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IGF2BP2 promotes colorectal cancer cell proliferation and survival through interfering with *RAF-1* degradation by miR-195

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Insulin-like growth factor 2 (*IGF2*) mRNA-binding protein 2 (IGF2BP2) is a post-transcriptional regulatory factor implicated in mRNA localization, stability, and translational control. However, the role of IGF2BP2 regulation in colorectal cancer (CRC) and its underlying mechanism remain elusive. In this study, we found that IGF2BP2 expression is markedly increased in CRC tissues. Notably, IGF2BP2 overexpression strikingly enhanced the proliferation and survival of CRC cells *in vitro*, whereas its shRNA-mediated silencing resulted in the opposite. Molecular function analyses revealed that IGF2BP2 regulates *RAF1* expression through blocking its degradation by miR-195. These results identify IGF2BP2 as a post-transcriptional regulatory mRNA-binding factor that contributes to CRC carcinogenesis.

Keywords: colorectal cancer; IGF2BP2; miR-195; RAF1; RNA-binding proteins

Colorectal cancer (CRC) is the third most common malignancy in the world and the fourth leading cause of cancer-related death in China [1]; however, the mechanism driving CRC progression remains unclear. Emerging evidence indicates that post-transcriptional regulatory mechanisms may play an important role in the development and progression of cancer. During this process, RNA-binding proteins (RBPS) control gene expression by modulating the maturation, stability, transport, or translation of RNA transcripts [2–4]. Several hundred RBPS have been identified in the vertebrate genome [5], but only a limited number of studies have characterized RPBS-mediated gene regulation. Thus, the identification of novel RBPS may help to better understanding the mechanism of carcinogenesis in CRC.

The *IGF2* mRNA-binding family member, insulin-like growth factor 2 (*IGF2*) mRNA-binding protein 2 (IGF2BP2), is implicated in post-transcriptional gene regulation [6]. IGF2BP2 binds RNA through its six characteristic RNA-binding domains, consisting of two RNA recognition motifs (RRM1 and RRM2) and four KH domains (KH1–KH4) [7]. IGF2BP2 binds to and regulates the translation of a set of mRNA including

Abbreviations
CRC, colorectal cancer; GF2, insulin-like growth factor 2; IGF2BP2, insulin-like growth factor 2 mRNA-binding protein 2; RBPS, RNA-binding proteins; RIP, RNA-binding protein immunoprecipitation; RRM, RNA recognition motifs.

IGF2, neuroblastoma RAS viral oncogene homolog (*NRAS*), *PINCH2*, and *MURF-3,* which are responsible for carcinogenesis and cellular mobility [8–10]. And several studies have reported that IGF2BP2 gene polymorphisms are associated with the risks of type 2 diabetes and cancers [7,11,12]. A recent publication has identified IGF2BP2 knock-out mice resist obesity through regulation of mRNA encoding mitochondrial proteins [13]. Although a higher frequency of autoantibody response to IGF2BP2/p62 was identified in colon cancer [14], the mechanisms regulating *IGF2BP2* mRNA specificity and its role in CRC carcinogenesis are still unknown.

In this study, we confirmed that IGF2BP2 is overexpressed in CRC tumors when compared to match normal control tissues. *In vitro* differential expression analyses revealed that IGF2BP2 promotes CRC cell proliferation and contributes to cancer progression. We also determined that IGF2BP2 binds to oncogenic *RAF1* mRNA to inhibit its degradation by miR-195. Thus, our analyses of the molecular functions suggest that IGF2BP2 acting as a post-transcriptional regulatory factor promotes colorectal cancer cell growth.

Materials and methods

Tissue microarray and immunohistochemistry

Ninety matched pairs of primary colorectal tumor and peritumoral normal tissues were used for tissue microarrays (Shanghai Biochip Co., Ltd., Shanghai, China). Rabbit monoclonal IGF2BP2 antibody (ab124930, 1:500; Abcam, Cambridge, MA, USA) was used to detect IGF2BP2 protein expression in the cytoplasma. IGF2BP2 intensity was classified as high or low expression based on the mean area of positive staining of tumor cells in CRC tissue, while epithelial cells in peritumoral normal tissue. Two wells without tissue were used as negative controls. High expression was defined as ≥76% positive staining, moderate expression as 26–50% staining, and low expression as ≤25% staining.

Cell lines and cell culture

The SW480 and SW620 CRC cell lines were purchased from The Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Carlsbad, CA, USA) supplemented with 10% FBS at 37 °C in a 5% CO_2 atmosphere.

Immunocytochemistry

The cells were fixed with 4% paraformaldehyde (PFA) and permeabilized with 100% cold methanol. After blocking with 3% BSA, the cells were incubated with anti-IGF2BP2 antibodies in blocking buffer for 2 h at room temperature and rinsed three times in PBS. The primary antibody was detected with secondary antibody conjugated with FITC (Santa Cruz, CA, USA). Nuclei were stained with DAPI (Roche, Mannheim, Germany). The levels of IGF2BP2 were observed with a LSM 510 fluorescence microscope (Hamamatsu, Bridgewater, NJ, USA).

Quantitative real-time polymerase chain reaction

RNA was extracted from cell lines with an RNeasy Mini Kit (Qiagen, Dusseldorf, Germany), and cDNA was synthesized with iScript cDNA synthesis reagent according to the manufacturer's instructions (BioRad, Hercules, CA, USA) with primers designed in Primer5 (Softonic, Barcelona, Spain). Primer sequences are provided in S1 Table.

Western blot analysis

Approximately 30 μg of protein lysate was separated by SDS/PAGE (Invitrogen, Carlsbad, CA, USA), and analyzed by western blotting and ECL staining (Pierce Biotechnology Inc., Rockford, IL, USA). Primary antibodies included IGF2BP2 (ab124930, Abcam), RAF-1 (ab32025, Abcam), CCNB1 (1496-1, Epitomics, Burlingame, CA, USA), NRAS (sc-31, Santa Cruz Biotechnology, Inc., Dallas, TX, USA), and β-Actin (A5441, Sigma-Aldrich, St. Louis, MO, USA). Primary and secondary antibodies were used at 1 : 1000 and 1 : 2000 dilutions, respectively.

IGF2BP2 overexpression and shRNA knockdown

For overexpression experiments, human IGF2BP2 cDNA (NM_001007225.1) was subcloned into the pLenti6.3_MCS_IRES2-EGFP lentiviral expression vector (Invitrogen). Lentiviral particles were produced in 293T cells, filtered, titered, and infected into SW620 cells. Cells were cultured with 5 μg/mL blasticidin S HCl (Invitrogen) to select stable infectants. For shRNA-mediated knockdowns, lentiviral-based IGF2BP2 shRNA and negative control shRNA were purchased from GeneChem (Shanghai, China) and were prepared and stored according to the manufacturer's instructions. SW480 cells were infected with shRNA lentiviral particles and those with stable expression selected with 5 μg/mL puromycin (Sigma-Aldrich).

Cell proliferation assays

Cell viability assays were performed using Cell Counting Kit-8 (Dojindo Laboratories, Tokyo, Japan). Absorbance at 450 nm was read to determine cell viability.

Colony formation assays

Stable cells (2×10^3) were seeded into 100-mm culture dishes and incubated at 37 °C with 5% CO_2 for 10 days. Surviving colonies (>50 cells) were then stained with crystal violet and counted.

Apoptosis assays

Early and late apoptosis were quantified by flow cytometry (Cytomics FC500; Beckman Coulter, Brea, CA, USA) after staining with Annexin V and propidium iodide (PI, Tianjin Sungene Biotech Co., Ltd., Tianjin, China). The stable infected cell populations were counted as viable (Annexin V^-/PI^-), early (Annexin V^+/PI^-), and late apoptotic and necrotic cells (Annexin V^+/PI^+).

Flow cytometry analysis of cell cycle distribution

For cell cycle assay, the stable infected cells were incubated in 70% ethanol at 4 °C for ≥24 h, and stained with DNA Prep stain (Beckman Coulter) before flow cytometry.

RNA-binding protein immunoprecipitation assay

RNA-binding protein immunoprecipitation (RIP) was performed using an EZ-Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, Billerica, MA, USA). CRC cells were lysed in RIP lysis buffer, and then the protein-RNA lysate, IGF2BP2 antibody (ab128175, Abcam) or normal mouse IgG, and protein A magnetic beads were incubated with rotating for overnight at 4 °C. The protein/bead conjugate samples were placed on the magnetic separator and the supernatant discarded. After washing, the IGF2BP2-bound RNA complexes were eluted, quantified, reverse-transcribed, and analyzed by quantitative real-time polymerase chain reaction (qRT-PCR).

Luciferase assays

Human wild-type and mutant RAF1-3′UTR were subcloned into the pmirGLO-Luciferase-Renilla (Promega, Madison, WI, USA) expression vector, respectively. SW620 cells infected with IGF2BP2- or GFP-expressing lentivirus were plated in 24-well plates and transfected with 100 ng of the luciferase vector using Lipofectamine 2000 (Invitrogen). The cells were collected and luciferase assays performed using a Dual-Glo® Luciferase Assay System Kit (Promega) on a luminometer 72 h after transfection.

MicroRNA oligonucleotides and transfection

MiR-195 mimics and negative control duplex (NC) were purchased from Genepharma (Shanghai, China) and infections performed using Lipofectamine 2000 (Invitrogen).

Statistical analysis

All data were presented as mean ± standard deviation (SD). ANOVAs were used to evaluate the statistical significance of differences between experimental groups. *P*-values <0.05 were considered to be statistically significant. All statistical analyses were performed with the SPSS 21.0 software (IBM Software, Armonk, NJ, USA).

Results

IGF2BP2 is highly expressed in human CRC

To examine IGF2BP2 protein expression in CRC, we performed a tissue microarray with 88 pairs of tumors and matched adjacent tissue. The results showed that 54 (61.4%) samples had high IGF2BP2 expression (IGF2BP2+++), 25 (28.4%) with moderate IGF2BP2 expression (IGF2BP2++), and 9 (10.2%) with low IGF2BP2 expression (IGF2BP2+). Representative IHC images of IGF2BP2 protein expression in CRC and adjacent normal tissue were shown in Fig. 1A–D. The statistical analysis showed that IGF2BP2 is highly expressed in human CRC tissue when compared to the adjacent tissue (Fig. 1E). Wild-type and *IGF2BP2* knockdown SW480 cells were used for immunocytochemistry analysis to determine the specificity of IGF2BP2 antibody in IHC (Fig. 1F).

IGF2BP2 is required for SW480 cells proliferation and evading growth suppression

SW480 cells, originating from a surgical specimen of a primary colorectal adenocarcinoma, express the high levels of *IGF2BP2* (S2 Figure). To investigate whether IGF2BP2 is required for CRC cell proliferation, SW480 cells were stably infected with lentiviral-based IGF2BP2 shRNA (shIGF2BP2). The *IGF2BP2* mRNA expression was strikingly knocked down compared to that of shRNA control cells (Fig. 2A). Then, we performed the loss-of-function experiments in SW480 cells. Cell viability assays revealed that *IGF2BP2* knockdown significantly inhibited the proliferation of SW480 cells (Fig. 2B). We also performed colony formation assays to further examine the role of IGF2BP2 in cell proliferation. Consistent with our previous results, these analyses indicated that *IGF2BP2* expression positively correlated with colony-forming ability and proliferation (Fig. 2C). To determine whether IGF2BP2 was a growth regulatory factor, we further analyzed cell cycle progression in *IGF2BP2* shRNA SW480 by flow cytometry. As expected, *IGF2BP2*-knockdown caused a striking decrease in

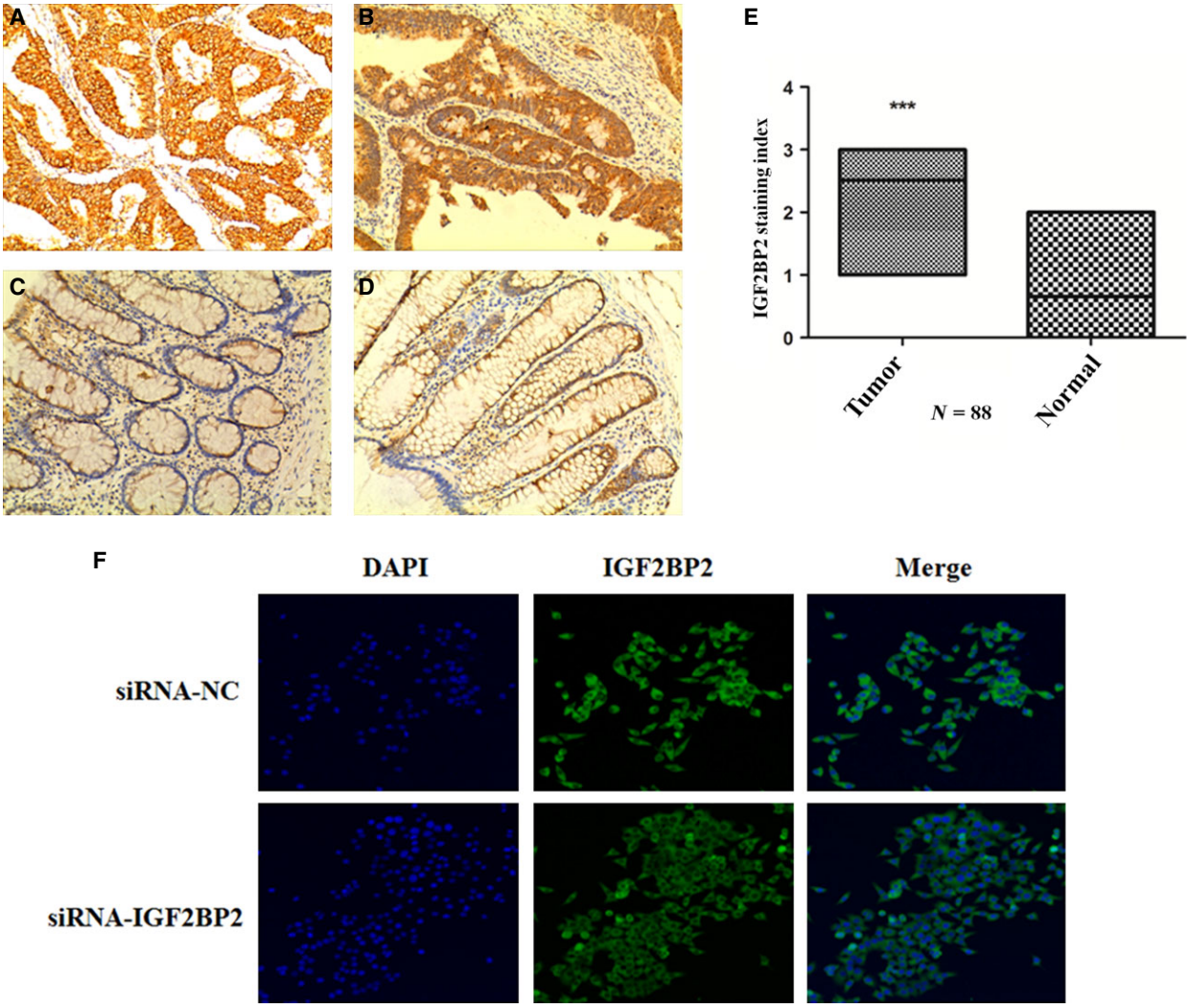

Fig. 1. IGF2BP2 is highly expressed in human colorectal cancer (CRC) samples. (A) Representative images of IGF2BP2 protein expression in CRC and adjacent normal tissue determined by immunohistochemistry (IHC). (A) and (B) show IGF2BP2 protein expression in the CRC tissues; while (C) and (D) show IGF2BP2 protein expression in the paracarcinoma tissues; the original magnifications, 100×. (E) IGF2BP2 protein was upregulated significantly in primary tumor specimens compared with the adjacent nontumor tissues by IHC (***$P < 0.001$, $n = 88$). High expression was defined as ≥76% positive staining, moderate expression as 26–50% staining, and low expression as ≤25% staining within tumor cells (0, none; 1, 1–25% positive staining cells; 2, 26–50% positive staining cells; 3, >76% positive staining cells). (F) IGF2BP2 was silenced in SW480 cells transfected with shRNA, and immunocytochemistry was performed to determine the specificity of IGF2BP2 antibody used in IHC assay. Typical photographs are presented, original magnification, 200×.

CRC cell growth (Fig. 2D). Therefore, the loss-of-function study suggests that IGF2BP2 is required for CRC cell proliferation and evasion of growth suppression.

IGF2BP2 is also required for SW620 cell proliferation and evading growth suppression

Both SW620 and SW480 cells were obtained from the same patient, the former originated from lymph node metastatic derivatives from the latter. The mRNA expression experiment showed that *IGF2BP2* downregulation in SW620 cells was carried out using qRT-PCR (S2 Figure). To further confirm IGF2BP2 expression which is required for CRC cell proliferation, SW620 cells were stably infected with lentiviral-based *IGF2BP2* for overexpression. IGF2BP2 protein expression was significantly elevated in the SW620 cells with exogenous IGF2BP2 overexpression (Fig. 3A). Cell viability assays revealed that restoration of

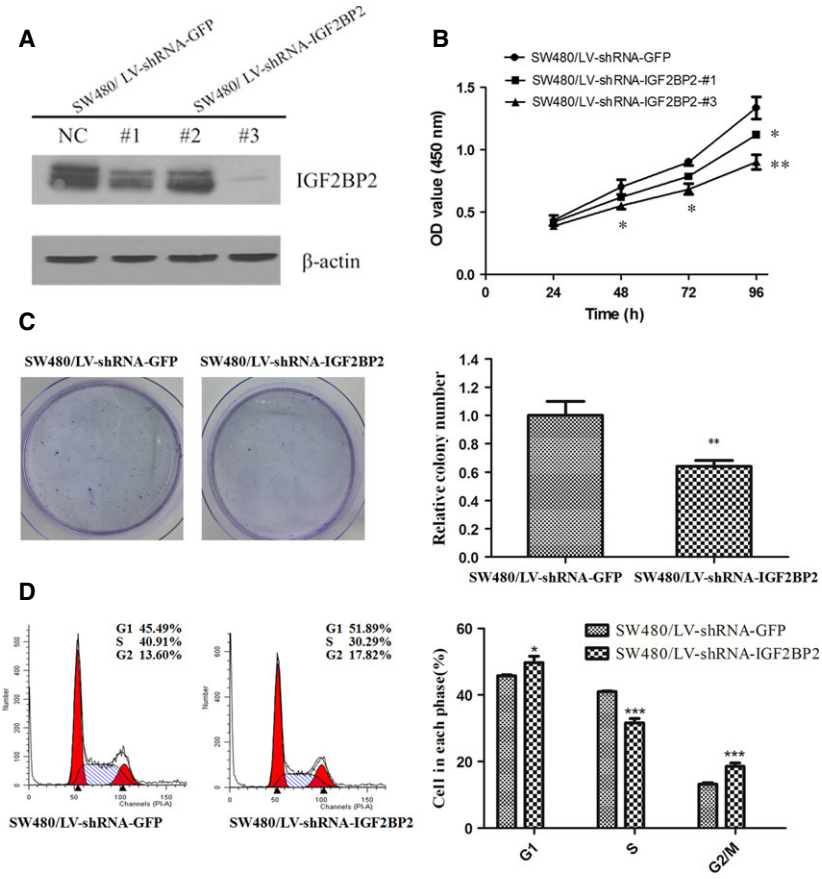

Fig. 2. IGF2BP2 is required for SW480 proliferation and evading growth suppression. (A) shRNA-mediated IGF2BP2 protein knockdown in SW480 cells. NC shRNA control or three independent shRNA (1#, 2#, 3#) targeting IGF2BP2 were used. All three IGF2BP2 shRNA significantly reduced IGF2BP2 levels, in comparison to the NC shRNA. (B) The 1# and 3# shRNA was used for the cell proliferation assay. Knockdown of IGF2BP2 by both shRNA in SW480 cells deduced cell viability. Quantitative analysis of OD value in SW480 cells with LV-shRNA-IGF2BP2 1# and 3# compared to LV-shRNA-GFP (*$P < 0.05$, **$P < 0.01$). (C) Knockdown of IGF2BP2 by 3# shRNA in SW480 cells significantly decreased colony formation. Quantitative analysis of colony numbers in SW 480 cells with LV-shRNA-IGF2BP2 compared to LV-shRNA-GFP (**$P < 0.01$). (D) Knockdown of IGF2BP2 by 3# shRNA in SW480 cells significantly reduced cell growth by cell cycle analysis. Quantitative analysis of cell cycle distribution in SW480 cells with LV-shRNA-IGF2BP2 compared to LV-shRNA-GFP (*$P < 0.05$, ***$P < 0.001$).

IGF2BP2 promoted the proliferation of SW620 cells (Fig. 3B). We then performed colony formation assays to further examine the role of IGF2BP2 in cell proliferation. Consistent with our previous results, these analyses indicated that IGF2BP2 expression positively correlated with colony-forming ability (Fig. 3C). Moreover, we analyzed cell cycle progression in IGF2BP2-overexpressing SW620 cells by flow cytometry. As expected, exogenous IGF2BP2 expression enhanced cell cycle progression in SW620 cells (Fig. 3D). Therefore, the gain-of-function study also suggests that IGF2BP2 is required for CRC cells proliferation and evasion of growth suppression.

IGF2BP2 promotes cell survival in both SW480 and SW620

To determine the function of IGF2BP2 in apoptotic cell death, shIGF2BP2 or exogenous IGF2BP2 CRC cells were double stained with Annexin V-APC and PI and then analyzed by flow cytometry. These results indicated that IGF2BP2-knockdown induced apoptosis in the SW480 cells (Fig. 4A), whereas exogenous IGF2BP2 promoted cell survival in the SW620 cells (Fig. 4B).

IGF2BP2 regulates RAF1 expression

A public database search revealed that exogenously overexpressed IGF2BP2 binds thousands of mRNA in HEK293 cells [5], the majority of which are involved in regulating cell proliferation and growth (S3 Table). To confirm whether endogenous IGF2BP2 bound these target mRNA in CRC cells, RIP was performed to pull down IGF2BP2-bound mRNA in SW480 cell using a specific IGF2BP2 antibody. From this, we were able to identify several bound mRNA including *MAPK1*, *EFF2F*, *RAF1*, *SP1*, *CCNB1*, *CCNA2*, *NUCKS1* and *IGF2*, while negative control *GAPDH* was not detected (Fig. 5A).

To determine whether IGF2BP2 affects mRNA translation, western blots were performed to examine the expression of candidate proteins in shIGF2BP2 SW480 cells, which showed that RAF1 expression was significantly lower in the absence of IGF2BP2 (Fig. 5B).

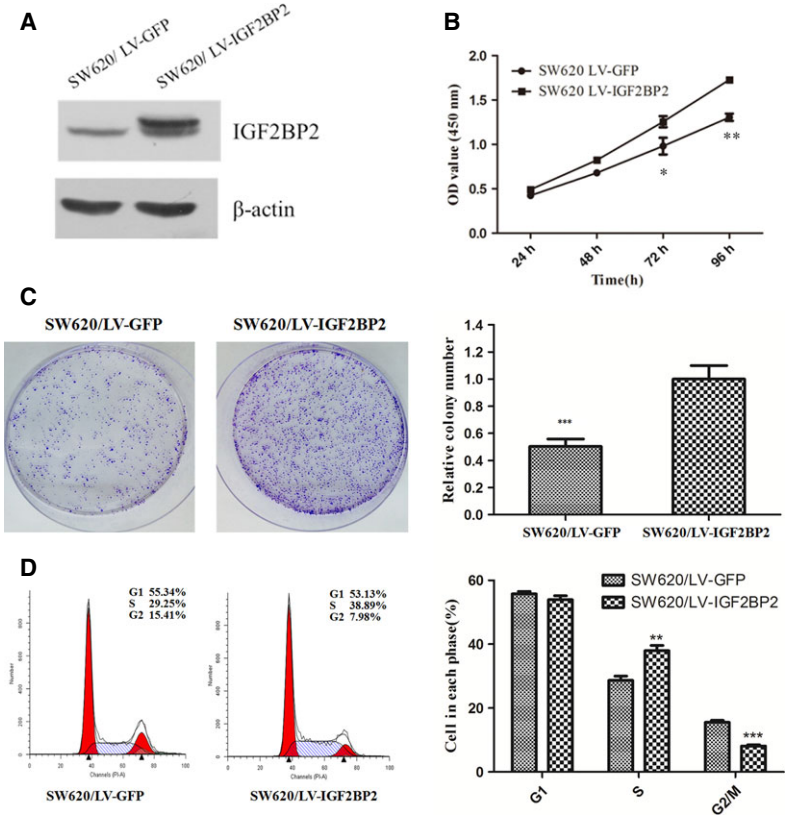

Fig. 3. IGF2BP2 promotes SW620 proliferation and cell cycle progression. (A) Ectopic IGF2BP2 protein expression in SW620 cells was confirmed by western blot analysis. (B) Ectopic IGF2BP2 protein expression in SW620 cells increased cell viability. Quantitative analysis of OD value in SW620 cells with LV-IGF2BP2 compared to LV- GFP (*$P < 0.05$, **$P < 0.01$). (C) Ectopic IGF2BP2 protein expression in SW620 cells significantly increased colony formation. Quantitative analysis of colony numbers in SW 620 cells with LV-IGF2BP2 compared to LV-GFP (***$P < 0.001$). (D) Ectopic IGF2BP2 protein expression significantly increased the growth of SW620 cells by cell cycle analysis. Quantitative analysis of cell cycle distribution in SW620 cells with LV-IGF2BP2 compared to LV-GFP (**$P < 0.01$, ***$P < 0.001$).

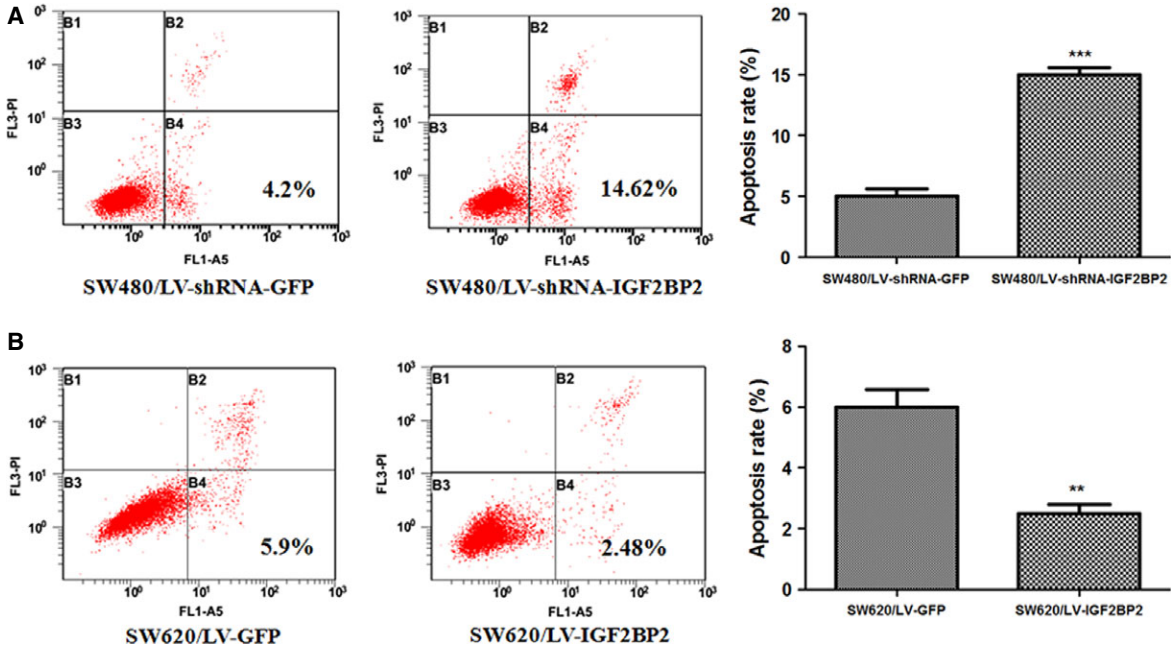

Fig. 4. IGF2BP2 promotes cell survival. (A) Knockdown of IGF2BP2 significantly induced SW480 cells apoptosis by flow cytometry. Quantitative analysis of early and late apoptosis in SW480 cells with LV-shRNA-IGF2BP2 compared to LV-shRNA-GFP (***$P < 0.001$). Apoptosis rate was calculated as Annexin V-APC staining positively. Both Annexin and PI results were used to discriminate the early and late apoptosis. (B) Ectopic IGF2BP2 protein expression significantly suppresses SW620 cells apoptosis by flow cytometry. Quantitative analysis of early and late apoptosis in SW620 cells with LV-IGF2BP2 compared to LV-GFP (***$P < 0.001$).

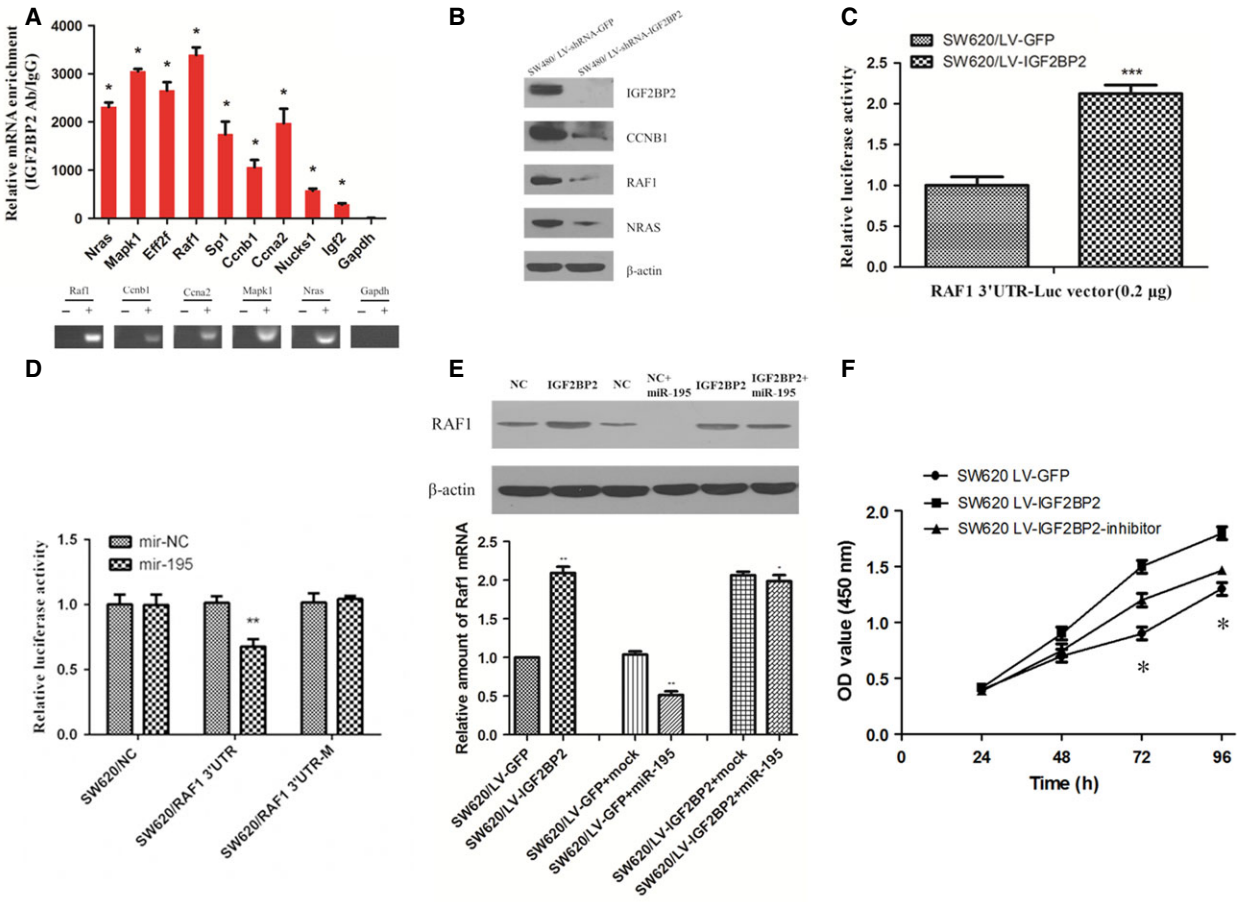

Fig. 5. IGF2BP2-mediated post-translational regulatory mechanism. (A) IGF2BP2 binds to mRNA of *Nras*, *Mapk1*, *Eff2f*, *Raf1*, *Sp1*, *Ccnb1*, *Ccna2*, *Nucks1* and *Igf2*, but not *Gapdh* (*$P < 0.05$). IGF2BP2 antibody versus IgG control. IGF2BP2-binding mRNA including *Raf1*, *Ccnb1*, *Ccna2*, *Mapk1*, and *Nras* were determined by RT-PCR, *Gapdh* as control. (B) Knockdown of IGF2BP2 in SW480 cells reduced the expression of proteins including CCNB1, RAF1, and NRAS, measured by western blot. (C) IGF2BP2 stabilizes RAF1 3′ UTR-luciferase protein in SW620 cells with ectopic IGF2BP2 protein expression (***$P < 0.001$). (D) MiR-195 directly regulated wild-type RAF1 3′ UTR instead of mutant RAF1 3′ UTR in SW620 cells, measured by luciferase reporters (*$P < 0.05$). (E) IGF2BP2 stabilizes *RAF1* mRNA by blocking miR-195-mediated degradation. RAF1 mRNA and protein expression were examined in SW620 cells infected with IGF2BP2, miR-195, and both by qRT-PCR and western blot, respectively. (*$P < 0.05$, **$P < 0.01$). (F) Raf-1 inhibitor sorafinib could partially attenuate the SW620 cells proliferation induced by IGF2BP2 overexpression. Quantitative analysis of OD value in SW620 cells with LV-IGF2BP2 compared to LV- IGF2BP2 combine with Raf-1 inhibitor sorafinib (*$P < 0.05$).

IGF2BP2 regulates RAF1 mRNA stability by blocking miR-195-mediated degradation

MicroRNA are post-transcriptional regulators that destabilize mRNA by duplexing with the 3′UTR of mRNA. Previous studies determined that miR-195 binds to the *RAF1* 3′UTR to regulate mRNA stability and subsequent protein expression in breast cancer cells [15]. We speculated that IGF2BP2 bound to the *RAF1* 3′UTR to hinder its miR-195-mediated degradation in CRC cells. Luciferase assays demonstrated that IGF2BP2 strongly bound the *RAF1* 3′UTR, as IGF2BP2 overexpression enhanced luciferase activity in SW620 cells ($P < 0.001$) (Fig. 5C). And we confirmed that miR-195 bound to and regulated the *RAF1* 3′UTR using Luciferase assay (Fig. 5D). Moreover, exogenous miR-195 attenuated *RAF1* mRNA expression in SW620 transfectants, but was restored in miR-195 and IGF2BP2 cotransfected cells (Fig. 5E). Similar results were also found for RAF1 protein levels by western blot analysis (Fig. 5E). Interestingly, Raf-1 inhibitor, sorafinib (5 nM, much lower than IC50 of other targets of this compound), effectively attenuated the proliferation induced by IGF2BP2 overexpression in SW620 cells. Altogether, IGF2BP2 may regulate *RAF1* expression by preventing its miR-195-directed

degradation in SW620 cells, which contribute to CRC cell proliferation and survival.

Discussion

Insulin-like growth factor 2 mRNA-binding protein family members are post-transcriptional regulatory factors that regulate the localization, stability, and translation of target mRNA, and have known roles in both normal tissue and cancer [10,16,17]. IGF2BP2 is expressed in liver cancer, serous endometrial adenocarcinomas, gonads, and testicular neoplasms [18–20]. David and his colleague found that HMGA2-IGF2BP2-NRAS signaling pathway is required for embryonic rhabdomyosarcoma, and the mechanism is IGF2BP2 essential for mRNA and protein stability of NRAS [18–20]. Our study demonstrated that IGF2BP2 protein expression was significantly increased in CRC tissues compared with matched adjacent normal tissue, suggesting that IGF2BP2 might drive CRC progression through its post-transcriptional regulatory effects. So we selected two cell lines originating from one patient to investigate the loss and gain function of IGF2BP2 in CRC. Among them, we found that IGF2BP2 overexpression promoted SW620 cell proliferation, whereas silencing inhibited the growth of SW480 cells. Moreover, IGF2BP2 knockdown induced cell cycle arrest at the G0/G1 and G2/M checkpoints in SW480 cells, while exogenous IGF2BP2 expression enhanced cell cycle progression in SW620 cells. Flow cytometry analysis also revealed that IGF2BP2 silencing increased early and late apoptosis in SW480 cells, while restoration of IGF2BP2 decreased early and late apoptosis in SW620 cell. Collectively, these results confirmed that IGF2BP2 functions as a tumor promoter to potentiate CRC cell proliferation and survival.

To explore the post-transcriptional regulatory mechanism of IGF2BP2, we first determined that IGF2BP2 bound several mRNA including *RAF1* [21], *CCNB1* [22], *NRAS* [9] and *MAPK1*, among others. Additionally, we found that IGF2BP2 could modulate *RAF1* mRNA stability and protein expression by binding the 3′UTR of *RAF1* mRNA. RAF1 is an essential component of the mitogen-activated protein kinase (MAPK) pathway [23]. Upon activation, RAF1 phosphorylates and activates the mitogen-activated protein kinase (MEK)1/2, which in turn phosphorylate and activate extracellular-related kinase (ERK)1/2 [24]. ERK1/2 can then regulate downstream gene regulatory pathways involved in cell proliferation and survival [21]. A previous study has reported that inhibition of the *RAF1* decreases the cell growth and increases apoptosis in colon cancer cells [25]. In this study, inhibition of Raf-1 reduced the effect induced by IGF2BP2 overexpression in SW620 cells. Therefore, we found the evidence that IGF2BP2 can bind the *RAF1* mRNA to regulate CRC cell proliferation and survival, and then contribute to CRC progression.

A number of studies report that the *RAF1* 3′UTR has rich locus targeted by multiple miRNAs, including miR-195 and miR-7 [15,26,27], which regulate *RAF1* mRNA stability. We verified miR-195 bound to and regulated the *RAF1* 3′UTR in this study, which was consistent with earlier studies [15,28]. Moreover, IGF2BP2 is known to modulate mRNA stability and translation, which may be attributed to interference with microRNA machinery [29]. This suggests that both IGF2BP2 and microRNA are post-transcriptional regulatory factors that may interact to regulate mRNA stability and translation. Here, we confirmed that IGF2BP2 regulated *RAF1* mRNA stability by preventing its miR-195-mediated degradation.

In conclusion, our findings demonstrated that IGF2BP2-dependent post-transcriptional regulation is critical for CRC cell proliferation and survival, and identified a novel mechanism of *RAF1* mRNA regulation. Altogether, our data support that targeting the IGF2BP2–miR-195–RAF1 axis may provide novel approaches for CRC diagnosis and therapy.

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Author contributions

Conceived and designed the experiments: LY; performed the experiments: SY, WS and LY; analyzed

the data: XYZ and XGX; funding support: LY and SY; wrote the manuscript: LY and WS.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. The mRNA expression of IGF2BP2 in SW480 and SW620 cell lines was quantified using qRT-PCR. The mRNA expression of IGF2BP2 in SW480 was higher than in SW620.
Table S1. Top IGF2BP2-binding mRNA with known regulatory functions in cell proliferation and growth in human 293 cells.
Table S2. Sequences of primers used for quantitative real-time PCR.
Sequence S1. *RAF1* 3′UTR wild-type and mutant sequence.