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Original Research

LINC00673-V4 Promotes Colorectal Cancer Cell Proliferation by Interacting With FUS and Restoring the Expression of Hippo-YAP Target Genes

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Abstract

Background: Colorectal cancer (CRC) is a leading cause of cancer-related morbidity and mortality worldwide; thus, understanding its molecular mechanisms is critical for developing novel therapeutic targets. Long non-coding RNAs (lncRNAs) play crucial isoform-specific roles in cancer. While oncogenic lncRNA LINC00673 is known to be involved in multiple malignancies and possesses five distinct transcript variants, the functional role of LINC00673-V4—a highly expressed transcript variant in CRC—remains largely unexplored. This study investigated how LINC00673-V4 drives CRC proliferation by modulating the Hippo-Yes-associated protein (Hippo-YAP) signaling pathway. **Methods:** Survival analysis was performed using the GSE39582 dataset to assess the prognostic value of LINC00673 in patients with CRC. The expression of LINC00673 transcript variants in CRC cell lines was detected by quantitative PCR. Cell Counting Kit-8 and 5-ethynyl-2′-deoxyuridine (EdU) incorporation assays were used to assess cell proliferation upon LINC00673-V4 knockdown or overexpression. Western blotting and immunofluorescence staining were utilized to analyze the expression of Hippo-YAP target genes. To confirm the interaction between LINC00673-V4 and fused in sarcoma (FUS), RNA immunoprecipitation (RIP) was performed, while co-IP was used to investigate potential protein–protein interactions. **Results:** High LINC00673 expression was observed in CRC cell lines and was associated with a poor prognosis in patients with CRC. Among the five LINC00673 transcript variants, LINC00673-V3 and LINC00673-V4 were the predominantly expressed isoforms; however, only LINC00673-V4 significantly promoted CRC cell proliferation. LINC00673-V4 inhibited YAP phosphorylation at Ser127, promoted YAP nuclear translocation, and upregulated the expression of Hippo-YAP target genes (connective tissue growth factor, cysteine-rich angiogenic inducer 61, survivin). RIP assays confirmed an association between LINC00673-V4 and FUS, while co-immunoprecipitation (co-IP) assays revealed that FUS interacted with both YAP and large tumor suppressor 1 (LATS1), suggesting FUS may facilitate LATS1-mediated YAP phosphorylation. FUS was found to promote LATS1-mediated YAP Ser127 phosphorylation, induce YAP cytoplasmic retention, and down-regulate Hippo-YAP target gene expression. Rescue experiments showed that LINC00673-V4 reversed the FUS-induced suppression of Hippo-YAP target gene expression. **Conclusions:** Our study identified LINC00673-V4 as an isoform-specific oncogenic lncRNA in CRC. By associating with FUS, LINC00673-V4 sequesters FUS away from the LATS1/YAP complex; thereby inhibiting FUS-mediated YAP phosphorylation and enhancing Hippo-YAP target gene expression. These findings expand the landscape of lncRNA isoform-specific regulation in cancer, highlighting LINC00673-V4 as a potential prognostic biomarker and therapeutic target for CRC.

Keywords: LINC00673-V4; RNA-binding protein FUS; Hippo signaling pathway; colorectal cancer

1. Introduction

Colorectal cancer (CRC) is a leading cause of cancer mortality worldwide. Data from the Global Cancer Observatory (GLOBOCAN) indicates that it is one of the most frequently diagnosed malignant tumors. With approximately 1.92 million new cases and over 900,000 deaths annually, CRC is the third most common cancer and the second most common cause of cancer-related mortality globally [1]. Therefore, there is an urgent need to elucidate the molecular mechanisms underlying the progression of CRC to identify novel therapeutic targets.

More than 90% of transcripts in the human genome lack protein-coding function and are referred to as non-coding RNAs (ncRNAs) [2]. Long ncRNAs (lncRNAs) are ncRNAs longer than 200 nucleotides, which play crucial roles in various biological processes, including tumorigenesis, tumor metastasis, and drug resistance [3]. In CRC, diverse lncRNAs drive biological processes through multiple

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molecular pathways. For example, specific lncRNAs such as LASTR [4] and AC093895.1 [5] function as competing endogenous RNAs by acting as sponges that sequester microRNAs (miRNAs), thereby indirectly regulating downstream target mRNA expression. By binding to proteins or serving as RNA scaffolds, lncRNAs act as critical regulators of cellular signaling. For example, in CRC, the lncRNA LINC00183 binds to enolase 1 to boost glycolysis. This metabolic shift leads to H3K18 lactylation, which ultimately triggers transcription of the oncogenic gene growth differentiation factor 15 [6]. In addition, alternative splicing enables the generation of multiple transcript variants or isoforms from a single gene [7]. Through the formation of structurally and functionally distinct transcript variants, lncRNAs impart remarkable complexity and precision to regulatory networks [8]. The lncRNA nuclear enriched abundant transcript 1 (NEAT1) is frequently dysregulated in various cancers. Its two primary isoforms, NEAT1_1 and NEAT1_2, exert distinct molecular mechanisms and isoform-specific functions in cancer progression [9,10]. LINC00673, also known as SLNCR or ERRLR01, is significantly upregulated in various cancers and promotes cancer progression [11,12,13,14]. We previously reported that LINC00673 promotes the epithelial-mesenchymal transition and cancer metastasis by sponging miR-150-5p, which indirectly upregulates zinc finger E-box binding homeobox 1 expression [15]. Although the *LINC00673* gene produces five transcript variants (V1 through V5), most studies have focused on the molecular function of its canonical transcript variant LINC00673-V3. For instance, Ni et al. [16] reported that LINC00673-V3 promotes autophagy by enhancing SMAD3-mediated light chain 3B transcription in non-small cell lung cancer; However, the molecular function of other LINC00673 transcript variants remains largely unexplored. A study by Guan et al. [17] showed that LINC00673-V4 promotes lung cancer progression by facilitating the interaction between DEAD-box polypeptide 3 (DDX3) and casein kinase I isoform epsilon (CK1ε), which subsequently triggers the downstream WNT/β-catenin signaling pathway. Therefore, it is important to elucidate how LINC00673 transcript variants regulate CRC.

The Hippo-Yes-associated protein (Hippo-YAP) signaling pathway is an important pathway that regulates organ size, cell proliferation, and cell survival [18,19] and plays critical roles in the progression of various cancers including CRC [20]. When the Hippo-YAP signaling pathway is activated, mammalian STE20-like protein kinases 1 and 2 phosphorylate and activate large tumor suppressor 1/2 (LATS1/2). Subsequently, these activated LATS kinases phosphorylate YAP, a modification that traps YAP in the cytoplasm and prevents it from entering the nucleus. When the Hippo-YAP signaling pathway is inhibited, YAP remains unphosphorylated and becomes hyperactive, driving the expression of proliferative genes such as connective tissue growth factor (CTGF) and cysteine-rich angiogenic inducer 61 (CYR61), which are frequently dysregulated in CRC [21,22].

In the current study, we explored the biological function and molecular mechanisms through which LINC00673-V4 activates the Hippo-YAP signaling pathway to facilitate CRC cell proliferation. Our findings highlight LINC00673-V4 as a promising therapeutic target for CRC.

2. Materials and Methods

2.1 Cell Culture and Reagents

Human CRC cell lines SW620 (SCSP-5234), DLD1 (SCSP-5241), HCT116 (SCSP-5076), LOVO (SCSP-514), SW480 (SCSP-5033), and HT29 (SCSP-5032) were purchased from the Shanghai Cell Collection (Shanghai, China) and cultured in mycoplasma-free RPMI 1640 medium (Sangon Biotech, Shanghai, China) supplemented with 10% fetal bovine serum (Gibco, Waltham, MA, USA) and 1% penicillin/streptomycin (Yeasen Biotechnology, Shanghai, China) under 37 °C and 5% CO_2. All cell lines were validated shortly before use by short tandem repeat profiling and tested negative for mycoplasma. Fused in sarcoma (FUS), YAP, LATS1, CTGF, CYR61, survivin, β-actin, lamin B, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primary antibodies were purchased from Proteintech (Rosemont, IL, USA). The primary antibodies, phosphorylated YAP (S127; 4911S, Cell Signaling Technology, Danvers, MA, USA) and phosphorylated YAP (S397; ImmunoWay Biotechnology, San Jose, CA, USA), were used. Horseradish peroxidase (HRP)-linked secondary antibodies were purchased from Huabio (Woburn, MA, USA). TED-347, an irreversible, allosteric, and covalent inhibitor of the YAP-TEAD PPI, was purchased from MedChemExpress (Monmouth Junction, NJ, USA).

2.2 Plasmids and Small Interfering RNA Transfection

Transfection of plasmids (final concentration 1 μg/mL) and small interfering RNA (siRNA) (final concentration of 15 nM) was performed using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The LINC00673-V3 and LINC00673-V4 overexpression constructs were generated by inserting the respective sequences into the pcDNA3.1 (+) vector (GenScript, Nanjing, China). The FUS overexpression plasmid was purchased from YouBio Biology (Changsha, China). The negative control (NC) siRNA and siRNAs targeting specific genes were provided by Tsingke Biology (Beijing, China), and their sequences are listed below:

si-NC sense: UUCUCCGAACGUGUCACGU(dT)(dT);

si-NC anti-sense: ACGUGACACGUUCGGAGAA(dT)(dT);

si-V3&V4-1 sense: AAGAGGAUGGGAAGGACUGAU;

si-V3&V4-1 anti-sense: AUCAGUCCUUCCCAUCCUCUU;

si-V3&V4-2 sense: AAGAAAGAGGAUGGGAAGGAC;

si-V3&V4-2 anti-sense: GUCCUUCCCAUCCUCUUUCUU;

si-FUS-1 sense: CGGACAUGGCCUCAAACGA(dT)(dT);

si-FUS-1 anti-sense: UCGUUUGAGGCCAUGUCCG(dT)(dT);

si-FUS-2 sense: UUGGGUGAUCAGGAAUUGGAA;

si-FUS-2 anti-sense: CCAAUUCCUGAUCACCCACUU.

2.3 Western Blotting and Protein Co-Immunoprecipitation

Cells (5×10^5) were collected and lysed with RIPA lysis buffer (Beyotime, Beijing, China) supplemented with 1 mM PMSF and complete protease inhibitor cocktail (Selleck Chemicals, Houston, TX, USA). A nuclear-cytoplasmic fractionation kit (Beyotime) was used to extract nuclear and cytoplasmic proteins. Protein co-immunoprecipitation (co-IP) was performed using cell lysates extracted with NP40 lysis buffer (Beyotime) supplemented with complete protease inhibitor cocktail (Selleck). Equal amounts of protein were incubated with the indicated primary antibodies or control immunoglobulin G (IgG) (1 µg) at 4 ℃ overnight with rotation. Then, protein A/G magnetic beads (Selleck) were added and incubated for an additional 2–4 h at 4 ℃. After washing with lysis buffer, the immunoprecipitates were eluted by boiling in protein loading buffer.

A BCA kit (Yeasen) was utilized to quantify the protein concentration, and equal amounts of total protein (20 µg) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis on 10–12% gels. Protein was electrotransferred to polyvinylidene fluoride (PVDF) membranes, and membranes were blocked in 5% bovine serum albumin (BSA) (Beyotime) and incubated with primary antibodies (1:1000 dilution) overnight at 4 °C, followed by incubation with HRP-linked secondary antibodies for 1 h. Proteins were detected by enhanced chemiluminescence (Yeasen) and visualized using the ChemiScope 3300 Mini Chemiluminescence Imaging System (Clinx Science Instruments, Shanghai, China).

2.4 Cell Proliferation Assays

The Cell Counting Kit-8 (CCK-8) assay (Yeasen) was performed according to the manufacturer's instructions. In brief, cells were exposed to the indicated treatment, and approximately 2000 cells were seeded into 96-well plates. After cells adhered, 10 µL CCK-8 solution was added at 0, 24, 48, and 72 h and incubated at 37 ℃ for 1 h. Optical density was measured at 450 nm using a microplate reader to generate cell proliferation curves (sample size = 3).

We used the Cell-Light EdU Apollo 567 *In Vitro* Imaging Kit (Ribobio, Guangzhou, China) to perform the EdU assay. In brief, cells were exposed to the indicated treatment, and approximately 5000 cells were seeded into 96-well plates at 37 ℃ for 24 h. Then, medium containing 50 µM EdU was added, and cells were incubated at 37 ℃ for 2 h. Cells were fixed in 4% paraformaldehyde and stained with Hoechst and Apollo reaction cocktail. Images were randomly captured using a fluorescence microscope and merged using Adobe Photoshop software. EdU-positive cells and total cells were counted within each field, and the proportion of EdU-positive cells was calculated (sample size = 3).

2.5 RIP

LncRNA interacting with FUS was identified using an RIP kit (BersinBio, Guangzhou, China). Briefly, 2×10^7 cells were lysed on ice in polysome lysis buffer supplemented with protease and RNase inhibitors. After DNA removal, cell lysates were incubated with anti-FUS or control IgG antibodies (2 µg) at 4 °C for 16 h. Immunoprecipitates were captured using protein A/G beads. RNA was then extracted from both immunoprecipitates and input samples via Trizol reagent, and RNA was subjected to further quantitative PCR (qPCR) analysis (sample size = 3).

2.6 RNA Extraction and qPCR

Total RNA was extracted using Trizol reagent (Takara Bio, San Jose, CA, USA) according to the manufacturer's instructions, and it was reverse transcribed to cDNA using the PrimeScript RT Reagent Kit with gDNA Eraser (Takara). qPCR was performed to detect the relative expression of mRNA or lncRNA. All qPCR reactions were performed using SYBR green premix in the LightCycler480 Real-Time PCR System (Roche, Basel, Switzerland). Agarose gel electrophoresis was used to show the qPCR product lengths of the LINC00673 transcript variants. We normalized LINC00673 transcript variants to LINC00673-V3 using $2^{-\Delta CT}$ normalization within a specific cell line. The expression levels of LINC00673 transcript variants among different CRC cell lines were calculated using $2^{-\Delta\Delta CT}$ normalization, and the β-actin (ACTB) gene was used as an internal control (sample size = 3). Primers for qPCR are listed below:

ACTB-FP: GTTGTCGACGACGAGCG;

ACTB-RP: GCACAGAGCCTCGCCTT;

LINC00673-V1-FP: ATTTTTCCCTCTCCACCCTGG;

LINC00673-V1-RP: CCAGCCCTGTAGAGGTCCTCA;

LINC00673-V2-FP: CAAGCTGGAGGTGGAATCAGAGG;

LINC00673-V2-RP: AGGAGGTGGCACCTCTTTCTTG;

LINC00673-V3-FP: ATTTTTCCCTCTCCACCCTGG;

LINC00673-V3-RP: GTCCTTCCCATCCTCTTTCTTG;

LINC00673-V4-FP: CAAGCTGGAGGTGGAATCAGAGG;

LINC00673-V4-RP: GTCCTTCCCATCCTCTTTCTTG;

LINC00673-V5-FP: AGATCAGGTTCAAGTTGCCAG;

LINC00673-V5-RP: CTGCAGGGCCGGGAGGAGAGG.

2.7 Immunofluorescence Staining

Cells (1×10^5/mL) were seeded onto sterile coverslips in 6-well plates (sample size = 3). After treatment, cells were fixed in 4% paraformaldehyde and permeabilized in phosphate-buffered saline with Tween-20 (PBST) containing 0.5% Triton X-100 (Sangon Biotech). After blocking in 5% BSA in PBST for 1 h, samples were incubated with primary antibodies overnight at 4 °C and then incubated with fluorochrome-conjugated secondary antibodies (HuaBio) for 1 h at room temperature. Nuclei were stained with DAPI (Sangon Biotech). Images were captured using a fluorescence microscope (Nikon, Tokyo, Japan).

2.8 Public Dataset Analysis and Statistical Analyses

The Cancer Cell Line Encyclopedia (CCLE) database was used to show the expression levels of LINC00673 across various cancer cell lines [23]. Survival analysis on the GSE39582 dataset (sample size = 558) was performed. Using X-tile software to determine the optimal cutoff value, we categorized patients into high and low LINC00673 expression groups [24]. Survival differences were evaluated using Kaplan-Meier survival curves and log-rank tests. All experiments included at least three independent biological replicates, with three technical replicates per sample. Statistical analyses were performed using GraphPad Prism, and data are expressed as the mean ± standard deviation. Two-group comparisons were performed using Student's *t*-test, whereas differences among multiple groups were determined using one-way analysis of variance and Dunnett's post-hoc analysis. $p < 0.05$ was considered statistically significant.

3. Results

3.1 LINC00673 is an Unfavorable Prognostic Factor for CRC

The CCLE database contains microarray expression matrix data for a variety of cancer cell lines. We found that the expression level of LINC00673 (measured as the sum of transcript variants V1–V5) is among the highest in all cancer cell lines (Fig. 1A). To investigate whether LINC00673 expression predicts survival in CRC, we analyzed the GSE39582 dataset from the Gene Expression Omnibus database. This dataset contains microarray expression data from the tumor tissues of 558 patients with stage I–IV CRC. High LINC00673 expression was significantly linked to poorer overall survival (OS) (hazard ratio [HR] = 1.44, 95% confidence interval [CI]: 1.07–2.12; $p = 0.021$) and recurrence-free survival (RFS) (HR = 1.64, 95% CI: 1.20–2.49; $p = 0.003$) compared to low expression (Fig. 1B,C). We further conducted a subgroup analysis of patients with stage III–IV CRC. Compared with the low LINC00673 expression group, the high LINC00673 expression group showed a trend towards worse OS (HR = 1.26, 95% CI: 0.80–2.08; $p = 0.307$) and significantly worse RFS (HR = 1.75, 95% CI: 1.17–3.12; $p = 0.011$) (Fig. 1D,E). These results suggest that high LINC00673 expression is an unfavorable prognostic factor for patients with CRC.

3.2 Relative Expression of LINC00673 Transcript Variants in CRC Cells

Since LINC00673 expresses five transcript variants (V1–V5), we used qPCR primers based on the exon-junction regions of each transcript for quantification (Fig. 2A). Fig. 2B presents the primer sequences and product length. Using agarose gel electrophoresis, we verified that all primers specifically amplified their respective LINC00673 transcript variants. Furthermore, the lengths of the PCR products matched their predicted sizes (Fig. 2C). Then, we detected the relative expression of LINC00673 transcript variants in six CRC cell lines (SW620, DLD1, HCT116, LOVO, SW480, and HT29). We normalized the LINC00673 expression data across all transcript variants to LINC00673-V3, which is the most abundantly expressed variant. In all CRC cell lines, LINC00673-V3 and LINC00673-V4 were highly expressed, whereas LINC00673-V2 and LINC00673-V5 were expressed at low levels (Fig. 2D). Using the expression of LINC00673 variants in SW620 cells as a baseline, we observed the high expression of LINC00673-V3 and LINC00673-V4 in SW480 cells, and relatively low expression in DLD1 cells (Fig. 2E).

3.3 LINC00673-V4 Promotes the Proliferation of CRC Cells

Since LINC00673-V3 and LINC00673-V4 are highly expressed in CRC cells, we further explored the biological function of these two transcript variants. Because LINC00673-V4 shares exons 1, 3, and 5 with all other LINC00673 variants, it is impossible to design an siRNA specific to this variant without unintentionally targeting the others. We designed siRNAs (si-V3&V4-1 and si-V3&V4-2) to target the junction between exon 3 and exon 5 of LINC00673, simultaneously knocking down both LINC00673-V3 and LINC00673-V4 variants in SW480 cells (Fig. 3A,B). In addition, we used plasmid transfection to individually overexpress LINC00673-V3 and LINC00673-V4 in DLD1 cells, which resulted in a 4- to 5-fold overexpression efficiency (Fig. 3C,D). Simultane-

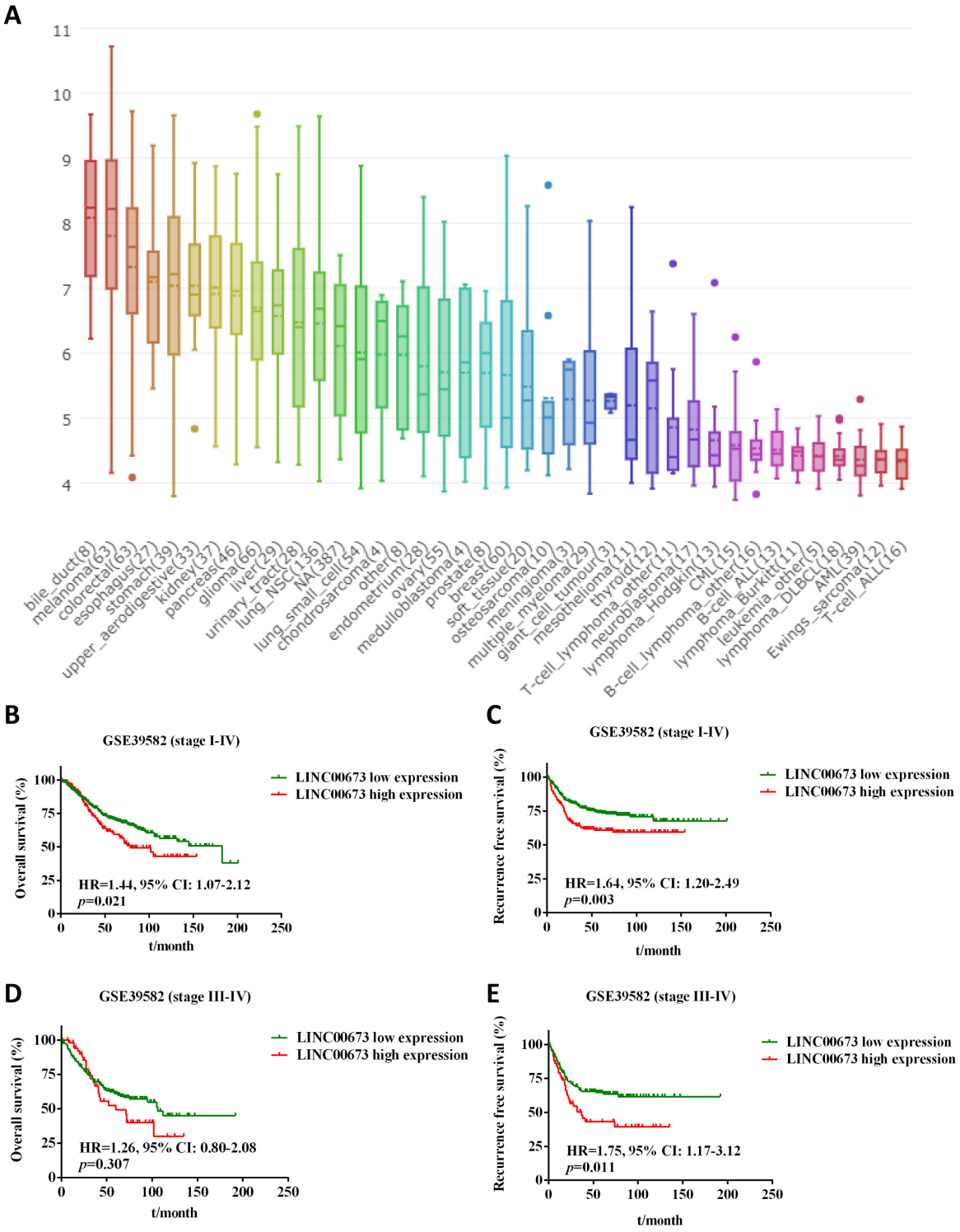

Fig. 1. LINC00673 is an unfavorable prognostic factor for CRC. (A) The CCLE database showed that LINC00673 is highly expressed in CRC cell lines. (B,C) Survival analysis on the GSE39582 dataset. The optimal cutoff value of LINC00673 expression was determined by X-tile software (cutoff value: 3.50 for OS and 3.47 for RFS), and patients were divided into LINC00673 high and low expression groups. Kaplan-Meier survival curves and log-rank tests were used. (D,E) Subgroup analysis in patients with stage III–IV CRC. CRC, colorectal cancer; CCLE, Cancer Cell Line Encyclopedia; OS, overall survival; RFS, recurrence-free survival.

ous knockdown of LINC00673-V3 and LINC00673-V4 in SW480 cells significantly inhibited cell proliferation, as demonstrated by the CCK-8 and EdU assays (Fig. 3E,G,I). In DLD1 cells, overexpression of LINC00673-V4 significantly increased cell proliferation (by 13.7% in the CCK-8 assay and 35% in the EdU assay), whereas overexpression

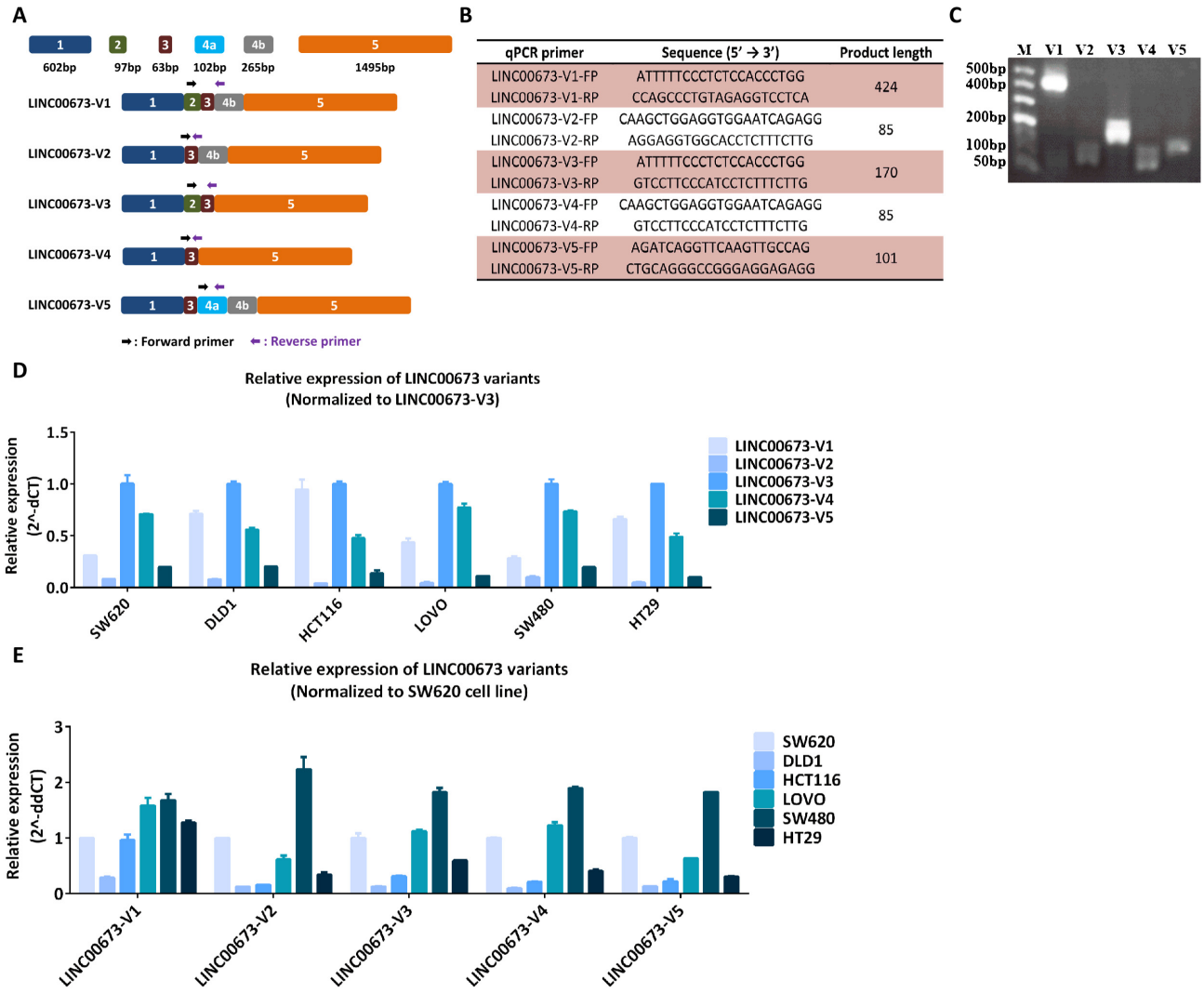

Fig. 2. Relative expression of LINC00673 transcript variants in CRC cells. (A) The LINC00673 gene generates five transcript variants (V1–V5) and consists of six exons (1, 2, 3, 4a, 4b, and 5). (B) qPCR primers used for quantifying LINC00673 transcripts. (C) Agarose gel electrophoresis of the PCR products was consistent with the predicted lengths. (D) qPCR was used to detect the relative expression of LINC00673 transcript variants in six CRC cell lines. We normalized LINC00673 transcript variants to LINC00673-V3 using the $2^{-\Delta CT}$ method. (E) qPCR was used to detect the expression levels of LINC00673 transcript variants among different CRC cell lines, with expression normalized to the ACTB housekeeping gene in SW620 cells using the $2^{-\Delta\Delta CT}$ normalization method. Data are presented as the mean ± standard deviation. qPCR, quantitative PCR; ACTB, β-actin.

of LINC00673-V3 had no such effect (Fig. 3F,H,J). These results suggest that LINC00673-V4, but not LINC00673-V3, drives the proliferation of CRC cells.

3.4 LINC00673-V4 Promotes the Nuclear Translocation of YAP and Upregulates the Expression of Hippo-YAP Target Genes

The Hippo-YAP signaling pathway is a fundamental regulator of cell proliferation and organ size. We further investigated how LINC00673-V4 regulates this pathway. Overexpression of LINC00673-V4 in DLD1 cells significantly increased the protein expression levels of Hippo-YAP target genes (CTGF, CYR61, and BIRC5/survivin), and markedly reduced the phosphorylation level of YAP at Ser127, which is responsible for YAP cytoplasmic retention. By contrast, total protein levels of YAP and LATS1, as well as YAP phosphorylation at Ser397, remained unchanged (Fig. 4A). Consistently, knockdown of LINC00673-V4 in SW480 cells significantly decreased the protein expression levels of Hippo-YAP target genes (CTGF, CYR61, and BIRC5/survivin), and markedly increased the phosphorylation level of YAP at Ser127 (Fig. 4B).

In SW480 cells, nuclear-cytoplasmic fractionation showed that knocking down LINC00673-V4 significantly decreased the amount of YAP entering the nucleus (Fig. 4C). Using immunofluorescence assays, we fur-

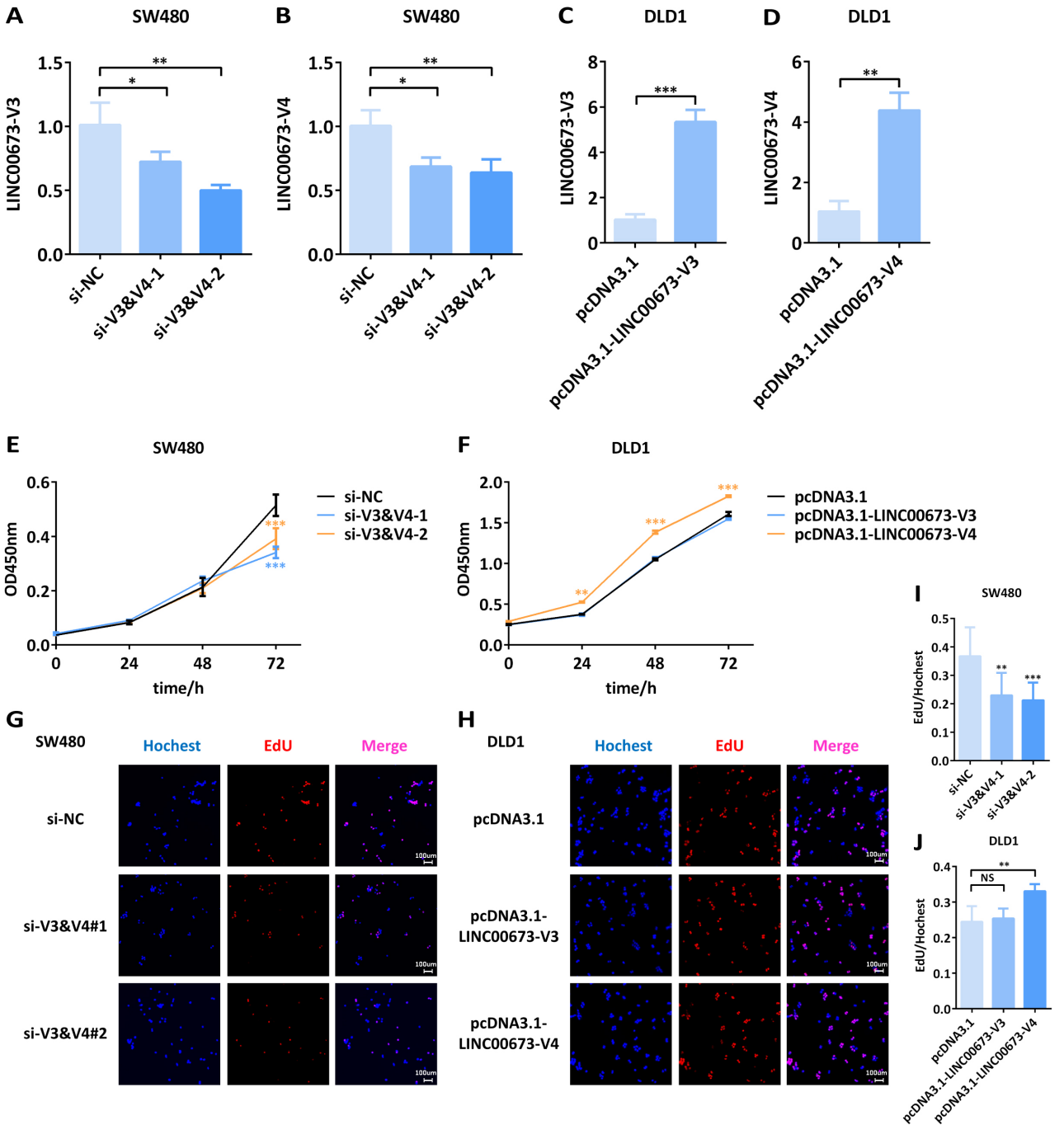

Fig. 3. LINC00673-V4 promoted the proliferation of CRC cells. (A,B) qPCR was performed to verify the knockdown efficiency of siRNA against LINC00673-V3 and LINC00673-V4 in SW480 cells. (C,D) qPCR was performed to verify the overexpression efficiency of LINC00673-V3 and LINC00673-V4 in DLD1 cells. (E,G,I) CCK-8 and EdU assays were performed to assess the effect of LINC00673-V3 and LINC00673-V4 knockdown on the proliferation of SW480 cells, scale bar =100 μm. (F,H,J) CCK-8 and EdU assays were performed to assess the effect of LINC00673-V3 and LINC00673-V4 overexpression on the proliferation of DLD1 cells, scale bar = 100 μm. Data are presented as the mean ± standard deviation, *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$. NS, not significant; CCK-8, Cell Counting Kit-8; EdU, 5-ethynyl-2′-deoxyuridine.

ther demonstrated that upregulating LINC00673-V4 drives YAP into the nucleus in DLD1 cells. Conversely, silencing LINC00673-V4 prevents YAP nuclear translocation in SW480 cells (Fig. 4D,E). In DLD1 cells, treatment with the YAP-TEAD inhibitor TED-347 reversed the LINC00673-V4-induced upregulation of Hippo-YAP target genes (CTGF, CYR61, and BIRC5/survivin) (Fig. 4F). In addition, we found that the mRNA expression levels of

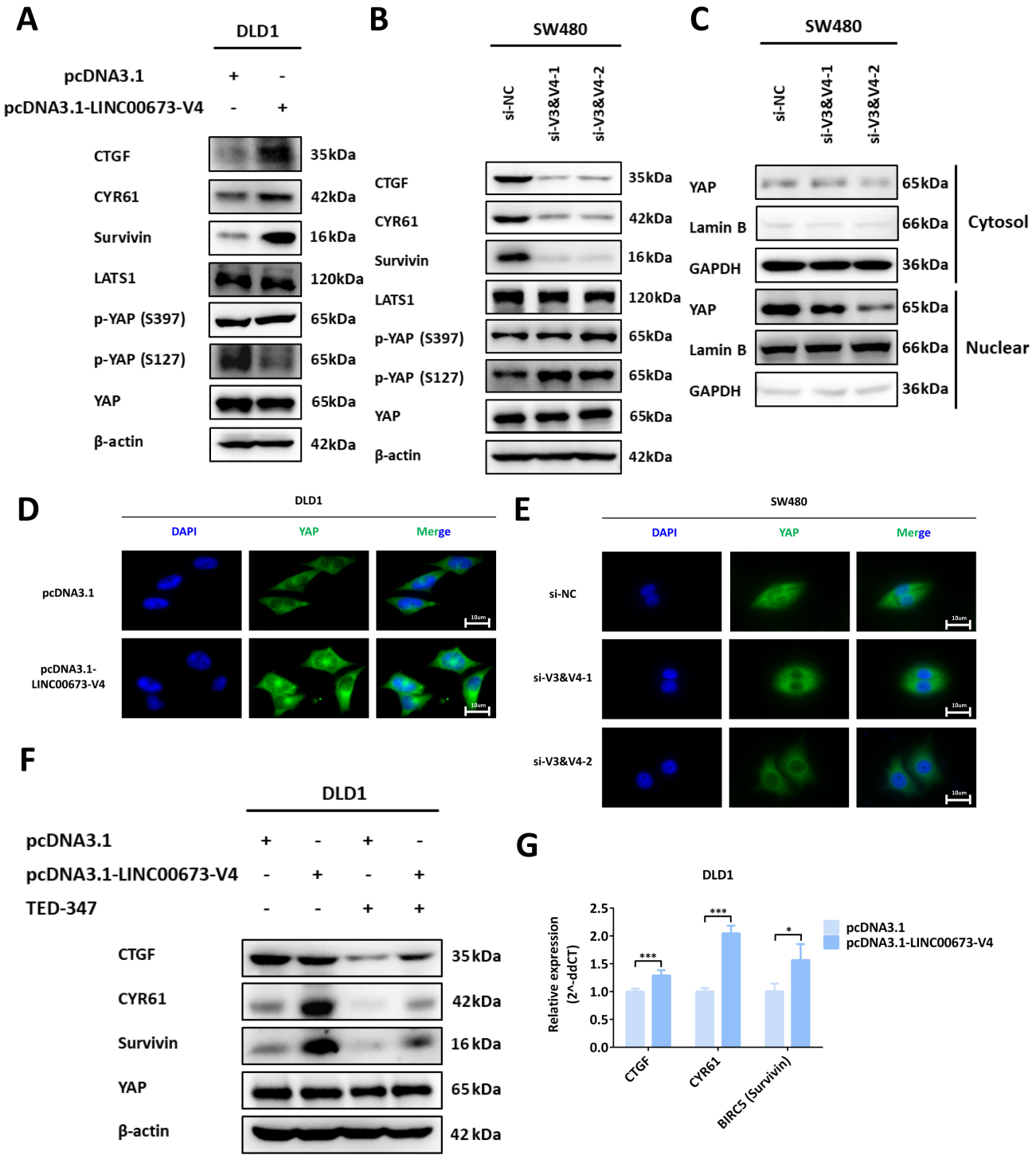

Fig. 4. LINC00673-V4 promoted the nuclear translocation of YAP and upregulated the expression of Hippo-YAP target genes. (A,B) LINC00673-V4 was overexpressed in DLD1 cells and knocked down in SW480 cells. Western blot analysis was used to detect the phosphorylation level of YAP and the expression of Hippo-YAP target genes. (C) Nuclear-cytoplasmic fractionation was used to examine the distribution of YAP in the nucleus and cytoplasm after LINC00673-V4 knockdown in SW480 cells. (D,E) Immunofluorescence was used to detect the subcellular localization of YAP after LINC00673-V4 knockdown or overexpression, scale bar: 10 μm. (F) LINC00673-V4 was overexpressed in DLD1 cells with the addition of the YAP-TEAD inhibitor TED-347. Western blotting was used to detect the expression of Hippo-YAP target genes. (G) After LINC00673-V4 overexpression in DLD1 cells, qPCR was performed to detect the mRNA transcription levels of downstream Hippo-YAP target genes. Data are presented as the mean ± standard deviation, $*p < 0.05$, $***p < 0.005$. YAP, Yes-associated protein.

Hippo-YAP target genes were also significantly elevated (Fig. 4G). These results suggest that LINC00673-V4 inhibited the phosphorylation of YAP at Ser127, thereby promoting the nuclear translocation of YAP and upregulating the expression of Hippo-YAP target genes.

3.5 LINC00673-V4 is Associated With the RNA-Binding Protein FUS

Interactions between lncRNAs and proteins represent one of the major mechanisms by which lncRNAs regulate gene expression and cellular phenotypes. A previous study reported that LINC00673-V4 promotes lung cancer progression by interacting with DDX3 and CK1ε [17]; however, the interacting proteins of LINC00673-V4 in CRC remain unexplored. To identify interacting proteins of LINC00673-V4 in CRC, we used the RNA–protein interaction prediction software catRAPID to screen a series of proteins that potentially interact with LINC00673-V4. Table 1 details the 10 proteins most likely to interact with LINC00673-V4, each yielding an interaction discriminant score and strength exceeding 85%. We identified the RNA-binding protein FUS as a key candidate that interacts with lncRNA ST18-AS1, stabilizes ST18 mRNA, and suppresses pancreatic cancer proliferation [25]. Furthermore, the RNA recognition motif of FUS spans amino acids 285–371, indicating that LINC00673-V4 likely binds to this specific region.

Table 1. Top 10 proteins predicted to interact with LINC00673-V4 by catRAPID.

Proteins	Interaction discriminant score (%)	Interaction strength (%)
TIA1	99	100
RBM4	98	100
ELAV2	97	99
LN28B	97	100
FUS	97	99
TIAR	96	99
HNRPF	93	99
HNRH2	89	100
PTBP1	89	99
SRSF2	89	99

RIP assays validated the RNA–protein interaction predictions, demonstrating that LINC00673-V4 binds to FUS (Fig. 5), whereas LINC00673-V3 does not, consistent with our previous phenotypic results.

3.6 FUS Interacts With YAP and LATS1 and Negatively Regulates the Expression of Hippo-YAP Target Genes

To further explore the downstream biological effects of the interaction between LINC00673-V4 and FUS, we used the BioGRID PPI database to search for proteins that potentially interact with FUS. We found that many of the candidate interacting proteins of FUS were transcription factors and transcriptional co-regulators. Notably, FUS was predicted to interact with YAP, the transcriptional co-activator of the Hippo-YAP signaling pathway (Fig. 6A). We further performed co-IP assays in SW480 and DLD1 cells and confirmed that FUS interacted with both YAP and its upstream kinase LATS1 (Fig. 6B,C).

We further knocked down FUS using siRNA in DLD1 cells, and found that the phosphorylation level of YAP at Ser127 was significantly decreased, while the protein expression levels of the Hippo-YAP target genes CTGF, CYR61, and BIRC5/survivin were markedly increased (Fig. 6D). By contrast, total protein levels of YAP, its upstream kinase LATS1, and Ser397-phosphorylated YAP remained unchanged (Fig. 6D). Consistent results were obtained by overexpressing FUS in SW480 cells, and overexpression of FUS significantly increased YAP phosphorylation at Ser127 and decreased the protein expression levels of Hippo-YAP target genes (CTGF, CYR61, and BIRC5/survivin) (Fig. 6E). Immunofluorescence assays showed that overexpression of FUS in SW480 cells significantly increased the nuclear translocation of YAP (Fig. 6F). We used siRNA to knockdown FUS in DLD1 cells, and qPCR assays revealed that the mRNA expression levels of Hippo-YAP target genes were significantly increased (Fig. 6G). CCK-8 cell proliferation assays demonstrated that overexpression of FUS significantly inhibited the proliferation of both SW480 and DLD1 cells (Fig. 6H,I). To further investigate how LINC00673-V4 and FUS co-regulate the Hippo-YAP signaling pathway, we performed rescue experiments. We found that overexpressing LINC00673-V4 in DLD1 cells reversed two effects caused by FUS overexpression: the increased phosphorylation of YAP at Ser127 and the downregulation of Hippo-YAP target genes (Fig. 6J).

4. Discussion

In this study, we identified LINC00673-V4 as a key tumor-promoting lncRNA in CRC. LINC00673-V4 exerts its biological function by interacting with the FUS RNA-binding protein to upregulate Hippo-YAP target genes. These findings broaden our understanding of how specific LINC00673 isoforms are regulated in CRC, while uncovering a novel regulatory mechanism in the Hippo-YAP signaling pathway.

Consistent with previous studies identifying LINC00673 as an oncogenic lncRNA [12,13,26,27], we found that elevated levels predict poor survival in patients with CRC—particularly reduced RFS in stage III–IV cases. In addition, genetic analysis revealed that the LINC00673 rs11655237 C>T polymorphism increases the risk of several malignancies, including pancreatic cancer, gastric cancer, cervical cancer, hepatoblastoma, and neuroblastoma [28].

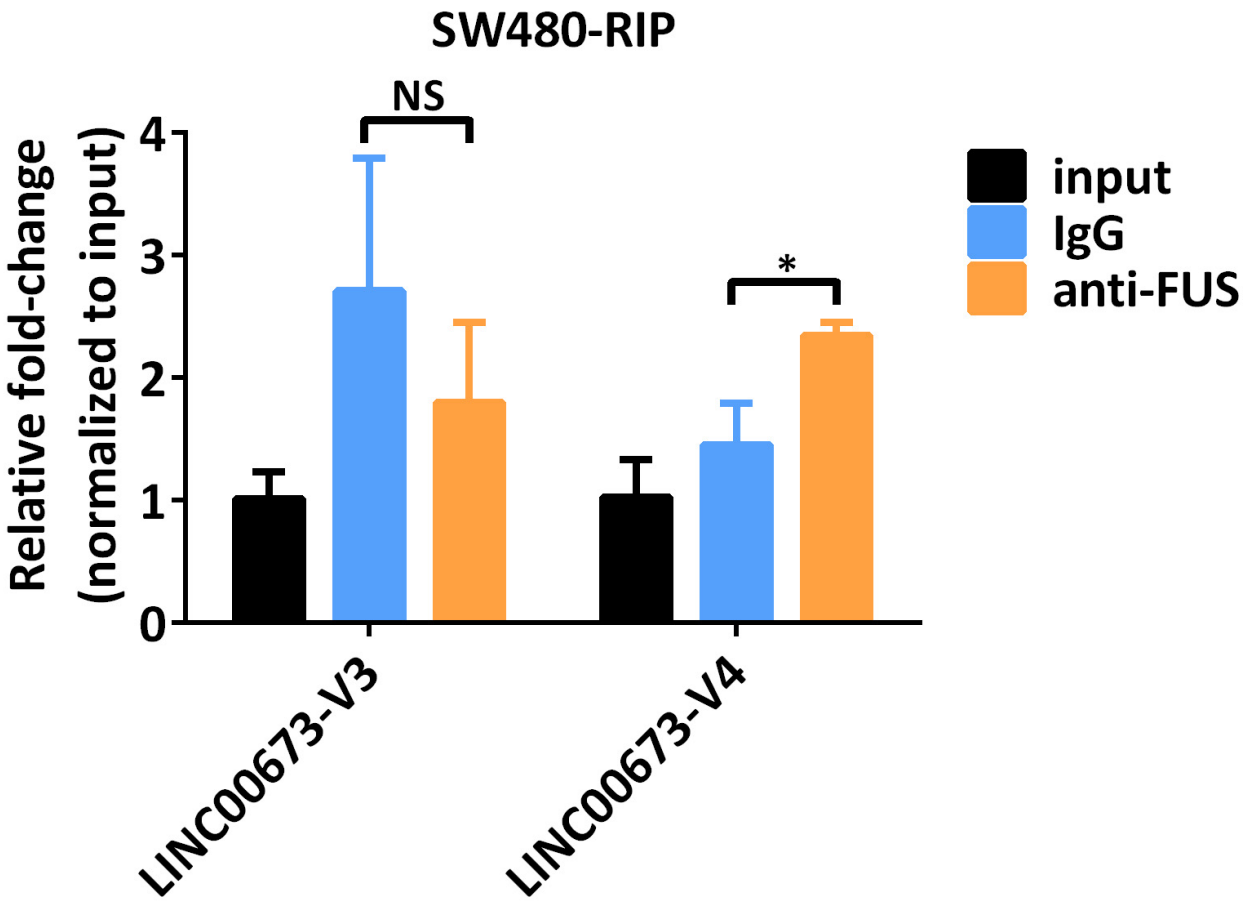

Fig. 5. LINC00673-V4 is associated with the RNA-binding protein FUS. RIP assays were performed in SW480 cells to detect the enrichment of LINC00673-V3 and LINC00673-V4 in complexes IP'd with anti-FUS antibodies, compared to IgG negative controls and input samples. The y-axis represents relative fold change normalized to input. Data are presented as the mean ± standard deviation, *$p < 0.05$. NS, not significant; FUS, Fused in sarcoma; RIP, RNA immunoprecipitation; IP, immunoprecipitation.

It is noteworthy that while prior research has focused on LINC00673-V3, our analysis of five LINC00673 transcript variants in CRC cell lines demonstrated that both LINC00673-V3 and LINC00673-V4 are primary isoforms, but only LINC00673-V4 promotes CRC cell proliferation. Our results are consistent with previous research showing that LINC00673-V4 is the most abundant LINC00673 transcript and drives lung cancer progression by activating the WNT/β-catenin signaling pathway [17]. This isoform-specific functional divergence underscores that different LINC00673 transcript variants may play distinct roles in cancer biology, which may vary by cancer type.

Our study presents the novel discovery that LINC00673-V4 binds to FUS, demonstrating for the first time how this interaction regulates Hippo-YAP signaling in CRC. We found that FUS interacts with both YAP and its upstream kinase, LATS1. By promoting LATS1-mediated phosphorylation of YAP at Ser127, FUS acts as a positive regulator of the Hippo-YAP cascade. This traps YAP in the cytoplasm, preventing it from activating target genes and ultimately reducing cell proliferation. Critically, LINC00673-V4 acts as an oncogenic sponge that sequesters FUS from the LATS1/YAP complex, reversing YAP phosphorylation and restoring the expression of Hippo-YAP target genes. This functional antagonism between LINC00673-V4 and FUS uncovers a novel mechanism of Hippo-YAP pathway regulation. Despite the sequence similarity between LINC00673-V3 and LINC00673-V4, LINC00673-V3 does not interact with FUS, which explains its inability to promote the proliferation of CRC cells. Our study has important clinical value for CRC. Because elevated LINC00673 expression correlates with shorter OS and RFS, it serves as a valuable prognostic biomarker to improve patient risk stratification and guide clinical decision-making for patients with CRC. In addition, the LINC00673-V4/FUS/Hippo-YAP regulatory axis provides a novel therapeutic target for CRC. Strategies aimed at silencing LINC00673-V4 or disrupting its interaction with FUS, such as antisense oligonucleotides (ASOs) or small molecule inhibitors, may effectively inhibit YAP-mediated tumor growth and hold promise for future clinical translation.

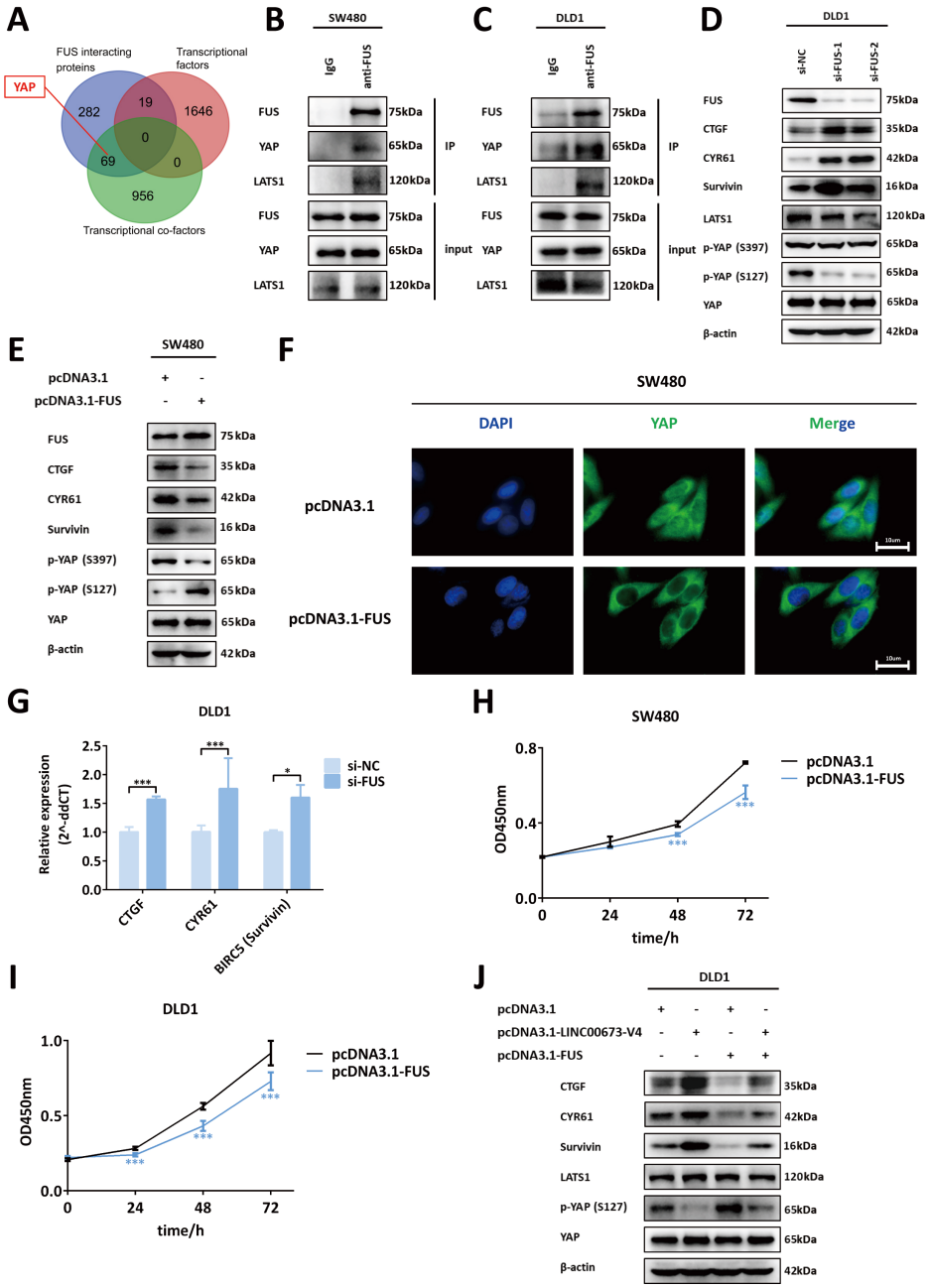

Fig. 6. FUS interacted with YAP and LATS1 and negatively regulated the expression of Hippo-YAP target genes. (A) Venn diagram shows that a subset of proteins potentially interacting with FUS includes transcription factors and transcriptional co-regulators. Since YAP functions as a transcriptional co-activator in the Hippo-YAP signaling pathway, it may also interact with FUS. (B,C) Co-IP assays were used to validate the interactions of FUS with YAP and LATS1 in SW480 and DLD1 cells. (D,E) FUS expression was knocked down using siRNA in DLD1 cells and overexpressed via plasmid transfection in SW480 cells. Western blotting was used to detect the protein expression levels of FUS, Hippo-YAP target genes, total YAP, p-YAP (S127), p-YAP (S397), and LATS1. (F) Overexpression of FUS was performed in SW480 cells, and immunofluorescence assays were used to examine the subcellular distribution of YAP in the nucleus and cytoplasm, scale bar: 10 μm. (G) FUS was knocked down by siRNA in DLD1 cells, and the mRNA expression levels of Hippo-YAP target genes were detected by qPCR. (H,I) FUS was overexpressed in SW480 and DLD1 cells, and the CCK-8 assay was used to assess cell proliferation. (J) Plasmids were used to overexpress FUS and LINC00673-V4 in DLD1 cells, and Western blotting was used to detect the protein expression levels of Hippo-YAP target genes, YAP, p-YAP (S127), and LATS1. Data are expressed as the mean ± standard deviation, $*p < 0.05$, $***p < 0.005$. LATS1, large tumor suppressor 1.

5. Limitations

While our study provides novel mechanistic insights, it had several limitations that warrant future investigation. First, our experiments were conducted *in vitro*; thus, *in vivo* validation is needed to confirm the oncogenic role of LINC00673-V4 in regulating the Hippo-YAP signaling pathway. Second, we performed the RIP assay to confirm that LINC00673-V4 binds to FUS; however, the precise binding region remains unknown. Mapping this interaction will enable the design of ASOs or targeted inhibitors to disrupt the LINC00673-V4/FUS complex for CRC treatment. Third, although this study explored the influence of LINC00673-V4 on the Hippo-YAP signaling pathway and cell proliferation, future studies should focus on determining whether LINC00673-V4 regulates other hallmarks of cancer, such as metastasis and chemoresistance. The biological functions of other LINC00673 transcript variants (e.g., LINC00673-V1, LINC00673-V2, and LINC00673-V5) remain undefined. Although they are relatively scarce in CRC, these variants may still play important roles in other cancers.

6. Conclusions

FUS suppresses Hippo-YAP target genes by driving LATS1 to phosphorylate YAP at Ser127, which sequesters YAP in the cytoplasm. By associating with FUS, LINC00673-V4 sequesters FUS away from LATS1/YAP complex, LINC00673-V4 blocks FUS's ability to promote YAP phosphorylation, up-regulates Hippo-YAP target gene expression and promotes colorectal cancer proliferation.

Availability of Data and Materials

The data and materials in the current study are available from the corresponding author on reasonable requests.

Author Contributions

WL: Writing-original draft, Investigation, Funding acquisition, Conceptualization. JMZ: Investigation, Data curation, Methodology. YXZ: Visualization, Writing-review & editing. HHC: Conceptualization, Investigation, Writing-review & editing, Supervision. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflicts of Interest

The authors declare no conflicts of interest.

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