

circ_0004662 contributes to colorectal cancer progression by interacting with hnRNPM

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Abstract. Circular (circ)RNAs participate in colorectal cancer (CRC) occurrence and progression. However, the role of hsa_circ_0004662 (circ_0004662) in CRC remains unknown. Reverse transcription-quantitative PCR noted high expression of circ_0004662 in CRC compared with normal colorectal epithelial cells. circ_0004662 knockdown inhibited migration of CRC cells *in vitro* and *in vivo*; wound healing and Transwell assays showed that circ_0004662 overexpression contributed to CRC migration. Nuclear cytoplasmic analysis and fluorescence *in situ* hybridization revealed localization of circ_0004662 in the nucleus and cytoplasm. CircRNADB databases predicted that circ_0004662 exhibited translational potential and liquid chromatography-mass spectrometry (LC-MS) of circ_0004662 pull-down products suggested that circ_0004662 bound to multiple ribosomal subunits. However, peptide products of 149aa translated by circ_0004662, with a molecular weight of ~17 kDa were not detected. Nevertheless, LC-MS analysis indicated that circ_0004662 bound multiple proteins. Immunoprecipitation of RNA-binding proteins revealed that circ_0004662 bound to heterogeneous nuclear ribonucleoprotein M (hnRNPM) and that hnRNPM interference decreased circ_0004662 expression, thereby affecting CRC progression. In summary, circ_0004662 was significantly upregulated in CRC. As a non-coding RNA, it may promote

CRC progression by binding to hnRNPM, which may serve as a potential target for treating CRC.

Introduction

Colorectal cancer (CRC) is one of the common types of malignant tumors, as the third most common malignancy and the second most deadly cancer, CRC accounts for 10% of global cancer incidence and 9.4% of cancer deaths in 2020 (1). Recently, its incidence has been continuously increasing, posing a serious threat to lives and health (2). Therefore, exploring underlying mechanisms of CRC progression and identifying novel therapeutic biomarkers for CRC are essential.

As a type of endogenous RNA, circular RNAs (circRNAs) are generated by the back-splicing of pre-mRNAs, forming a covalently closed loop structure. circRNAs lack the traditional 5'-end cap structure and 3'-end poly A tail; this makes them more stable and resistant to exonucleases compared with linear RNA (3). Accumulating evidence suggests the key role of circRNAs in cancer development and progression, with some (such as circRNA_102231 and circRNA_CDR1as) identified as available biomarkers for predicting cancer diagnosis, treatment and prognosis (4-6). Multiple circRNAs with different fragment lengths and sequences can be derived from the same parental gene, such as different circRNAs are generated from ubiquitin-associated protein 2 (UBAP2) and three domain family 33 (7-10), which may exert various biological function. Circular RNA UBAP2 contributed to malignant properties of prostate cancer and osteosarcoma, however, it inhibited proliferation and metastasis of clear cell renal cell carcinoma. Previous study showed that manganese superoxide dismutase (SOD2) could modulate colorectal tumorigenesis (11), however, the role of its circRNAs in CRC remains unreported.

The present study aim to explore the circRNAs derived from the SOD family using databases and clarify its role in colorectal cancer.

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Materials and methods

Clinical specimens. Paired adjacent normal tissue (distance from cancer tissue greater than 5 cm) and CRC tissue (n=18) was collected from Anhui Provincial Cancer Hospital (Hefei, China) from January 2023 to December 2023. Two pathologists histologically confirmed the final diagnosis of CRC. Only patients with pathologically diagnosed CRC were included, the exclusion criterion was a history of malignant tumor treatment in other parts of the body. Table SI summarizes the clinical characteristics of 18 patients with CRC, with patients age ranging from 41 to 84 years, and the median age was 58. The surgically excised specimens were stored at -80°C until used. The Medical Ethics Committee of the Anhui Provincial Cancer Hospital approved the study (approval no. 2023081). All participants provided written informed consent according to the Declaration of Helsinki.

Cell lines. Human normal colon epithelial cells FHC (cat. no. FH1283, Cellosaurus Accession no. CVCL_3688) were purchased from Fuheng Biology. CRC cell lines HT-29 (cat. no. C5083, CVCL_0320), SW480 (cat. no. C5233, CVCL_0546), DLD-1 (cat. no. C5060, CVCL_0248), HCT116 (cat. no. C5229, CVCL_0291), LOVO (cat. no. C5178, CVCL_0399), COLO205 (cat. no. C5053, CVCL_0218) and Caco-2 (cat. no. C5224; CVCL_0025) were purchased from Zhejiang Baidi Biotechnology Co., Ltd. All cells were cultured in high-glucose Dulbecco's modified Eagle's medium (cat. no. 10566016) supplemented with 1% penicillin and streptomycin (cat. no. 15140122, both Gibco; Thermo Fisher Scientific, Inc.) and 10% fetal bovine serum (FBS, cat. no. 40131ES76, Shanghai Yeasen Biotechnology Co., Ltd.). Cells were cultured in a humidified environment with 5% CO₂ at 37°C. All the cell lines were verified via short tandem repeat analysis.

RNA extraction and reverse transcription-quantitative (RT-q) PCR. Eastep Super Total RNA Extraction kit (cat. no. LS1040, Promega Corporation) was used to extract total tissues/cell RNA. RNA was converted to cDNA using GoScript Reverse Transcription Mix (cat. no. A2800, Promega Corporation) according to the manufacturer's instructions. The GoTaq qPCR Master Mix (cat. no. A6002, Promega Corporation) was used to perform RT-qPCR. 18S rRNA was used as an endogenous control to measure the levels of circRNA (Table SII) and mRNA. Thermocycling conditions were as follows: Initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 30 sec. The specificity of amplification was confirmed via melting curve analysis. The 2^{-ΔΔC_q} method was used to assess gene expression (12). Primers are listed in Table SIII. Primers of circ_0004662 and circ_0078541 were designed based on the sequence of back splicing site and the primers of circRNAs in the present study were designed and validated by Guangzhou Genesee Biotech. Co., Ltd.

RNase R treatment. Total RNA (5 μg) extracted from CRC cells was treated in the presence or absence of 5 U/μg RNase R (cat. no. R0300, Guangzhou Genesee Biotech. Co., Ltd.) at 37°C for 30 min. Then, RNase R was inactivated at 70°C for 10 min. Finally, RNA was reverse-transcribed using a random primer.

Actinomycin D treatment. CRC cells were treated with 2 μg/ml actinomycin D (cat. no. 15021S, Cell Signaling Technology, Inc.) at 37°C for 0, 4, 8 and 12 h. Then, total cell RNA was extracted, followed by RT-qPCR to measure the stability of circRNAs. The gene expression at 0 h was considered the baseline.

Construction of plasmid vectors and cell transfection. To silence circ_0004662, short hairpin RNA (shRNA) targeting the junction sites of circ_0004662 was designed by Guangzhou RiboBio Co., Ltd. (Table SIV). The pLshRNA vector (Shanghai Calm Biotechnology Co., Ltd.) was used as the shRNA plasmid; empty vector was used as sh-negative control (NC). cDNA sequence of human circ_0004662 was synthesized and cloned into pLC5-ciR vector (Guangzhou Genesee Biotech. Co., Ltd.) to generate overexpression plasmids. Sanger sequencing was performed to confirm all constructs. The 2.5 μg constructed plasmids, 1.88 μg PSPAX2 vector (Shanghai Calm Biotechnology Co., Ltd.) and 0.625 μg PMD2G vector (Shanghai Calm Biotechnology Co., Ltd.) were then co-transfected into 3rd generation 293T cells (Zhejiang Baidi Biotechnology Co., Ltd.) using Lipofectamine 3000 (cat. no. L3000008, Thermo Fisher Scientific, Inc.) to package lentivirus according to manufacturer's instruction. After a 48 h incubation at 37°C, 2 ml viral supernatant was collected and filtered with a 0.22 μm filter. Then viral supernatant was added for 8 h, after which the medium was replaced. The cells were then subjected to selection using 2 μg/ml puromycin for 3 days, with 1 μg/ml puromycin used for maintenance. Subsequent experiments were then started. The sequence used in the present study are as follows: circ_0004662-siRNA#1 (TATGCTGAGAGATGTTACA); circ_0004662-siRNA#2 (CGATCGTTATGCTGAGAGA); circ_0004662-siRNA#3 (TCGTTATGCTGAGAGATGT).

Cell proliferation and migration assay. Wound healing and Transwell assays were performed to assess migration ability of CRC cells. For wound healing assay, 1x10⁶ transfected CRC cells (DLD-1, SW480, HCT116) were added to complete DMEM supplemented with 10% FBS in 24-well plates. A 200-μl sterile tip was used to make a scratch in the monolayer (confluence ~100% at the start of the assay, followed by culture in medium containing 2-5% FBS as aforementioned). Cells were photographed at 0 and 48 h (cat. no. IX2-SLP, Olympus) with magnification is 100X. ImageJ software (version 1.54 g, National Institutes of Health) was used to measure relative wound healing area.

For Transwell assay, serum-free medium containing 1x10⁵ transfected CRC cells (DLD-1 and SW480) was added to upper 24-well Transwell chambers (cat. no. 3422, Corning, Inc.), a complete DMEM (cat. no. 10566016, Gibco; Thermo Fisher Scientific, Inc.) containing 10% FBS was added to lower chamber in 24-well plates. Cells were then incubated in a CO₂ incubator at 37°C. After 48 h, cells were fixed with 4% paraformaldehyde for 10 min at room temperature, and stained with 0.1% crystal violet at room temperature for 10 min. The migrated cells in the bottom chambers were photographed by invert light microscope (cat. IX2-SLP, Olympus Corporation) with magnification 200X, and counted by ImageJ software (version 1.54 g, National Institutes of Health).

Subcellular fractionation. Cytoplasmic and Nuclear RNA Purification kit (cat. no. 21000, Norgen Biotek Corp.) was used to extract cytoplasmic and nuclear RNA. Briefly, CRC cells were treated with lysis buffer for 15 min and centrifuged at 4°C, 12,000 x g for 5 min. Nuclear RNA was isolated from the pellets and supernatant was collected to extract cytoplasmic RNA according to the manufacturer's instructions. Subsequently, reverse transcription and PCR were performed as aforementioned. GAPDH was utilized as the cytoplasmic reference gene and U6 as the nuclear reference gene.

Fluorescence in situ hybridization (FISH). RNA FISH probes to target the splicing site of circ_0004662 (5'CY3-TGTAAC ATCTCTCAGCATAACG-3'CY3) were designed and synthesized by Guangzhou Genesee Biotech. Co., Ltd. A total of 5x10⁴ DLD-1 cells were seeded on slides at the bottom of 24-well plates. When the cell density reaches 70%, slides were washed with PBS at room temperature for 5 min, then 4% paraformaldehyde was used to fix cells at room temperature for 5 min and washed with PBS for 5 min. TritonX-100 (cat. no. P0096, Beyotime Institute of Biotechnology) was added to incubate slides at room temperature for 15 min, and wash with PBS three times for 5 min each time. Then anhydrous ethanol was added, and the ethanol was aspirated after 1 min of action at room temperature. circ_0004662-specific Cy3 probes were added to cells at 37°C overnight. The nuclei were counterstained with DAPI (cat. no. C1002, Beyotime Institute of Biotechnology) at room temperature for 5 min. FISH kit (cat. no. H0101, Guangzhou Genesee Biotech. Co., Ltd.) was used according to the manufacturer's instructions. Probe signals were imaged under a laser scanning confocal microscope (cat. no. TCS SP2 AOBs, Leica GmbH; magnification is 400X).

Western blotting. Briefly, cells were lysed using RIPA lysis buffer containing protease and phosphatase and protease inhibitor cocktails (cat. nos. P0013B, P1009 and P1050, respectively; all Beyotime Institute of Biotechnology). Protein concentration was determined by BCA assay. A One-Step PAGE Gel Fast Preparation kit (8%; cat. no. E302-1, Vazyme Biotech Co., Ltd.) was used to separate equal amounts of protein (20 µg/lane). The separated proteins were transferred to polyvinylidene fluoride membranes (cat. no. IPVH00010, MilliporeSigma). Non-specific binding was blocked at room temperature for 1 h using Quick Block Blocking Buffer (cat. no. P0252, Beyotime Institute of Biotechnology). Then, the membranes were incubated with antibodies overnight at 4°C as follows: Flag-tagged recombinant rabbit monoclonal (cat. no. 30504ES50, Shanghai Yeasen Biotechnology Co., Ltd.), recombinant anti-SOD2 (1:1,000, cat. no. ab68155, Abcam), S100 calcium binding protein A9 (S100A9) polyclonal (1:1,000, cat. no. 26992-1-AP), phosphoglycerate kinase 1 (PGK1) polyclonal (1:1,000, cat. no. 17811-1-AP), heterogeneous nuclear ribonucleoprotein M (hnRNP) polyclonal (1:1,000, cat. no. 26897-1-AP) and β-tubulin monoclonal (1:1,000, cat. no. 30301ES60, all Proteintech Group, Inc.). After the membranes were washed with 1X TBST (cat. no. ST673, Beyotime Institute of Biotechnology), they were incubated with horseradish peroxidase-labeled goat anti-mouse or anti-rabbit IgG (1:1,000, cat. nos. A0216 and A0208, respectively; Beyotime Institute of Biotechnology) for

1 h at 25°C. Finally, ECL substrate kit (cat. no. 36222ES60, Shanghai Yeasen Biotechnology Co., Ltd.) was used to visualize the membranes. A chemiluminescence imaging system (Tano4600, Shanghai Tianneng Technology) was used to visualize strips and to perform semiquantitative analysis.

circ_0004662 pull-down assay and liquid chromatography-mass spectrometry (LC-MS) analysis. MS2 bacteriophage coat protein (MS2-CP) circRNA pull-down assay was performed to detect RNA-binding proteins (RBPs) that bind to circ_0004662. MS2 tagging technique is based on the natural binding between a stem-loop structure of MS2 and MS2-CP (13-15). Briefly, overexpression vectors (Guangzhou Genesee Biotech. Co., Ltd.) carrying circ_0004662-MS2 were constructed and tagged with green fluorescent protein (GFP); MS2-CP-Flag was tagged with red fluorescent protein (m-Cherry; Guangzhou Genesee Biotech. Co., Ltd.). The vectors were co-transfected into 293T cells as aforementioned. Cell protein was isolated by 500 µl lysis buffer as aforementioned. A total of 450 µl lysate was used for each IP reaction using RNA Immunoprecipitation Kit (cat. no. P0101, Guangzhou Genesee Biotech. Co., Ltd.) according to manufacturer's illustration. A total 5 µg anti-Flag antibody (cat. no. A00170, Nanjing Kingsray Biotechnology Co., Ltd.) and 100 µl protein A/G immunoprecipitation magnetic beads (cat. no. B23201, Selleck) were used to pull down MS2-CP-MS2-circ_0004662 complex. Lysate extracted from cells without the MS2 tagging system was used as NC. After detecting captured lysates via RT-qPCR and western blotting as aforementioned. The complexes were isolated by magnetic grate. LC-MS was performed to analyze the circ_0004662 pull-down complex and controls. Briefly, the peptide segments were dissolved in sample solution [0.1% formic acid (cat. no. 64186, Sigma), 2% acetonitrile (cat. no. A/0626/17, Fisher), and centrifuged at 4°C, 14,000 x g for 20 min, supernatant was collected and performed mass spectrometry identification. The liquid phase parameters was as follows: (a) Column information: 300 µm x 5 mm, Acclaim PepMap RSLC C18, 5 µm, 100A (cat. no. 160454, Thermo); Acclaim PepMap 75 µm X150 mm, C18, 3 µm, 100A (cat. no. 160321, Thermo). (b) Mobile phase information: Mobile phase A (0.1% formic acid); Mobile phase B: 0.1% formic acid, 80% acetonitrile; Flow rate: 300 nl/min. (c) Analysis time: 45 min. The separated peptide segments are directly fed into the mass spectrometer (Q Exactive, Thermo Fisher Scientific, Inc.) for online detection, with a resolution: 70,000; AGC target: 3e6; Maximum IT: 40 msec; Scan range: 350 to 1,800 m/z. The raw mass spectrometry files were processed and converted by MM File Conversion software to obtain MGF format files, and then the Uniprot database was searched using MASCOT (<http://www.matrixscience.com/>).

Prediction and verification of open reading frames (ORFs). The human circular RNA database (circRNA DB) was used to predict ORFs in circ_0004662 (16). To verify the functionality of the predicted ORF sequence in circ_0004662, circ_0004662 sequence was cloned into pLC5-ciR [translation verification vector (T)0] to construct T1 (Guangzhou Genesee Biotech. Co., Ltd.). Further, a FLAG coding sequence (5'-GAC TACAAAGACCATGACGGTGATTATAAAGATCATGAC ATCGATTACAAGGATGACGATGACAAG-3') was inserted

upstream of the stop codon (TGA) present in the ORF sequence to construct T2 vector (17,18). As a result, once the potential ORF sequence was translated, a tagged protein was generated. Vector with start codon ATG of the predicted ORF was mutated (T3), and vectors containing Flag tag with deletion of circular elements were also constructed (T4; Guangzhou Genesee Biotech. Co., Ltd.). FLAG sequence was directly cloned into pLC5-ciR (Guangzhou Genesee Biotech. Co., Ltd.) and used as a positive control (T5). Cells were transfected with the aforementioned plasmids and harvested after 3 days as aforementioned. Western blotting was performed using anti-FLAG antibody (1:1,000, cat. no. 30504ES50; Shanghai Yeasen Biotechnology Co., Ltd.) to measure the flagged protein according to previous description.

RNA-binding protein immunoprecipitation (RIP). Briefly, 1×10^7 cells were treated with IP lysis buffer (cat. no. P0013, Beyotime Institute of Biotechnology), protease inhibitor, and RNase inhibitor for 10 min on ice. Cells were centrifuged at 4°C and $10,000 \times g$ for 10 min. A total of $5 \mu\text{g}$ IP hnRNPM (1:100), S1009A (1:100), PGK1 (1:50) and IgG control polyclonal antibody (cat. no. 30000-0-AP, Proteintech Group, Inc.) were each bound to $20 \mu\text{l}$ magnetic beads (cat. no. P2108, Beyotime Institute of Biotechnology) for 2 h at 4°C . The cell lysis supernatant was then incubated with the magnetic bead suspension at room temperature for 2 h. A magnetic separator was used to collect the magnetic beads. Finally, pellets were treated with RIPA lysis buffer or TRIZOL (cat. no. 15596018CN, Thermo Fisher Scientific, Inc.) for western blotting and RNA extraction.

Animal experiments. A total of 12 male BALB/c nude mice (age, 5-6 weeks 17-19 g) were purchased from Hangzhou Ziyuan Laboratory Animal Technology (Zhejiang, China). Mice were housed under specific pathogen-free conditions with a 12/12-h light/dark cycle and controlled temperature at $24 \pm 2^{\circ}\text{C}$, and the relative humidity range was $50 \pm 10\%$. The mice were allowed ad libitum access to water and food pellets. DLD-1 cells were transfected with sh-circ_0004662 or sh-NC and subcutaneously injected into the right dorsum to generate subcutaneous tumors (5×10^6 /mouse; $n=6$ /group). Mice were monitored daily and tumor volume was measured every 4 days. After 30 days, mice were anesthetized with 3% isoflurane (maintained with 1.5% isoflurane) and euthanized using cervical dislocation. Physiological indicators such as respiration, heartbeat and pupil response were monitored to confirm the death of mice, and subcutaneous tumors were removed. Tumors were subjected to histological analysis. Tumor volume was calculated as follows: $\text{Volume (mm}^3\text{)} = \text{width}^2 \times \text{length} / 2$. All experiments were approved and performed according to the guidelines approved by the Institutional Animal Care and Use Committee of the First Hospital Affiliated to the University of Science and Technology of China [approval no. 2022-N(A)-072].

Immunohistochemical analysis. The mouse xenograft tumors were cut to $5\text{-}\mu\text{m}$ thick sections, and immunohistochemistry was performed according to established protocols (19). Primary antibodies utilized included anti-Ki67 (original usage; cat. no. MAB-0672; Proteintech Group, Inc.),

anti-E-cad, anti-N-cad (1:5,000, cat. nos. 20874-1-AP; 22018-1-AP, Proteintech Group, Inc.) and anti-vimentin (1:300; cat. no. ab92547, Abcam). Images were captured using the Olympus CX41 system with cell Sens Standard software (Olympus, Tokyo, Japan). IHC score were measured using ImageJ software (version 1.54 g, National Institutes of Health). The percentage of positive cells was subdivided into four grades: 0, $<5\%$; 1 for 6-25%; 2 for 26-50%; 3 for 51-75% and 4 for $>75\%$. Staining intensity was assessed by four degrees: 0, negative; 1, weak; 2, moderate; and 3, strong. The IHC score is the cell staining intensity score multiplied by the percentage of positive cells score.

Statistical analysis. SPSS 24.0 software (IBM Corp.) and GraphPad Prism 8.0 (GraphPad Software, Inc.; Dotmatics) were used for statistical analysis. Data are presented as the mean $\pm$ SD. Kolmogorov-Smirnov test was used to determine the distribution of each group. Paired student's t-test was used to measure significance between adjacent tissues and CRC tissues, while unpaired t-test (two-tailed) was used to determine significance between two groups. One-way ANOVA followed by Bonferroni's post hoc test was used to analyze >2 groups. A paired t-test was used to analyze circRNAs between CRC and adjacent normal tissue. All experiments were repeated at least three times. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

circ_0004662 is expressed highly in CRC samples. circBank database was used to identify circRNAs derived from SOD family genes (SOD1, SOD2 and SOD3). In total, five potential circRNAs were generated, including two derived from SOD1 (hsa_circ_0061417 and hsa_circ_0115795) and three from SOD2 (hsa_circ_0004662, hsa_circ_0078541 and hsa_circ_0005472). No circRNAs were generated from SOD3 (Table SII). However, only two circRNAs (circ_0004662 and circ_0078541) were successfully validated. Sanger sequencing was performed to confirm back-splice sites of circ_0004662 and circ_0078541; this matched the information in circBase (Fig. 1A and B). These circRNAs were resistant to RNase R treatment, whereas linear SOD2 were considerably digested with RNase R (Fig. 1C and D). Moreover, compared with wild type (0 h), treatment with actinomycin D, which can block new transcription, revealed that circ_0004662 and circ_0078541 were more stable in comparison to SOD2 mRNA (Fig. 1E and F). This confirmed the circular structures of circ_0004662 and circ_0078541 (Fig. 1G and H). Further analysis revealed differential expression of circ_0004662 in cancerous tissues compared with adjacent normal tissues (Fig. S1A). However, no significant difference was noted in transcription levels of circ_0078541 (Fig. S1B). Collectively, these findings underscore the potential importance of circ_0004662 in CRC pathogenesis.

circ_0004662 promotes the malignant characteristics of CRC cells both in vitro and in vivo. circ_0004662 expression was significantly higher in most CRC cell lines (DLD-1, LOVO, COLO205, Caco-2, SW480) compared with normal colon epithelial cells (FHC; Fig. 2A). shRNAs targeting

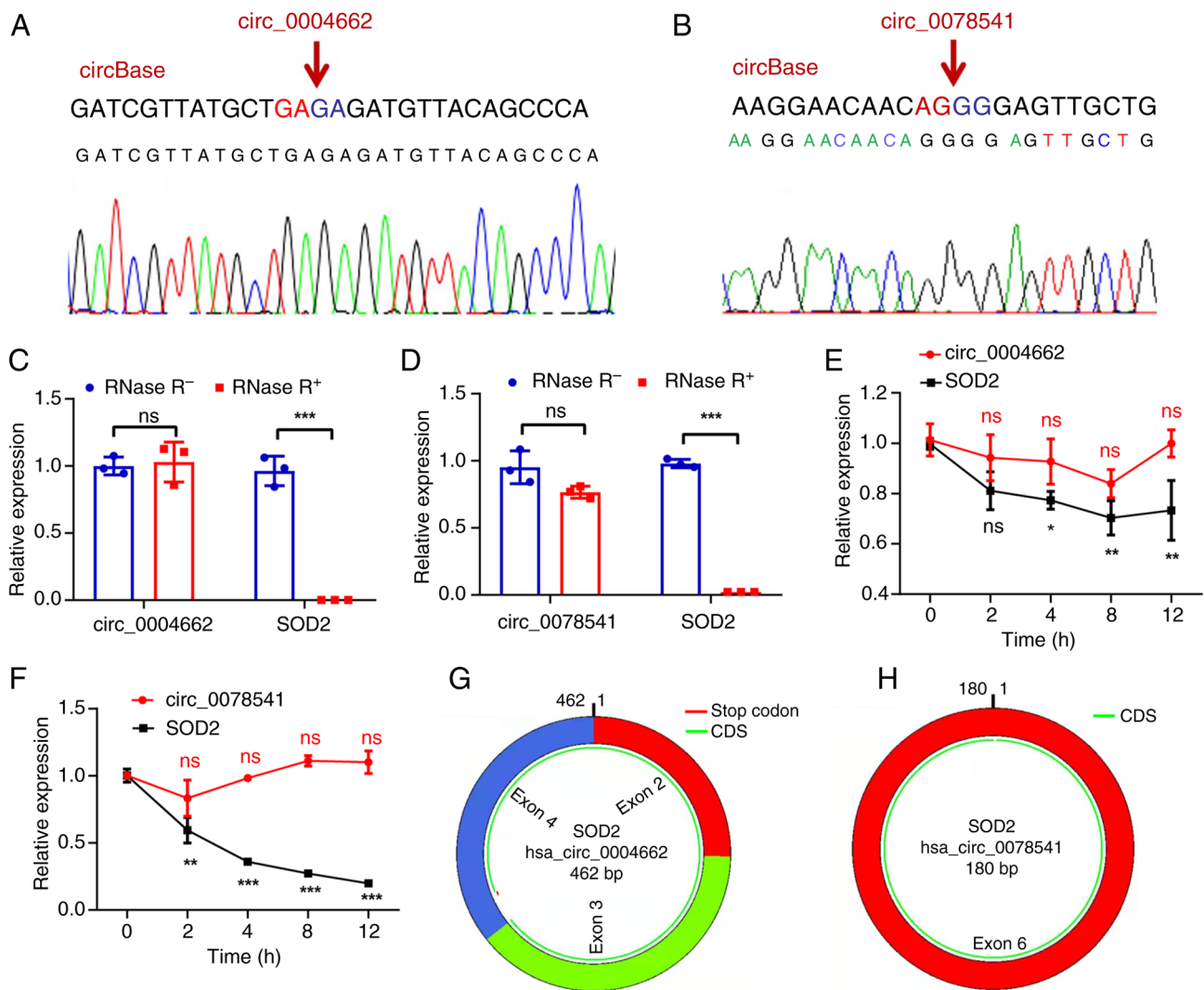


Figure 1. Characteristics of circSOD2. Reverse transcription-quantitative PCR products of (A) circ_0004662 and (B) circ_0078541 were analyzed via Sanger sequencing. Arrow indicates back-splicing sites. Expression of (C) circ_0004662, (D) circ_0078541 and SOD2 mRNA in CRC cells with or without RNase R treatment. Expression of (E) circ_0004662, (F) circ_0078541, and SOD2 mRNA in CRC cells following treatment with actinomycin D. Structure of (G) circ_0004662 and (H) circ_0078541 from circPrimer. *P<0.05, **P<0.01, ***P<0.001 vs. original group. circSOD2, circular RNAs derive from SOD2; CRC, colorectal cancer; CDS, coding sequence; ns, not significant.

circ_0004662 were transfected into DLD-1 and SW480 cells, whereas overexpression vector for circ_0004662 was transfected into HCT116 cells. circ_0004662 expression was significantly decreased in DLD-1 and SW480 cells (Figs. 2B and S2A) and circ_0004662 was significantly upregulated in the HCT116 cells (Fig. 2C).

Functional analysis revealed that circ_0004662 down-regulation markedly decrease migratory ability of CRC cells (Figs. 2D and S2B). By contrast, circ_0004662 over-expression contributed to migration ability of HCT116 cells (Fig. 2D, right). The Transwell assay also demonstrated that knockdown of circ_0004662 attenuated migration capacity in DLD-1 cells (Fig. 2E). circ_0004662 knockdown attenuated tumor growth *in vivo* (Fig. 3A-D). Immunohistochemistry showed that the proportion of Ki-67 in the knockdown group was lower than that in control group, indicating that knock-down of circ_0004662 decreased the proliferation ability of CRC cells *in vivo* (Fig. 3E). E-cadherin (E-cad), N-cad and vimentin (VIM) are metastasis biomarkers (20,21); E-cad was

upregulated, whereas N-cad were downregulated following knockdown of circ_0004662, while VIM expression was low in both groups (Fig. 3E).

circ_0004662 is localized in the nucleus and cytoplasm of CRC cells. Because circRNAs exert different functions depending on cellular localization (22,23), subcellular fractionation analysis was conducted to investigate localization of circ_0004662. circ_0004662 was present in the nucleus and cytoplasm of SW480 and HCT116 cells (Fig. 4A), which was further confirmed via FISH in DLD-1 cells (Fig. 4B).

circ_0004662 is a non-coding RNA in CRC cells. Using circDB database (reprod.njmu.edu.cn/circnadb), circ_0004662 was predicted to contain a potential ORF and a putative internal ribosome entry site sequence; this suggested that it can encode a 149-amino acid peptide (Fig. 5A). To investigate whether endogenous circ_0004662 can be translated into circ_0004662_149aa, Flag-coding sequence

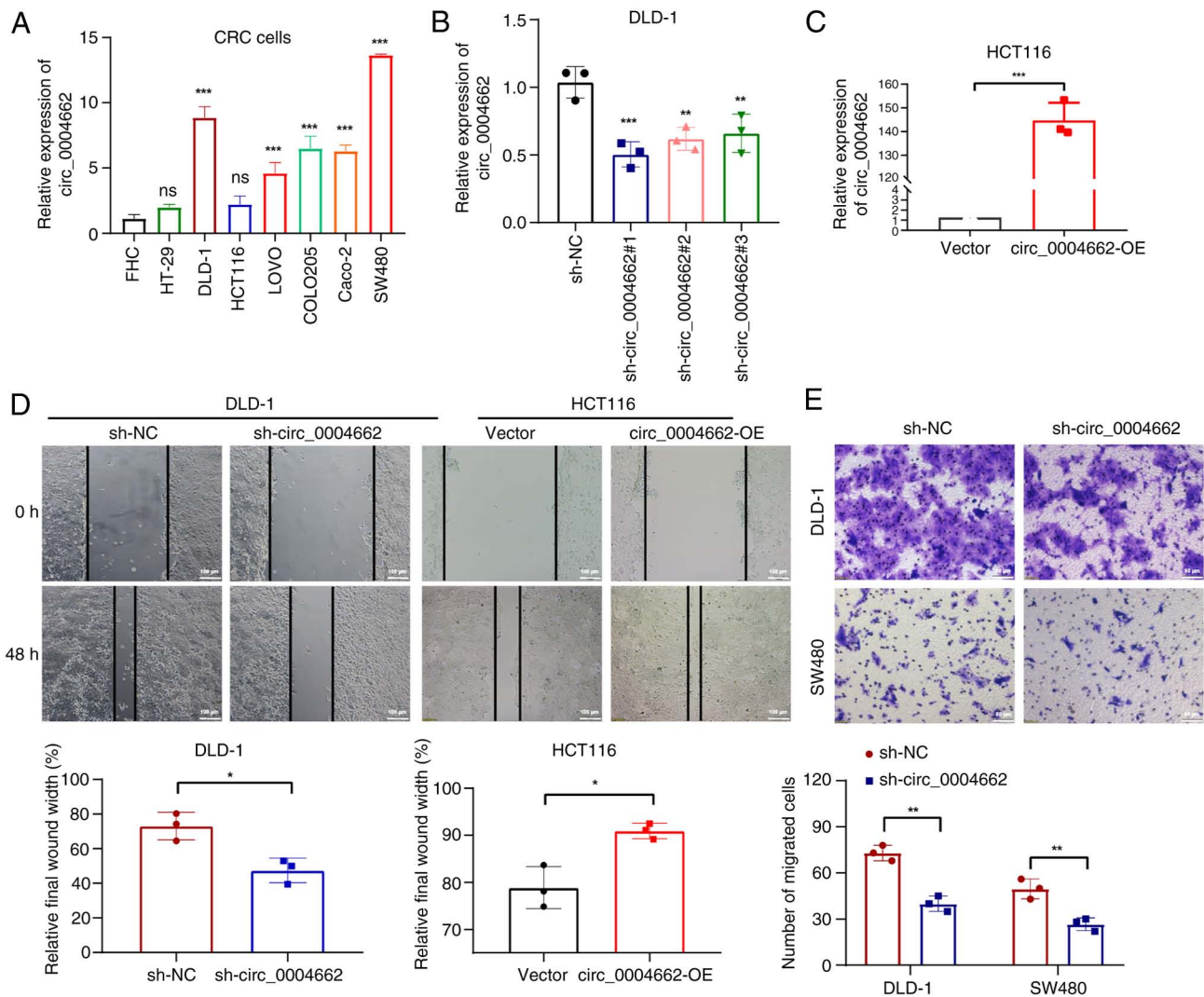


Figure 2. circ_0004662 promotes malignant biological characteristics *in vitro* and *in vivo*. circ_0004662 expression in (A) CRC cells and (B) DLD-1 cells treated with shRNAs against the junction sites of circ_0004662. (C) Overexpression efficiency of circ_0004662 was measured by reverse transcription-quantitative PCR in HCT 116 cells. (D) Migratory ability of circ_0004662-knockdown or overexpressing CRC cells was assessed via wound healing assay. Scale bar, 100 μ m. (E) Migration ability of circ_0004662-knockdown CRC cells was measured via Transwell assay. Scale bar, 50 μ m. * P <0.05, ** P <0.01, *** P <0.001 vs. control. circ, circular; CRC, colorectal cancer; sh, short hairpin; ns, not significant; NC, negative control.

was inserted upstream of the stop codon in the potential ORF sequence (Fig. 5B). Sanger sequencing confirmed the sequences of the plasmids (Fig. S3). However, in transfected cells, no FLAG-tagged proteins were detected at the predicted molecular weight size (Fig. 5C). Furthermore, immunoblotting using SOD2 antibody failed to identify circ_0004662_149aa at the expected size in 293T cells (Fig. 5D). Collectively, these findings confirm that circ_0004662 was a non-coding RNA.

circ_0004662 binds hnRNPM in CRC cells. MS2-CP-Flag circRNA pull-down assay was performed to discover the potential protein partners of circ_0004662 (Fig. 6A). Plasmids expressing circ_0004662-MS2-GFP and MS2-CP-Flag-mCherry were constructed. Using the circ_0004662-MS2 tagging system, RIP assay was conducted after co-transfecting circ_0004662 and MS2-CP-FLAG, resulting in pull-down of protein complexes between MS2 and MS2-CP by Flag antibodies (Fig. 6B). Sanger sequencing confirmed MS2-Flag insertion did not affect circRNA

circularization (Fig. S4A). RT-qPCR validated overexpression of circ_0004662 in the circ_0004662-MS2 tagging system (Fig. 6C). Label-free LC-MS analysis revealed that circ_0004662 may interact with multiple proteins, including several ribosomal proteins (ribosomal protein L36, ribosomal protein L35A, ribosomal protein S15A; Table SV), and genes (subtilisin-like Serine Protease 1, pyruvate kinase, lipocalin-1, S100 calcium binding Protein A9), that serve key roles in cancer progression (Table SVI). Based on previous literature, PGK1 (24,25), S100A9 (26,27), and hnRNPM (28,29) were selected for further validation. A RIP assay was conducted in DLD-1 cells using anti-hnRNPM, anti-PGK1 and anti-S100A9 antibodies, and found significant enrichment of circ_0004662 after anti-hnRNPM immunoprecipitation compared with IgG (Fig. 6D); immunoblotting confirmed this finding (Fig. S4B). hnRNPM can bind to circRNAs and regulate their generation, thereby affecting biological functions of cancer cells (29,30). Subsequent hnRNPM knockdown decreased circ_0004662 expression (Figs. 6E and F and S5)

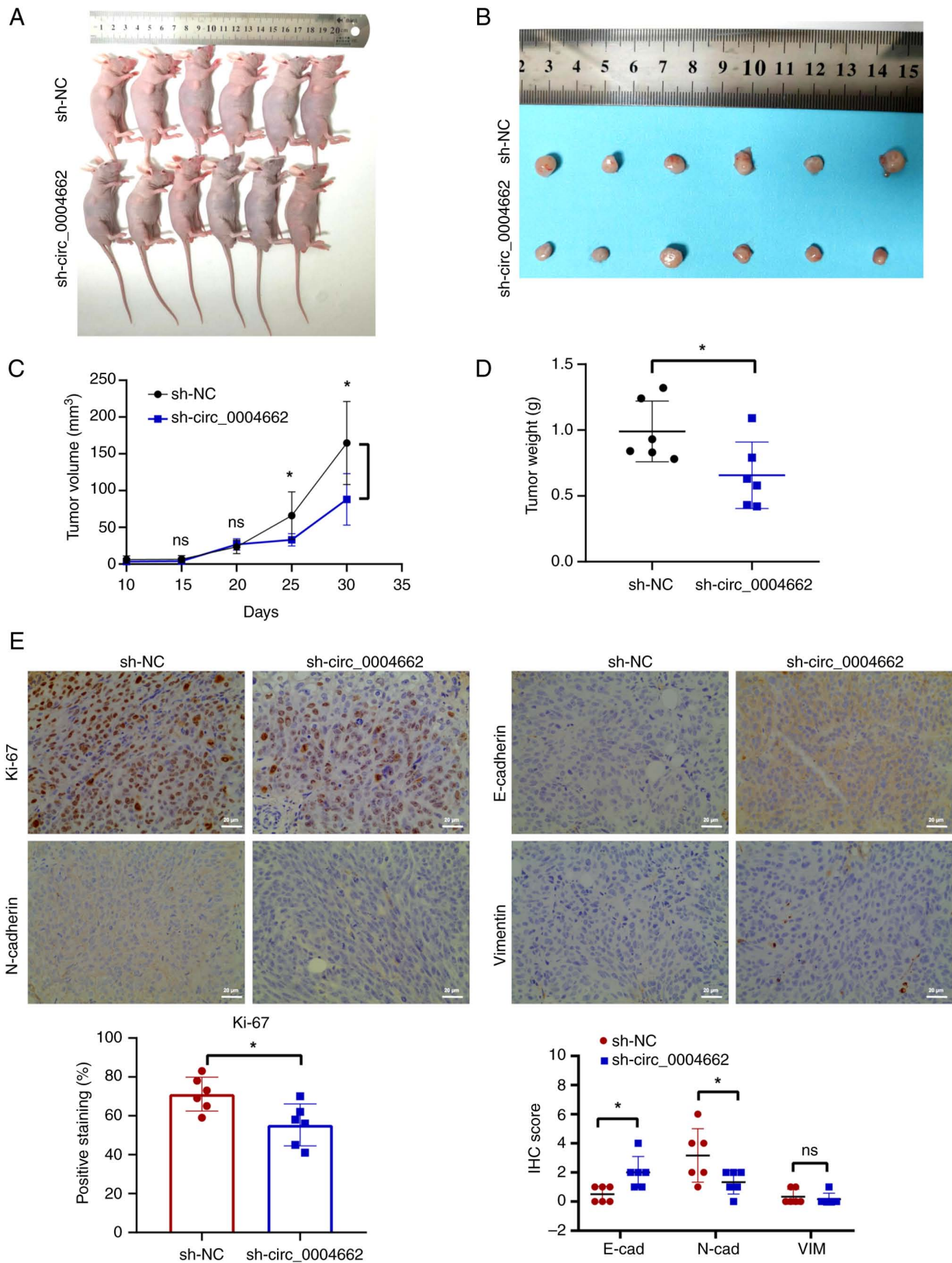


Figure 3. circ_0004662 contributes to proliferation and migration of colorectal cancer cells *in vivo*. (A) Nude mice after subcutaneous tumorigenesis. (B) Tumors were dissected from nude mice subcutaneously injected with circ_0004662-knockdown or control DLD-1 cells. (C) Volume and (D) weight of subcutaneous xenograft tumors. (E) Representative IHC analysis of Ki-67, E-cad, N-cad and VIM. Scale bar, 20 μ m. *P<0.05 vs. sh-NC group. circ, circular; ns, not significant; sh, short hairpin; IHC, immunohistochemistry; cad, cadherin; VIM, vimentin; NC, negative control.

in DLD-1 cells. The enhanced migration ability induced by circ_0004662 was reversed following silencing hnRNPM in HCT116 cells (Fig. 6G). In conclusion, these findings

suggested that circ_0004662 promotes CRC progression by interacting with hnRNPM, highlighting the regulatory role of the circ_0004662/hnRNPM interaction in CRC cells (Fig. S6).

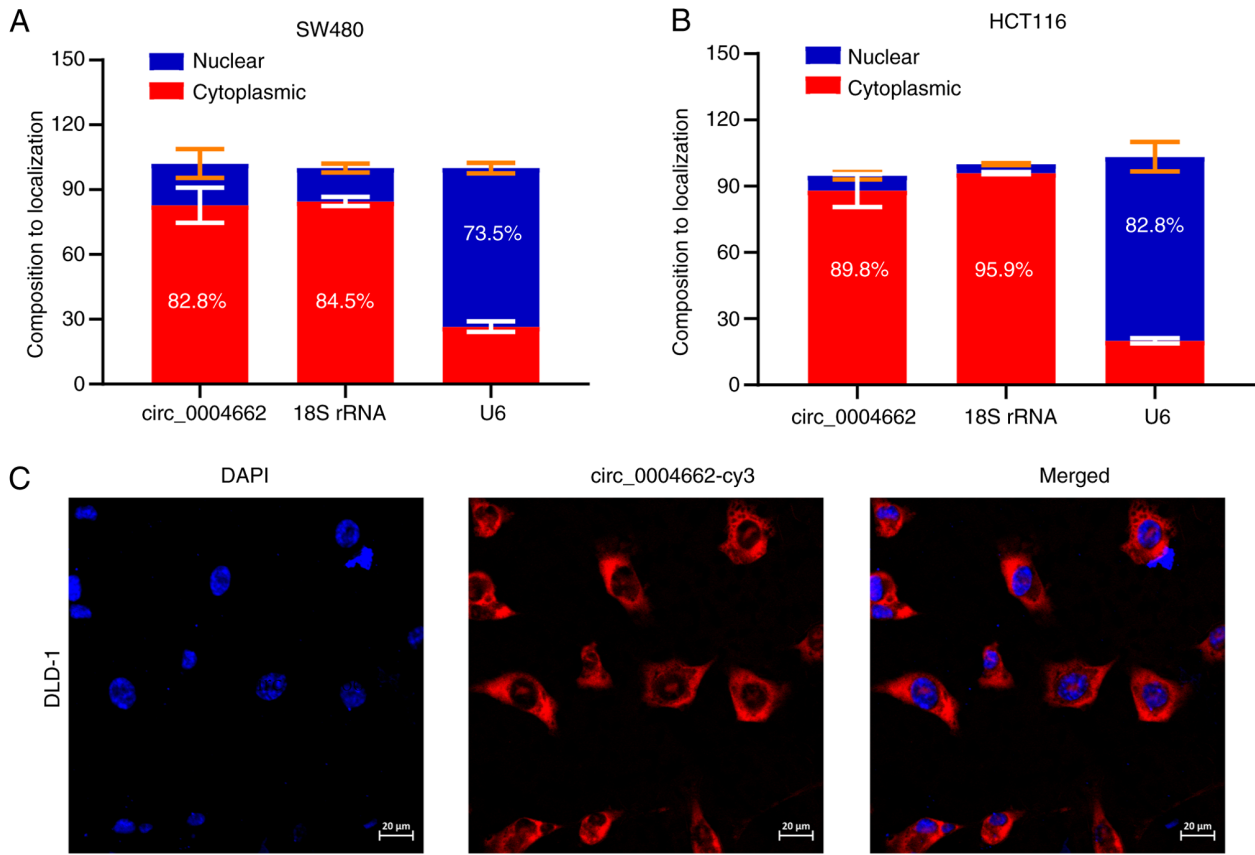


Figure 4. circ_0004662 is located in the cytoplasm and nucleus. Location of circ_0004662 was confirmed via subcellular fractionation analysis in (A) SW480 and (B) HCT116 cells. (C) Fluorescence *in situ* hybridization was performed to observe the cellular location of circ_0004662 in DLD-1 cells. circ_0004662 was labeled with Cy3 (red) and nuclei were counterstained with DAPI (blue). Scale bar, 20 μ m. circ, circular.

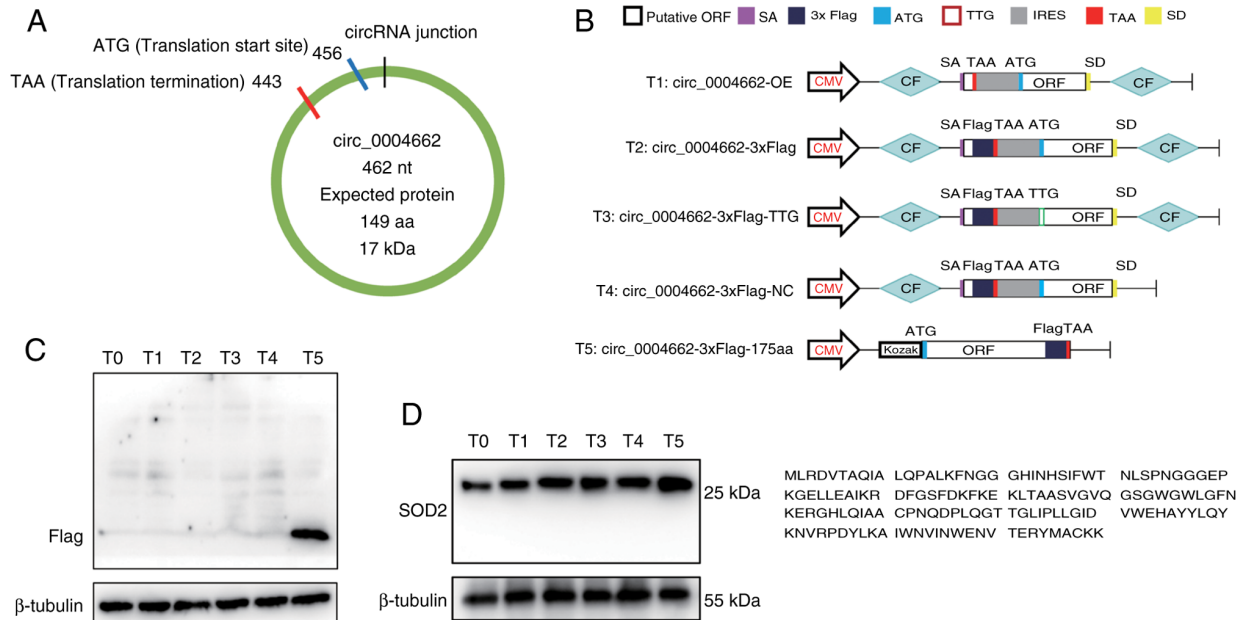


Figure 5. circ_0004662 is a non-coding RNA in colorectal cancer cells. (A) Putative ORF in circ_0004662. (B) Construction of Flag-tagged circ_0004662 and the predicted peptide sequence. Plasmids with start codon mutations and deletion of downstream flanking sequences could not generate SOD2-149aa. Positive control was constructed by cloning the linearized circ_0004662 ORF into a CMV-induced expression vector. (C) Measurement of flagged protein via western blotting. (D) Detection of SOD2-149aa using antibody identifying C or N terminal regions of SOD2. T0, empty vector; T1 circ_0004662-overexpressing vector; T2, circ_0004662-overexpressing vector containing 3x Flag tag; T3 was circ_0004662-overexpression vector containing 3x Flag tag, with ATG mutation; T4 was vectors containing 3x Flag tag, whose circular elements was deleted, forming a negative control that cannot form a circular RNA; T5 included the predicted full-length ORF without a cyclic framework, allowing normal translation and generating predicted peptides, which was a positive control. circ, circular; T, translation vector; OE, overexpression; CF, circular frame; ORF, open reading frame; IRES, internal ribosome entry site; SOD, superoxide dismutase; aa, amino acid; CMV, CMV promoter; SA, splice acceptor; SD, splicing donor.

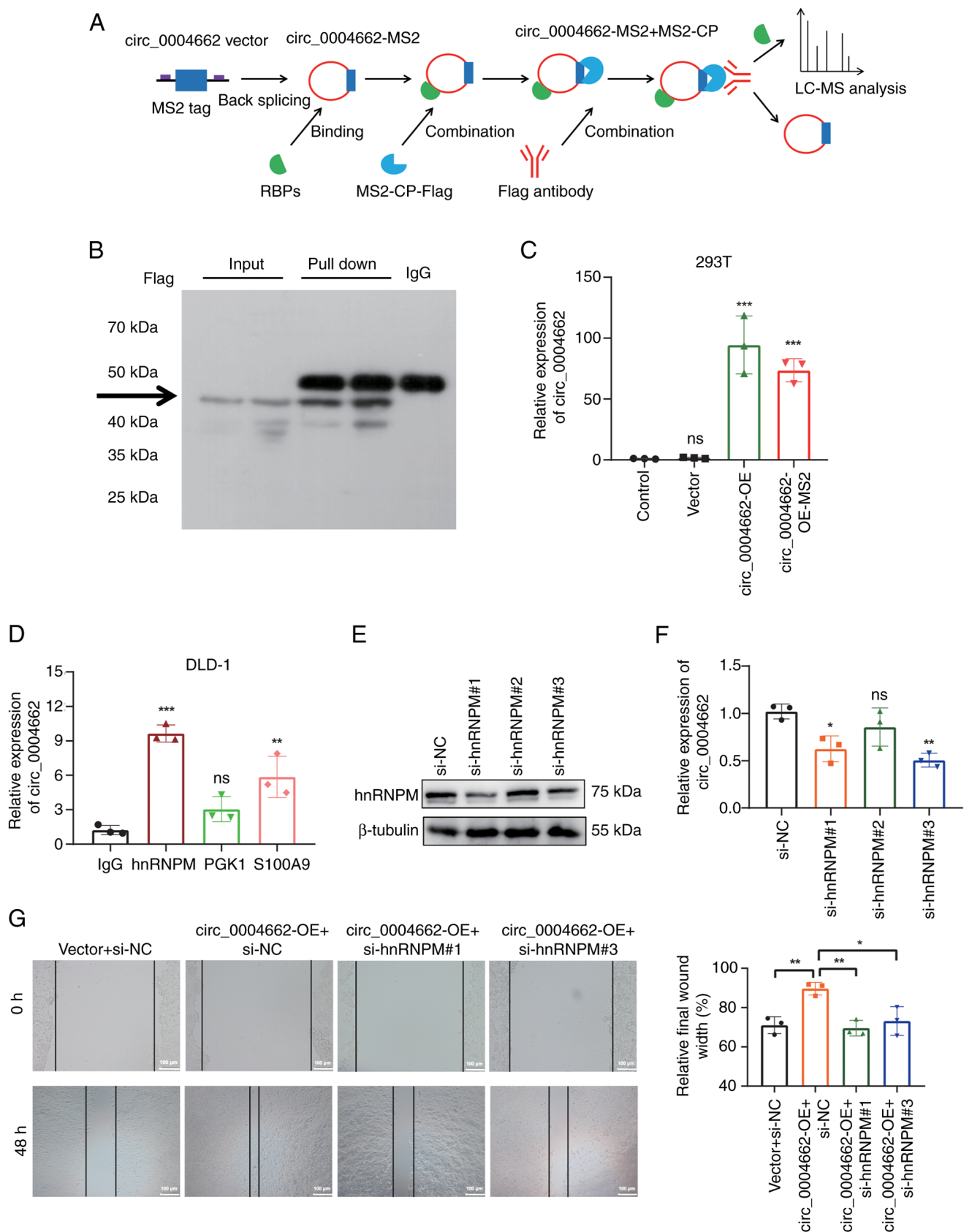


Figure 6. Interaction between circ_0004662 and hnRNPM in colorectal cancer cells. (A) circ_0004662 pull-down products using the MS2-tagging system. (B) MS2-CP-Flag was pulled down by anti-Flag antibodies and subjected to western blotting. (C) HEK-293T cells were transfected with circ_0004662, control or MS2-labelled circ_0004662 expression plasmids. Relative expression of circ_0004662 was measured via PCR. (D) RNA binding protein immunoprecipitation assay of DLD-1 cells using anti-hnRNPM, PGK-1 and S100A9 antibodies or control IgG. circ_0004662 expression was measured via RT-qPCR. (E) Interference efficiency of hnRNPM was measured via western blot. (F) circ_0004662 expression after knocking down hnRNPM. (G) Effect of hnRNPM interference in HCT116 cells was measured using the wound healing assay. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. vector group or IgG group or NC group. circ, circular; hnRNPM, heterogeneous nuclear ribonucleoprotein M; MS2-CP, MS2 bacteriophage coat protein; PGK, phosphoglycerate kinase; RBP, RNA binding protein; LC-MS, liquid chromatograph mass spectrometer; OE, overexpression; si, small interfering; NC, negative control; ns, not significant.

Discussion

Accumulating evidence indicates that non-coding RNAs (31,32), particularly circRNAs, serve key roles in CRC development (33–35). The present study characterized circRNAs derived from SOD gene family and identified upregulation of circ_0004662 in CRC cells and tissue. circ_0004662 was present both in the cytoplasm and nucleus. As a non-coding RNA, circ_0004662 binds hnRNP and promotes the migration of CRC cells, thereby offering potential novel therapeutic targets for personalized treatment of CRC.

Despite being formed by the same parental genes, circRNAs exhibit varying roles in cancer progression. For example, circUBAP2 facilitates malignant characteristics in prostate (7) and breast cancer (36), hepatocellular carcinoma (19) and osteocarcinoma (8); however, it inhibits proliferation and metastasis of clear cell renal cell carcinoma cells (10). Furthermore, linear Rho GTPase activating protein 35 (ARHGAP35) encodes a tumor suppressor, while circARHGAP35 translates into an oncogenic large protein to promote cancer progression (37). However, the specific role of circSODs in regulating biological characteristics of CRC cells remains unclear. circ_0004662 accelerates osteoarthritis progression via the microRNA (miR)-424-5p/VEGFA axis (38), and circ_0004662 drives progression of hepatocellular carcinoma by serving as a sponge for miR-502-5p and activating the JAK2/STAT3 signaling pathway (39). Furthermore, the Paired Box 5/circ_0004662/miR-532-3p axis serves an important role in promoting the proliferation, invasion and migration of clear cell renal cell carcinoma cells (40). Here, circ_0004662 contributed to the migration ability of CRC cells *in vitro*.

E-cadherin, N-cadherin, vimentin, matrix metalloproteinases, claudins, epithelial cell adhesion molecules and cytokeratins are common biomarkers for the detection of epithelial-mesenchymal transition (EMT), which is a vital phenotype in metastasis (20). Downregulated E-cad and upregulate N-cad and VIM can promote EMT, leading to increased invasiveness and metastasis in CRC (21). Here, E-cad was upregulated, whereas N-cad was downregulated following knockdown in circ_0004662. circRNA SOD2 promotes EMT in non-small cell lung cancer advancement via acting as miR-2355-5p competing endogenous RNA to mediate calmodulin-regulated spectrin associated proteins-2 (41).

Although most circRNAs are non-coding RNAs, many are translated to peptides and regulate biological characteristics of cancer cells, affecting tumor progression (42,43). For example, circAXIN1 encodes AXIN1-295aa and promotes gastric cancer progression by activating the Wnt/ β -catenin signaling pathway (18). A novel protein encoded by circ_0017272 promotes multiple myeloma progression by regulating the bone marrow microenvironment and circ_0133744 could encode proteins promote CRC proliferation and metastasis (44,45). The present study evaluated the translation potential of circ_0004662 and suggested that it interacts with multiple ribosomal proteins. However, the predicted 149aa peptide encoded by circ_0004662 was not detected. This warrants additional investigation considering the complex translation process.

With advances in high-throughput screening, multiple RBPs have been implicated in cancer progression (46,47). Interaction between circ_0004662 and RBPs in CRC remains

unexplored, the present study noted a direct interaction between circ_0004662 and hnRNP. Accumulating evidence indicates that hnRNP contributes to cancer cell metastasis in hepatocellular carcinoma (28,48) and breast cancer (49,50). Notably, as an RBP, hnRNP can interact with circRNAs and control their expression via the alternative splicing of circRNAs (51), which may impair the stability of target genes. For example, circ_0000921 directly interacts with hnRNP to modulate alternative splicing of genes involved in the process of cell migration, thus regulating gastric cancer metastasis (29). Further, combination of hnRNP with circ_0003764 enhances the ability of hnRNP to maintain the stability of IL-6 mRNA and further activates the STAT3 signaling pathway, promoting progression and sunitinib resistance in renal cell carcinoma (52). Clinical colon cancer specimens and mouse carcinogenesis model show that hnRNP is elevated during the development of CRC and is associated with poor prognosis (53), and genome-wide transcriptomics and translomics analyses have revealed a unique set of hnRNP-targeted genes involved in metabolic processes and cancer neoplasia are selectively translated under hypoxia (53). Further, hnRNP bind with carcinoembryonic antigen (CEA), which may participate in the antiapoptotic role of CEA and mediate the prometastatic properties of CEA in colon cancer cells, but needs future experiments (54). In the present study, hnRNP knockdown impaired circ_0004662 expression; this indicated the role of hnRNP in regulating circ_0004662 in CRC cells. However, target genes of hnRNP associated with CRC progression remain unclear. Therefore, the specific mechanisms of how circ_0004662 and hnRNP promotes CRC metastasis should be assessed in future research.

In conclusion, circ_0004662 promoted CRC cell migration by interacting with hnRNP. The present findings may provide novel insights into the potential strategies for personalized therapy for patients with CRC.

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Availability of data and materials

The data generated in the present study may be found in the iProX database under accession number IPX0009947000 or at the following URL: <https://www.iprox.cn/page/project.html?id=IPX0009947000>.

Authors' contributions

YZ conceived the study, conducted experiments and drafted and revised the manuscript. JW and RQ analyzed data and wrote the manuscript. LL conceived the study and performed experiments. YZ and LL confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Medical Ethics Committee of the Anhui Provincial Cancer Hospital (approval no. 2023081). All participants in this study gave written informed consent in accordance with the Declaration of Helsinki. All animal care and procedures were performed according to guidelines approved by the Institutional Animal Care and Use Committee of the Anhui Provincial Cancer Hospital, University of Science and Technology of China [approval no. 2022-N(A)-072].

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Xi Y and Xu P: Global colorectal cancer burden in 2020 and projections to 2040. *Transl Oncol* 14: 101174, 2021.
- Qu R, Ma Y, Zhang Z and Fu W: Increasing burden of colorectal cancer in China. *Lancet Gastroenterol Hepatol* 7: 700, 2022.
- Kristensen LS, Andersen MS, Stagsted LVW, Ebbesen KK, Hansen TB and Kjems J: The biogenesis, biology and characterization of circular RNAs. *Nat Rev Genet* 20: 675-691, 2019.
- Kristensen LS, Jakobsen T, Hager H and Kjems J: The emerging roles of circRNAs in cancer and oncology. *Nat Rev Clin Oncol* 19: 188-206, 2022.
- Yuan G, Ding W, Sun B, Zhu L, Gao Y and Chen L: Upregulated circRNA_102231 promotes gastric cancer progression and its clinical significance. *Bioengineered* 12: 4936-4945, 2021.
- Li R, Tian X, Jiang J, Qian H, Shen H and Xu W: CircRNA CDR1as: A novel diagnostic and prognostic biomarker for gastric cancer. *Biomarkers* 28: 448-457, 2023.
- Li X, Azhati B, Wang W, Rexiati M, Xing C and Wang Y: Circular RNA UBAP2 promotes the proliferation of prostate cancer cells via the miR-1244/MAP3K2 axis. *Oncol Lett* 21: 486, 2021.
- Ma W, Zhao X, Gao Y, Yao X, Zhang J and Xu Q: Circular RNA circ_UBAP2 facilitates the progression of osteosarcoma by regulating microRNA miR-637/high-mobility group box (HMGB) 2 axis. *Bioengineered* 13: 4411-4427, 2022.
- Zhang PF, Wei CY, Huang XY, Peng R, Yang X, Lu JC, Zhang C, Gao C, Cai JB, Gao PT, *et al*: Circular RNA circTRIM33-12 acts as the sponge of MicroRNA-191 to suppress hepatocellular carcinoma progression. *Mol Cancer* 18: 105, 2019.
- Sun J, Yin A, Zhang W, Lv J, Liang Y, Li H, Li Y and Li X: CircUBAP2 inhibits proliferation and metastasis of clear cell renal cell carcinoma via targeting miR-148a-3p/FOXK2 pathway. *Cell Transplant* 29: 963689720925751, 2020.
- Zhou C, Lyu LH, Miao HK, Bahr T, Zhang QY, Liang T, Zhou HB, Chen GR and Bai Y: Redox regulation by SOD2 modulates colorectal cancer tumorigenesis through AMPK-mediated energy metabolism. *Mol Carcinog* 59: 545-556, 2020.
- Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
- Bertrand E, Chartrand P, Schaefer M, Shenoy SM, Singer RH and Long RM: Localization of ASH1 mRNA particles in living yeast. *Mol Cell* 2: 437-445, 1998.
- Zhou M, Yang Z, Wang D, Chen P and Zhang Y: The circular RNA circZFR phosphorylates Rb promoting cervical cancer progression by regulating the SSBP1/CDK2/cyclin E1 complex. *J Exp Clin Cancer Res* 40: 48, 2021.
- Zheng R, Zhang K, Tan S, Gao F, Zhang Y, Xu W, Wang H, Gu D, Zhu L, Li S, *et al*: Exosomal circLPA1 functions in colorectal cancer diagnosis and tumorigenesis through suppressing BRD4 via METTL3-eIF3h interaction. *Mol Cancer* 21: 49, 2022.
- Chen X, Han P, Zhou T, Guo X, Song X and Li Y: circRNADb: A comprehensive database for human circular RNAs with protein-coding annotations. *Sci Rep* 6: 34985, 2016.
- Zhao Q, Liu J, Deng H, Ma R, Liao JY, Liang H, Hu J, Li J, Guo Z, Cai J, *et al*: Targeting mitochondria-located circRNA SCAR alleviates NASH via reducing mROS output. *Cell* 183: 76-93.e22, 2020.
- Peng Y, Xu Y, Zhang X, Deng S, Yuan Y, Luo X, Hossain MT, Zhu X, Du K, Hu F, *et al*: A novel protein AXIN1-295aa encoded by circAXIN1 activates the Wnt/ β -catenin signaling pathway to promote gastric cancer progression. *Mol Cancer* 20: 158, 2021.
- Lyu LH, Zhang CY, Yang WJ, Jin AL, Zhu J, Wang H, Liu T, Wang BL, Cheng JW, Yang XR and Guo W: Hsa_circ_0003945 promotes progression of hepatocellular carcinoma by mediating miR-34c-5p/LGR4/ β -catenin axis activity. *J Cell Mol Med* 26: 2218-2229, 2022.
- Yang M, Sun M and Zhang H: The interaction between epigenetic changes, EMT, and exosomes in predicting metastasis of colorectal cancers (CRC). *Front Oncol* 12: 879848, 2022.
- Vu T and Datta PK: Regulation of EMT in colorectal cancer: A culprit in metastasis. *Cancers (Basel)* 9: 171, 2017.
- Wang L, Long H, Zheng Q, Bo X, Xiao X and Li B: Circular RNA circRHOT1 promotes hepatocellular carcinoma progression by initiation of NR2F6 expression. *Mol Cancer* 18: 119, 2019.
- Ma Q, Yang F, Huang B, Pan X, Li W, Yu T, Wang X, Ran L, Qian K, Li H, *et al*: CircARID1A binds to IGF2BP3 in gastric cancer and promotes cancer proliferation by forming a circARID1A-IGF2BP3-SLC7A5 RNA-protein ternary complex. *J Exp Clin Cancer Res* 41: 251, 2022.
- He Y, Luo Y, Zhang D, Wang X, Zhang P, Li H, Ejaz S and Liang S: PGK1-mediated cancer progression and drug resistance. *Am J Cancer Res* 9: 2280-2302, 2019.
- Fu Q and Yu Z: Phosphoglycerate kinase 1 (PGK1) in cancer: A promising target for diagnosis and therapy. *Life Sci* 256: 117863, 2020.
- Chen Y, Ouyang Y, Li Z, Wang X and Ma J: S100A8 and S100A9 in cancer. *Biochim Biophys Acta Rev Cancer* 1878: 188891, 2023.
- Markowitz J and Carson WE III: Review of S100A9 biology and its role in cancer. *Biochim Biophys Acta* 1835: 100-109, 2013.
- Qiao L, Xie N, Li Y, Bai Y, Liu N and Wang J: Downregulation of HNRNPM inhibits cell proliferation and migration of hepatocellular carcinoma through MAPK/AKT signaling pathway. *Transl Cancer Res* 11: 2135-2144, 2022.
- Wang X, Li J, Bian X, Wu C, Hua J, Chang S, Yu T, Li H, Li Y, Hu S, *et al*: CircURI1 interacts with hnRNPM to inhibit metastasis by modulating alternative splicing in gastric cancer. *Proc Natl Acad Sci USA* 118: e2012881118, 2021.
- Ho JS, Di Tullio F, Schwarz M, Low D, Incarnato D, Gay F, Tabaglio T, Zhang J, Wollmann H, Chen L, *et al*: HNRNPM controls circRNA biogenesis and splicing fidelity to sustain cancer cell fitness. *Elife* 10: e59654, 2021.
- Sarraf JS, Puty TC, da Silva EM, Allen TSR, Sarraf YS, de Carvalho LEW, Adami F and de Oliveira EHC: Noncoding RNAs and colorectal cancer: A general overview. *Microna* 9: 336-345, 2020.
- Liu Z, Liu X, Li Y, Ren P, Zhang C, Wang L, Du X and Xing B: miR-6716-5p promotes metastasis of colorectal cancer through downregulating NAT10 expression. *Cancer Manag Res* 11: 5317-5332, 2019.
- Mao J and Lu Y: Roles of circRNAs in the progression of colorectal cancer: Novel strategies for detection and therapy. *Cancer Gene Ther* 31: 831-841, 2024.
- Zhang Y, Luo J, Yang W and Ye WC: CircRNAs in colorectal cancer: Potential biomarkers and therapeutic targets. *Cell Death Dis* 14: 353, 2023.
- Fang G, Xu D, Zhang T, Wang G, Qiu L, Gao X and Miao Y: Biological functions, mechanisms, and clinical significance of circular RNA in colorectal cancer. *Front Oncol* 13: 1138481, 2023.
- Wang L, Yang X, Zhou F, Sun X and Li S: Circular RNA UBAP2 facilitates the cisplatin resistance of triple-negative breast cancer via microRNA-300/anti-silencing function 1B histone chaperone/PI3K/AKT/mTOR axis. *Bioengineered* 13: 7197-7208, 2022.
- Li Y, Chen B, Zhao J, Li Q, Chen S, Guo T, Li Y, Lai H, Chen Z, Meng Z, *et al*: HNRNPL circularizes ARHGAP35 to produce an oncogenic protein. *Adv Sci (Weinh)* 8: 2001701, 2021.
- Xie W, Jiang L, Huang X, You W and Sun W: Hsa_circ_0004662 accelerates the progression of osteoarthritis via the microRNA-424-5p/VEGFA axis. *Curr Mol Med* 24: 217-225, 2024.

39. Zhao Z, Song J, Tang B, Fang S, Zhang D, Zheng L, Wu F, Gao Y, Chen C, Hu X, *et al*: CircSOD2 induced epigenetic alteration drives hepatocellular carcinoma progression through activating JAK2/STAT3 signaling pathway. *J Exp Clin Cancer Res* 39: 259, 2020.
40. Yao GS, Fu LM, Dai JS, Chen JW, Liu KZ, Liang H, Wang Z, Deng Q, Wang JY, Jin MY, *et al*: Exploring the oncogenic potential of circSOD2 in clear cell renal cell carcinoma: A novel positive feedback loop. *J Transl Med* 22: 596, 2024.
41. Lv C, Hu Y, Zhou X, Zhu Y, Wang J and Zhou F: CircRNA SOD2 motivates non-small cell lungs cancer advancement with EMT via acting as microRNA-2355-5p's competing endogenous RNA to mediate calmodulin regulated spectrin associated proteins-2. *Bioengineered* 13: 5756-5768, 2022.
42. Wang Y, Wu C, Du Y, Li Z, Li M, Hou P, Shen Z, Chu S, Zheng J and Bai J: Expanding uncapped translation and emerging function of circular RNA in carcinomas and noncarcinomas. *Mol Cancer* 21: 13, 2022.
43. Zhang L, Gao H, Li X, Yu F and Li P: The important regulatory roles of circRNA-encoded proteins or peptides in cancer pathogenesis (Review). *Int J Oncol* 64: 19, 2024.
44. Tang X, Deng Z, Ding P, Qiang W, Lu Y, Gao S, Hu Y, Yang Y, Du J and Gu C: A novel protein encoded by circHNRNPU promotes multiple myeloma progression by regulating the bone marrow microenvironment and alternative splicing. *J Exp Clin Cancer Res* 41: 85, 2022.
45. Xiong L, Liu HS, Zhou C, Yang X, Huang L, Jie HQ, Zeng ZW, Zheng XB, Li WX, Liu ZZ, *et al*: A novel protein encoded by circINSIG1 reprograms cholesterol metabolism by promoting the ubiquitin-dependent degradation of INSIG1 in colorectal cancer. *Mol Cancer* 22: 72, 2023.
46. Yang B, Wang YW and Zhang K: Interactions between circRNA and protein in breast cancer. *Gene* 895: 148019, 2024.
47. Huang A, Zheng H, Wu Z, Chen M and Huang Y: Circular RNA-protein interactions: Functions, mechanisms, and identification. *Theranostics* 10: 3503-3517, 2020.
48. Zhu GQ, Wang Y, Wang B, Liu WR, Dong SS, Chen EB, Cai JL, Wan JL, Du JX, Song LN, *et al*: Targeting HNRNPM inhibits cancer stemness and enhances antitumor immunity in Wnt-activated hepatocellular carcinoma. *Cell Mol Gastroenterol Hepatol* 13: 1413-1447, 2022.
49. Yang WH, Ding MJ, Cui GZ, Yang M and Dai DL: Heterogeneous nuclear ribonucleoprotein M promotes the progression of breast cancer by regulating the axin/ β -catenin signaling pathway. *Biomed Pharmacother* 105: 848-855, 2018.
50. Sun H, Liu T, Zhu D, Dong X, Liu F, Liang X, Chen C, Shao B, Wang M and Wang Y: HnRNPM and CD44s expression affects tumor aggressiveness and predicts poor prognosis in breast cancer with axillary lymph node metastases. *Genes Chromosomes Cancer* 56: 598-607, 2017.
51. Gu X, Li X, Jin Y, Zhang Z, Li M, Liu D and Wei F: CDR1as regulated by hnRNPM maintains stemness of periodontal ligament stem cells via miR-7/KLF4. *J Cell Mol Med* 25: 4501-4515, 2021.
52. Shou Y, Yue C, Wang Q, Liu J, Xu J, Miao Q, Liu D, Yang H, Liu Y and Zhang X: circPTPN12 promotes the progression and sunitinib resistance of renal cancer via hnRNPM/IL-6/STAT3 pathway. *Cell Death Dis* 14: 232, 2023.
53. Chen TM, Lai MC, Li YH, Chan YL, Wu CH, Wang YM, Chien CW, Huang SY, Sun HS and Tsai SJ: hnRNPM induces translation switch under hypoxia to promote colon cancer development. *EBioMedicine* 41: 299-309, 2019.
54. Laguinge L, Bajenova O, Bowden E, Sayyah J, Thomas P and Juhl H: Surface expression and CEA binding of hnRNP M4 protein in HT29 colon cancer cells. *Anticancer Res* 25: 23-31, 2005.



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