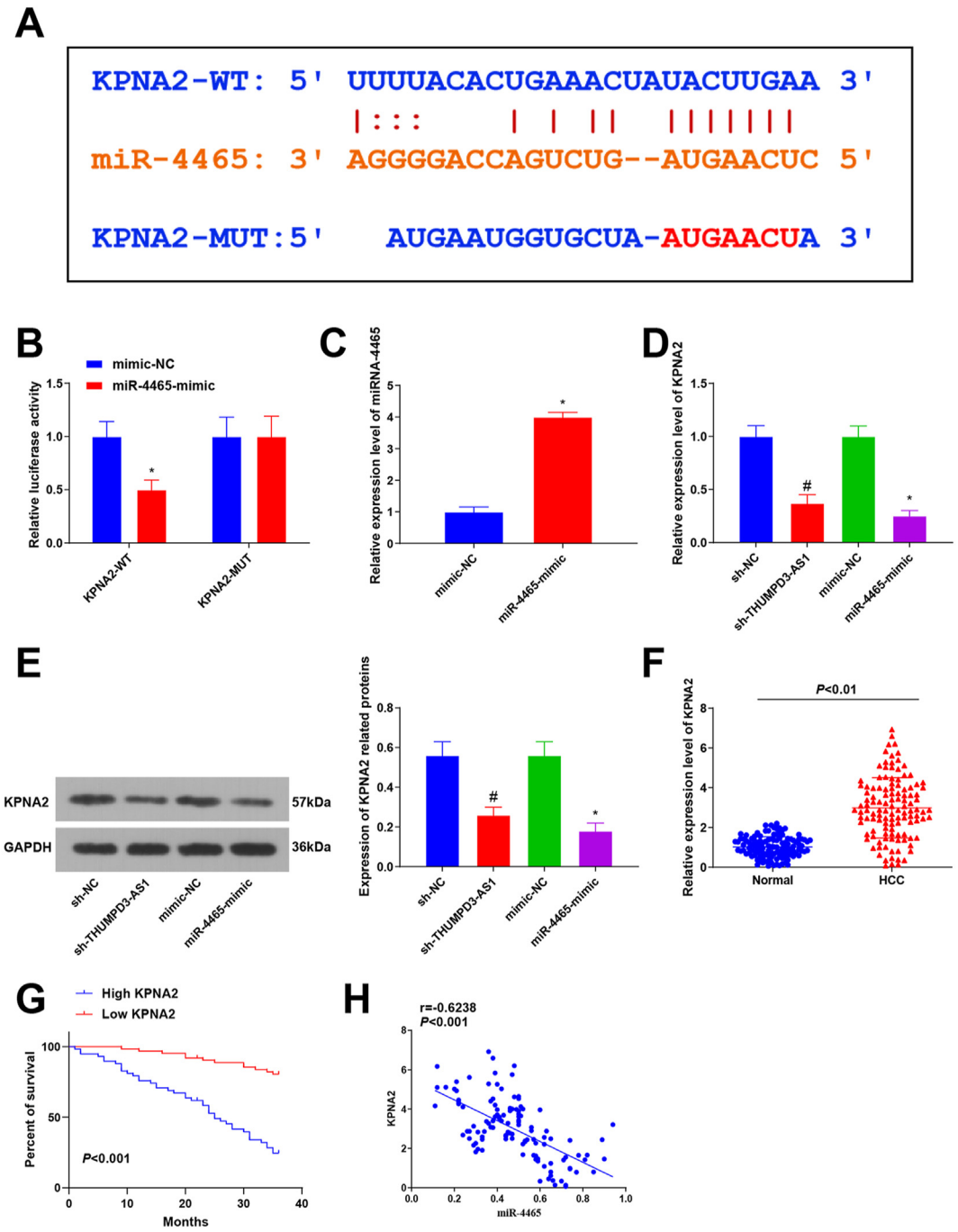

Fig. 3. THUMPD3-AS1 regulates the KPNA2 axis through miR-4465. A. The binding site and mutant site of miR-4465 to KPNA2; B. The binding relationship of miR-4465 to KPNA2 verified via the luciferase activity assay; C. Transfection efficiency of miR-4465 validated by RT-qPCR; D. KPNA2 expression after silence of THUMPD3-AS1 or elevation of miR-4465 in Huh-7 cells detected by RT-qPCR; E. KPNA2 expression after silence of THUMPD3-AS1 or elevation of miR-4465 in Huh-7 cells detected via Western blot analysis; F. KPNA2 expression in HCC and normal tissues detected via RT-qPCR; G. Correlation analysis of KPNA2 expression and HCC prognosis; H. The correlation of miR-4465 with KPNA2 in clinical samples via Pearson analysis; Figure F, H, n = 120. Cell experiments were repeated at least three times, * vs the mimic NC group, $P < 0.05$; # vs the sh-NC group, $P < 0.05$.

3.5. THUMPD3-AS1 advances epithelial mesenchymal transformation (EMT) of HCC through regulating miR-4465

To further explore the impact of the THUMPD3-AS1/miR-4465 axis on EMT, we first observed the cell morphology changes under a microscope, and the results revealed that the treatment of sh-THUMPD3-AS1 and miR-4465 mimic was able to inhibit the transformation from epithelial cell-like morphology to mesenchymal cell-like morphology (Fig. 5A). A study has shown that EMT are generally associated with E-cadherin, Vimentin, N-cadherin, ZEB1 and SNAIL [28]. The molecular phenotype of EMT in cell lines was assessed by RT-qPCR. It was manifested that in Huh-7 cells transfected with sh-THUMPD3-AS1 or miR-4465 mimic, E-cadherin expression was elevated but Vimentin, N-cadherin, ZEB1 and SNAIL expression was depressed. However, sh-THUMPD3-AS1-mediated expression trend of E-cadherin, Vimentin, N-cadherin, ZEB1 and SNAIL was mitigated by further transfection with miR-4465-inhibitor (Fig. 5B–D). In addition, we also tested E-cadherin

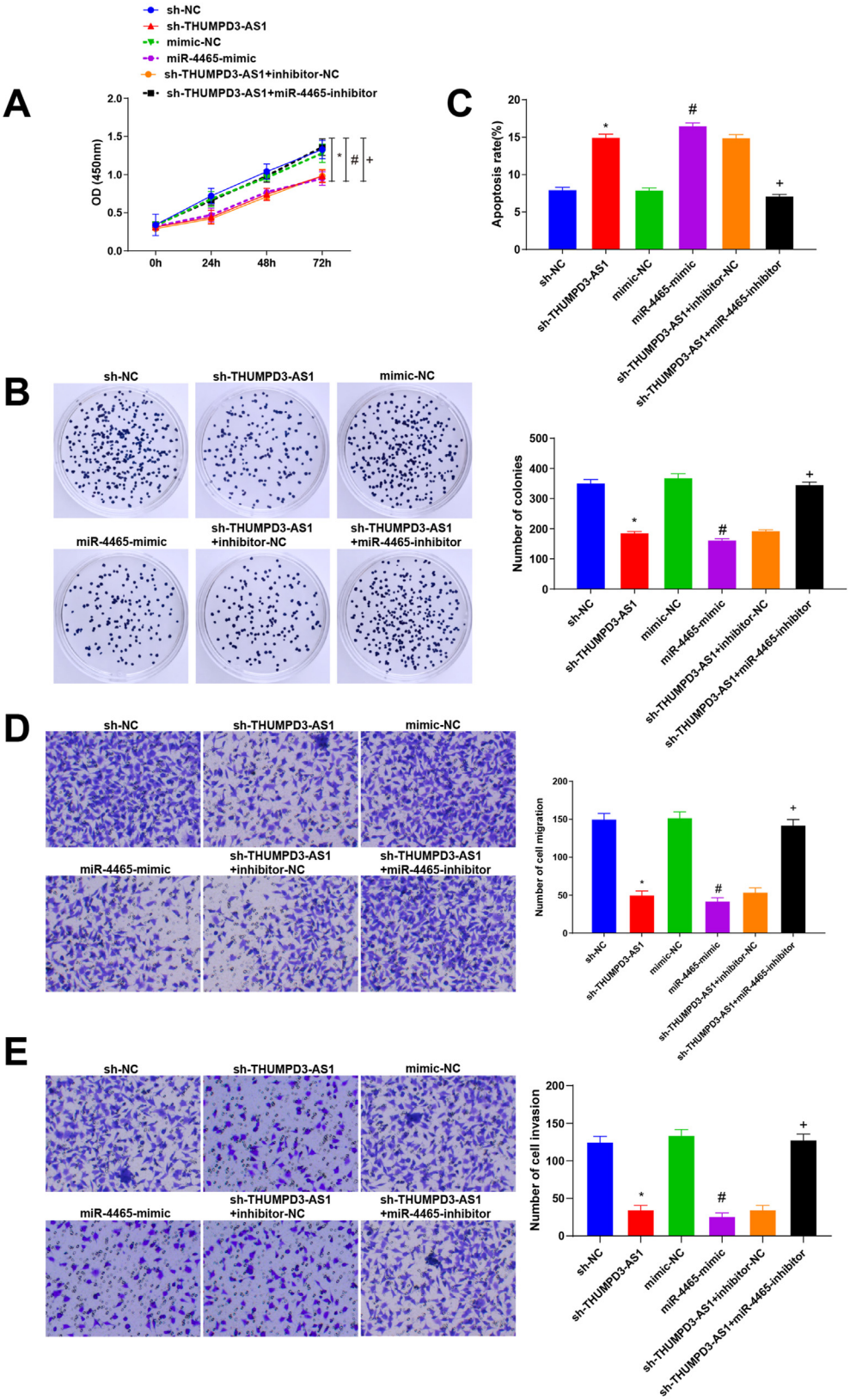

Fig. 4. THUMPD3-AS1 facilitates the growth of HCC cells by suppressing miR-4465. A. The proliferation ability of Huh-7 cells measured by CCK-8 assay; B. Cell proliferation was tested by colony formation assay; C. The apoptosis of Huh-7 cells tested by flow cytometry; D-E. The migration and invasion abilities of Huh-7 cells determined via Transwell assay; Cell experiments were repeated at least three times, * vs the sh-NC group, $P < 0.05$; # vs the mimic-NC group, $P < 0.05$; + vs sh-THUMPD3-AS1 + inhibitor-NC group, $P < 0.05$.

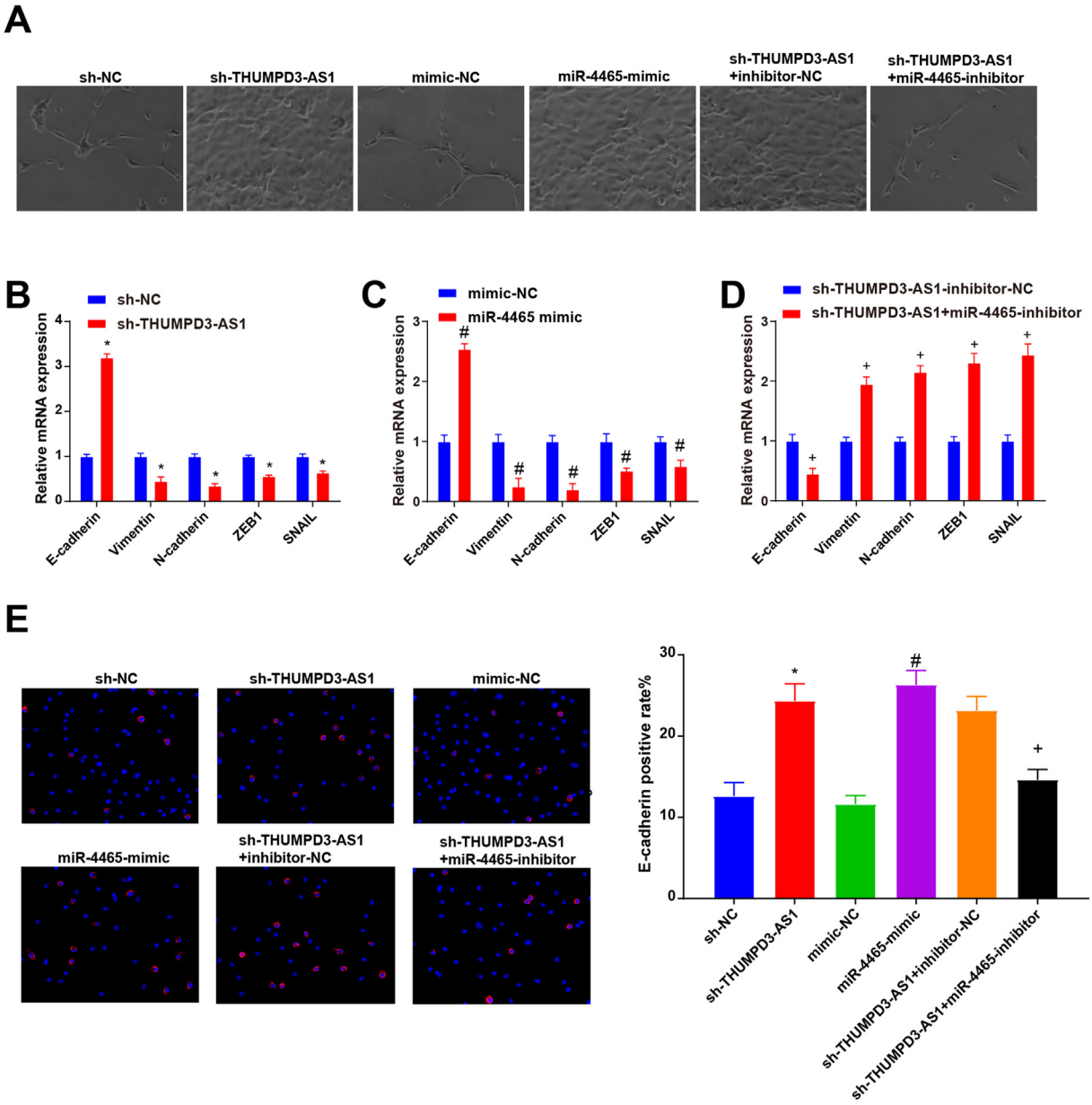

Fig. 5. THUMPD3-AS1 accelerates EMT of HCC through modulating miR-4465. A. Changes in cell morphology were observed by inverted microscope; B-D. E-cadherin, Vimentin, N-cadherin, ZEB1 and SNAIL expression in Huh-7 cells tested via RT-qPCR; E. E-cadherin protein expression was measured by immunofluorescence detection; Cell experiments were repeated at least three times, * vs the mimic-NC group, $P < 0.05$; # vs the sh-NC group, $P < 0.05$; + vs sh-THUMPD3-AS1 + inhibitor-NC group, $P < 0.05$.

protein expression by implementing immunofluorescence, and the results unearthed that E-cadherin expression was elevated after treatment of sh-THUMPD3-AS1 and miR-4465 mimic, and E-cadherin expression after sh-THUMPD3-AS1 + miR-4465-inhibitor treatment was reduced (Fig. 5E). Thus, it was implied that THUMPD3-AS1 promoted EMT of HCC through regulating miR-4465.

4. Discussion

HCC, the 4th main cause of cancer-linked deaths in the world, has drawn public attention. The data from previous work have verified that lncRNAs take part in the malignant processes of cancers. THUMPD3-AS1 has been manifested to perform as a tumor promoter in some cancers. Nevertheless, the concrete role of THUMPD3-AS1 in HCC requires deeper exploration. This work aimed to uncover the potency of THUMPD3-AS1 in accelerating the phenotype of HCC cells by regulating miR-4465 and KPNA2.

The main finding of this work manifested that over-expressed THUMPD3-AS1 exhibited in HCC clinical tissues and cells. In addition, silenced THUMPD3-AS1 inhibited the cell growth and EMT *in vitro* but promoted apoptosis in HCC. Plenty of studies are applied for validation. For instance, THUMPD3-AS1 has been revealed to be upregulated in ovarian cancer cells, and THUMPD3-AS1 knockdown impedes OVCAR3 and SKOV3 cell viability and strengthen apoptosis [9]. Another articles has discovered that THUMPD3-AS1 is upregulated in lung cancer tissues. In addition, overexpression and knockdown analysis has implied that THUMPD3-AS1 advances tumor progression by enhancing lung cancer cell proliferation and self-renewal [8]. A interesting finding of the work was that

THUMPD3-AS1 could bind to miR-4465. THUMPD3-AS1 has a targeting relationship with miRNAs. For example, a study has found that THUMPD3-AS1 suppresses ovarian cancer cell apoptosis via mediating the miR-320d/ARF1 axis [9]. Zhang et al. have supported that THUMPD3-AS1 exerts its functions on BCAT1 via competitive binding with miR-1297 [29]. While the connection of THUMPD3-AS1 with miR-4465 requires to be further researched.

The most glamorous finding was that reduced miR-4465 was present in HCC tissues and cells. Meanwhile, elevated miR-4465 suppressed the growth of HCC cells and EMT. As demonstrated before, similar results are gained in these studies. For example, Tang et al. have stated that miR-4465 expresses at a low level in cervical cancer, and gain-of-function assays have reflected that miR-4465 hinders cervical cancer growth and migration [30]. In Sun et al.'s article, they stated that the miR-4465 expression is decreased in lung cancer cells versus normal human bronchial epithelial cell lines, and elevation of miR-4465 impedes lung cancer cell invasiveness [11]. An obvious finding of our work was that miR-4465 had a targeting relationship with KPNA2. miRNAs have a targeting link with KPNA2. For instance, Tang et al. have stated that circ_0102171 elevates the level of CREBRF which is the downstream target of miR-4465 [30]. Mechanically, another paper has suggested that SDHAP1 upregulates EIF4G2 expression via sponging miR-4465 in ovarian cancer [12]. The relationship of miR-4465 with KPNA2 requires to be further researched.

A novel finding of the work was that KPNA2 was elevated in HCC tissues. KPNA2 is elevated in multiple cancers, which is related to poor prognosis. Besides, KPNA2 has been demonstrated to reinforce tumor formation and progression via involving cell differentiation, immune response, as well as viral infection [31]. It has been demonstrated that KPNA2 expression is higher in HCC tissues versus paracancerous tissues, which is identified as a indicator of poor prognosis and quick recurrence in HCC patients [14].

Altogether, the study stresses that THUMPD3-AS1 inhibits miR-4465 expression to induce EMT in HCC, thereby promoting HCC cell growth, which is of benefit to HCC treatment. The study provides promising potentials for HCC treatment. Such new molecular mechanisms which sustain HCC offer a new biomarker and treatment target for HCC and supply a molecular basis for tumor growth. Further studies should be constructed to verify the connection of THUMPD3-AS1, miR-4465 and KPNA2.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.reth.2025.01.010.

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