Introduction

Colorectal cancer (CRC) ranks as the third most frequent human cancer worldwide and causes serious deaths every year (1). With the development of CRC diagnosis and therapy, the 5-year survival rate has been improved. However, CRC patients’ prognosis is still not optimistic. A high incidence of distant metastasis and recurrence has been identified as the most crucial obstacle in treating this cancer (2-4). Thence, the inner mechanism underlying CRC should be further explored for searching of more effective diagnosis and treatment for CRC patients.

Circular RNAs (circRNAs) are characterized as a class of new noncoding RNAs and function in tumorigenesis of colorectal cancer (CRC). In our study, the molecule mechanism of circ_0022340 in CRC was investigated. For this aim, quantitative real-time polymerase chain reaction (RT-qPCR) was used to test gene expression in CRC cells. Cell function assays including 5-ethyl-20-deoxyuridine (EdU), colony formation and transwell investigated the proliferation and migration capacity in CRC cells. Luciferase reporter and RNA immunoprecipitation (RIP) assays determined the interaction between circRNA, miRNA and mRNA. Western blot was used to test protein expression. An immunohistochemistry assay was used to assess the tumor growth in vivo. Results showed that Circ_0022340 was highly expressed in CRC cells. Circ_0022340 was formed from exon 5 to 6 of the synaptotagmin 7 (SYT7). Silencing of circ_0022340 suppressed CRC cell proliferation and migration. Functionally, circ_0022340 recruited heterogeneous nuclear ribonucleoprotein C (HNRNPC) to stabilize EBF1 mRNA and thereby activated SYT7. Moreover, circ_0022340 targeted miR-382-5p to up-regulate ETS transcription factor ELK1 (ELK1). It is concluded that Circ_0022340 promoted colorectal cancer progression via recruiting HNRNPC to stabilize EBF1 mRNA and thereby activated SYT7 or miR-382-5p/ELK1 axis, which might provide a novel target for CRC treatment.

Circ_0022340 promotes colorectal cancer progression via HNRNPC/EBF1/SYT7 or miR-382-5p/ELK1 axis

Zhaohui Liu1, Xiaoping Xu1, Dong Chen1, Lei Zhang1, Yuan Pan1, Daocheng Liu1, Minmin Shen1, Min Chen2*

1 Department of Anorectal Surgery, First People’s Hospital of Linping District, Hangzhou, 311199, Zhejiang Province, China
2 Department of Anesthesia, First People’s Hospital of Linping District, Hangzhou, 311199, Zhejiang Province, China

**Corresponding author. Email: chen2022min@yandex.com**

Cellular and Molecular Biology, 2022, 68(7): 107-116
the cell invasion of hepatocellular carcinoma by modulating the expression of miR-141-3p(31). Despite these progress, the mechanistic functions of circRNAs in CRC have not been deeply investigated.

In summary, our study focused on investigating the role and potential mechanism of circ_0022340 in CRC. First, circ_0022340, which was formed from exon 5 to 6 of the synaptotagmin 7 (SYT7) and was highly expressed in CRC cells. Knockdown of circ_0022340 impeded CRC cell proliferation and migration in vitro and vivo. Mechanistically, circ_0022340 recruited heterogeneous nuclear ribonucleoprotein C (HNRNPC) to stabilize EBF1 mRNA and thereby activated SYT7 and acted as a ceRNA to target miR-382-5p/ETS transcription factor ELK1 (ELK1) axis. All these results suggested that circ_0022340 presented oncogenic properties, which might be a candidate for CRC diagnosis and treatment.

Materials and Methods

Cell lines and reagents

Human normal colorectal mucosal cell line (FHC) and human CRC cell lines (LOVO, HT-29, HCT116, SW480) in this study, were available from American Type Culture Collection (ATCC, Manassas, VA). FHC and LOVO cells were cultured in F-12K medium (Invitrogen, Carlsbad, CA, USA). HT-29 and HCT116 cells were cultured in ATCC-formulated McCoy’s 5a medium (Manassas, VA). SW480 cell was cultured in ATCC-formulated Leibovitz’s L-15 medium (Manassas, VA). All mediums with 10% FBS (Gibco) and 1% antibiotics were utilized for cell culture under the condition of 37°C and 5% CO₂. Besides, 3 U/ml of RNase R from Epicentre Technologies (Madison, WI) and 2 mg/ml of Actinomycin D from Sigma-Aldrich (St. Louis, MO) were used to treat cell samples of HCT116 and SW480.

Quantitative real-time polymerase chain reaction (RT-qPCR)

Total RNA was isolated from SW480 and HCT116 cells with TRIzol reagent (Invitrogen, Carlsbad, CA), then converted into cDNA as per the user manual (Takara, Shiga, Japan). SYBR Green PCR Master Mix (Invitrogen) was employed on Step-One Plus System (Applied Biosystems, Foster City, CA) to perform quantitative analysis. RNA level was calculated by the 2-ΔΔCT method, with GAPDH or U6 as an internal control. All experiments were in triplicate.

Plasmid transfection

The specific short hairpin RNAs (shRNAs) were designed by Genepharma Company (Shanghai, China) to stably silence circ_0022340, ELK1, EBF1 and HNRNPC in HCT116 and SW480 cells. Besides, the full-length cDNA sequences of ELK1 or SYT7 were subcloned into the pcDNA3.1 vector (Invitrogen) for gene overexpression. The miR-382-5p mimics/inhibitor and NC mimics/inhibitor were also procured from Genepharma. 48 h of plasmid transfection was implemented in presence of Lipofectamine 3000 (Invitrogen). All experiments were in triplicate.

5-ethynyl-20-deoxyuridine (EdU) staining assay

Cells were put on sterile coverslips in 96-well plates with 5 × 10⁴ cells in each well. EdU kit from Ribobio (Guangzhou, China) was used following guidebook. Cell nucleus was double-stained with EdU and DAPI dye (Beyotime, Shanghai, China). Images were captured by fluorescence microscope (Olympus, Tokyo, Japan). All experiments were in triplicate.

Coloncy formation

Clonogenic cells of HCT116 and SW480 in 6-well plates (500/well) were cultured for 14 days and then stained for 15 min with 0.5% crystal violet in 4% paraformaldehyde. Colonies containing more than 50 cells were counted. All experiments were in triplicate.

Cell migration assay

Cell samples suspended in serum-free medium were planted in the upper chamber of 24-well plates transwell insert (Corning Incorporated, Corning, NY) for migration assay. The lower chamber was supplemented with the conditioned culture medium. Migrated cells in the bottom were fixed in 4% paraformaldehyde and dyed with crystal violet, counted under a microscope. All experiments were in triplicate.

Subcellular fractionation

CRC cell samples (1 × 10⁶) were subjected to PARIS™ Kit as instructed by the supplier (Invitrogen) for collecting cell nuclear and cytoplasmatic fractions. RT-qPCR was performed for quantification of circ_0022340, U6 (nuclear control) and GAPDH (cytoplasmatic control). All experiments were in triplicate.

Fluorescent in situ hybridization (FISH)

The synthesized circ_0022340-FISH probe was procured from Ribobio for culturing with hybridization buffer in presence of the air-dried cell samples. After double-staining cell nuclei with DAPI, samples were visualized using a fluorescence microscope. All experiments were in triplicate.

RNA immunoprecipitation (RIP)

RIP assay in CRC cells was performed by using EZ-Magna RIP RNA Binding Protein Immunoprecipitation Kit based on the protocol (Millipore, Billerica, MA). Cell lysates from RIP lysis buffer were conjugated with human Ago2, EBF1 or HNRNPC antibodies in magnetic beads. A normal mouse IgG antibody was applied to the control group. All experiments were in triplicate.

RNA pull-down

RNA pull-down assay in CRC cells was achieved with Pierce Magnetic RNA-Protein Pull-Down Kit as instructed (Thermo Fisher Scientific, Waltham, MA). Cell protein samples from RIPA lysis buffer were mixed with the biotin-labeled RNA probes for circ_0022340, miR-382-5p or EBF1. After adding magnetic beads for 1 h, the pull-downs were assayed by RT-qPCR or western blot. All experiments were in triplicate.

Luciferase reporter assay

The circ_0022340 or ELK1 fragments covering miR-382-5p binding sites (wild-type and mutated) were applied for generating circ_0022340 WT/MUT and ELK1 WT/MUT by use of pmirGLO luciferase vector (Promega, Massachusetts, MA). After co-transfection with pmirGLO
linear transcripts generated from the same genes(33), therefore we speculated that circRNAs that were produced by extensive pre-mRNA back-splicing could also promote CRC progression. To identify which circRNA expression was abnormal in CRC cells, we applied RT-qPCR to detect different expressions of 8 circRNAs which were generated from precursor SYT7 back-splicing in four of CRC cells (LOVO, HT-29, HCT116 and SW480) and human normal cell (FHC). As shown in Figure 1A, the circ_0022340 level was obviously up-regulated compared with other circRNAs in CRC cells. Then we determined that circ_0022340 was formed from exons 5 to 6 of the SYT7 gene. The back-spliced junction of circ_0022340 was confirmed by Sanger sequencing (Figure 1B). Further vectors and miR-382-5p mimics or NC mimics, CRC cell samples were examined by Luciferase Reporter System (Promega). Besides, cells were co-transfected with pGL3-Basic luciferase vector (Promega) containing SYT7 promoter and the shRNAs for circ_0022340 or EBF1 for promoter analysis. All experiments were in triplicate.

**Western blot**

Total protein samples were obtained and loaded onto 12% SDS-PAGE and transferred to PVDF membrane (Millipore), blocked with 5% fat-free milk, followed by incubation with the primary antibodies against internal control GAPDH (CST, 1:1000) and ELK1 (Abcam, 1/500), SYT7 (Abcam, 1/1000), EBF1 (Abcam, 1/1000), PCNA (Abcam, 1/1000), Ki67 (Abcam, 1/5000) overnight. Afterward, proteins were hatched with the secondary antibodies for probing membranes after specific dilution. The final membranes were all visualized using an ECL detection system (Bio-Rad, Hercules, CA). All experiments were in triplicate.

**Chromatin immunoprecipitation (ChIP)**

ChIP assay in CRC cells was implemented utilizing Magna ChIP Kit as requested (Millipore). DNA-protein cross-links were sonicated to 200-500-bp fragments and immunoprecipitated with EBF1 or control IgG antibody. Precipitated chromatin was retrieved and assayed by RT-qPCR and western blot. All experiments were in triplicate.

**In vivo tumor growth assay**

Transfected HCT116 cells with sh-circ_0022340 or sh-NC were inoculated subcutaneously into BALB/c male nude mice (6-week old; the First Hospital of China Medical University). The animal-related assay was performed strictly in line with the protocol approved by the Animal Research Ethics Committee of the First Hospital of China Medical University. Tumor volume was calculated every 4 days. 4 weeks later, mice were sacrificed after which tumors were excised and weighed. All experiments were in triplicate.

**Immunohistochemistry (IHC)**

Paraffin-embedded tissues from in vivo tumor growth assay were sectioned at 4 μm thick and cultured with primary antibodies, including anti-Ki67, anti-ELK1, anti-SYT7 and anti-PCNA at 4°C overnight, then with secondary antibodies for 30 min at room temperature. All experiments were in triplicate.

**Statistical analyses**

Data were reported as mean ± standard deviation (SD) for three individual experiments. Prism 6 (GraphPad, San Diego, CA) was used for statistical analyses, with p < 0.05 as the threshold for significant level. Group differences were analyzed using Student’s t-test or ANOVA.

**Results**

**Circ_0022340 knockdown inhibits CRC cell proliferation and migration**

It has been reported that SYT7 is overexpressed in CRC cells and can promote cell proliferation in CRC(32). Furthermore, the expression of certain circRNAs was more than ten times higher than those of their canonical...
thermore, RT-qPCR confirmed that linear-SYT7 level was digested by adding RNase R to both HCT116 and SW480 cells, while circ_0022340 was counteractive to RNase R degradation (Figure 1C). In addition, circ_0022340 was further identified using either divergent primers that amplified only circ_0022340 or convergent primers that assessed linear-SYT7. Complementary DNA (cDNA) and genomic DNA (gDNA) from HCT116 and SW480 cells were as templates. The data showed that circ_0022340 was detected with divergent primers in cDNA but not gDNA (Figure 1D). Meanwhile, we also examined the stability of circ_0022340 in HCT116 and SW480 cells treated with Actinomycin D (Act D), an inhibitor of transcription. The result displayed a half-life of more than 12 h, implying that circ_0022340 was more stable than linear-SYT7 (Figure 1E). All these results suggested the presence of circ_0022340 in CRC cells. To investigate the potential mechanism of circ_0022340 in CRC cells, we carried out subcellular fractionation and FISH analysis and discovered that the subcellular location of circ_0022340 was major in the cytoplasm (Figure 1F and 1G), suggesting that circ_0022340 exerted functions in CRC cells via post-transcriptional regulation. Subsequently, we conducted loss-of-function assays to assess the role of circ_0022340 through stably silencing the level of circ_0022340 by transfecting two shRNAs into HCT116 and SW480 cells (Figure 1H). In EdU assays, we found that the EdU-positive cells were obviously decreased after the knockdown of circ_0022340 (Figure 1I). Likewise, the results from colony formation assays revealed that the colony forming efficiency was also inhibited by circ_0022340 deletion (Figure 1J). Besides, silencing of circ_0022340 led to a decrease in migration ability in CRC cells through transwell assays (Figure 1K). Totally, circ_0022340 is highly expressed in CRC cells and circ_0022340 knockdown inhibits cell proliferation and migration in CRC.

**EBF1 binds to SYT7 promoter in CRC cells**

TCGA database showed that SYT7 was obviously up-regulated in CRC cells (Figure 2A). Through luciferase reporter assay, we also discovered that the luciferase activity of SYT7 promoter was decreased when circ_0022340 was silenced in HCT116 and SW480 cells (Figure 2B), therefore we speculated that SYT7 might be regulated at the transcription level. Then we utilized the UCSC website (http://genome.ucsc.edu) to find 5 transcription factors combined with the SYT7 promoter. For further screening, we detected SYT7 expression in HCT110 and SW480 cells transfected with shRNAs targeting these five transcription factors, respectively. The results from Figure 2C showed that SYT7 expression was down-regulated by EBF1 deletion, while it had no change after the transfection of other transcription factors. Thus, we further guessed whether circ_0022340 combined with EBF1 to regulate the transcriptional level of SYT7. However, we found that the enrichment of circ_0022340 has no statistical significance in the EBF1 complex (Figure 2D). Furthermore, we stably down-regulated EBF1 expression and found that the mRNA level and protein level of SYT7 were markedly reduced by EBF1 deletion (Figures 2E and 2F). Through JASPAR software (http://jaspar.genereg.net/), we predicted the binding sites between the SYT7 promoter and EBF1 (Figures 2G and 2H). To verify the effectiveness of the binding sites, luciferase reporter assays were carried out. The data disclosed that the luciferase activity was obviously reduced in HCT116 and SW480 cells co-transfected with shRNAs targeting EBF1 and SYT7 promoter-WT, while there was no change in HCT116 and SW480 cells co-transfected with shRNAs targeting EBF1 and SYT7 promoter-MUT (Figure 2I). Additionally, ChIP assays further reflected that SYT7 was prominently abundant in Anti-EBF1 groups (Figure 2J). All these results indicate that EBF1 can activate SYT7 expression in CRC cells.

**Circ_0022340 recruits HNRNPC to stabilize EBF1 mRNA in CRC cells**

Due to the fact that circ_0022340 could not directly combine with EBF1 to regulate the SYT7 promoter, we doubted whether circ_0022340 recruited some certain protein to combine with EBF1 and thereby affected the SYT7 promoter in CRC cells. We next performed RNA pull-down, followed by SDS-PAGE and mass spectrometry assays to identify proteins associated with circ_0022340(Supplementary figure 1A). At the same time, starbase (http://starbase.sysu.edu.cn/) predicted 15
RBPs that could both bind to circ_0022340 and EBF1, among which only RBP was chosen for it was also specifically identified in mass spectrometry analysis (Supplementary figure 1B). Hence, we chose HNRNPC for further analysis. To further validate our assumption, we first inhibited the expression and protein level of HNRNPC (Figure 3A and 3B) and used RT-qPCR and western blot to analyze the mRNA level and protein level of EBF1 in CRC cells transfected with sh-HNRNPC#1 and sh-HNRNPC#2, respectively. The data showed that HNRNPC silencing dramatically decreased the mRNA level and protein level of EBF1 (Figure 3C and 3D). Besides, we found that circ_0022340 was highly enriched in Ago2 groups, while EBF1 was not, which excluded the existence of a ceRNA mechanism between circ_0022340 and EBF1 (Figure 3E). Meanwhile, RNA pull-down assays disclosed that HNRNPC was largely enriched in Bio-circ_0022340-WT groups and EBF1 groups, suggesting that HNRNPC could combine with both circ_0022340 and EBF1 in CRC cells (Figure 3F). Similarly, the RIP assay also demonstrated that circ_0022340 and EBF1 were both enriched in Anti-HNRNPC groups (Figure 3G). In addition, we found that the expression of EBF1 mRNA declined after circ_0022340 knockdown or HNRNPC deletion in CRC cells treated with Act D, suggesting that the stability of EBF1 was evidently reduced by the depletion of circ_0022340 or HNRNPC (Figure 3H). Conclusively, circ_0022340 recruits HNRNPC to stabilize EBF1 mRNA in CRC cells.

**Figure 3.** Circ_0022340 can recruit HNRNPC to stabilize EBF1 mRNA in CRC cells. A-D. RT-qPCR and western blot severally examined the expression and protein levels of HNRNPC and EBF1 in both HCT116 and SW480 cells transfected with shRNAs targeting HNRNPC. E. RIP assays displayed the enrichment of circ_0022340 and EBF1 in Ago2 groups. F. RNA pull down assays disclosed that the enrichment of HNRNPC in Bio-circ_0022340-WT group and EBF1 group. G. RIP assays demonstrated the enrichment of circ_0022340 and EBF1 in Anti-HNRNPC groups. H. RT-qPCR separately detected the EBF1 mRNA expression in HCT116 and SW480 cells transfected with sh-NC, sh-circ_0022340#1 or sh-HNRNPC#1 upon adding Actinomycin D. \^P<0.01. n.s.: no significance.

**Figure 4.** Circ_0022340 targets miR-382-5p to promote proliferation and migration of CRC cells. A-D. RIP assays analyzed the enrichment of circ_0022340 in Ago2 groups. B. RNA pull down assays analyzed the enrichment of Ago2 in circ_0022340 WT-bio probe groups and circ_0022340 MUT bio-bio groups. C. RNA pull down assays displayed the richness of miRNAs combined with circ_0022340 in Bio-circ_0022340-WT group and EBF1 group. G. Luciferase reporter assays analyzed the combination of circ_0022340 and miR-382-5p. H. EdU and colony formation assays detected the proliferation ability in HCT116 and SW480 cells transfected with miR-382-5p mimics. I. Transwell assays detected the migration ability in HCT116 and SW480 cells transfected with miR-382-5p mimics. \^P<0.01. n.s.: no significance.

**MiR-382-5p is the downstream gene of circ_0022340 in CRC cells**

Since circ_0022340 had been proven to play a promoting role in CRC cell proliferation and migration, we further determined its potential mechanism in CRC cells. Based on the previous mass spectrometry assay, we found that Ago2 protein was also pulled down by circ_0022340 (Supplementary Figure 1A). Therefore, we suspected that circ_0022340 might function as a ceRNA to regulate CRC progression at the post-transcriptional level. To validate our assumption, RIP assay and RNA pull-down assays were carried on to further verify the association between circ_0022340 and Ago2. For Ago2 is a part of RNA-induced silencing complex (RISC) which contains miRNA ribonucleoprotein complexes(34). The data showed that circ_0022340 was obviously enriched in Ago2 groups (Figure 3A and 3B), which proved our conjecture. Subsequently, we applied bioinformatics prediction to observe 16 miRNAs combined with circ_0022340 and performed RNA pull-down assay to filter the certain one that was targeted by circ_0022340 in CRC cells. The results dis-
closed that the enrichment of miR-382-5p was apparently increased in circ_0022340 WT bio-probe groups whereas other miRNAs had no change (Figure 4C). We also used bio-probe to mark miR-382-5p and observed that circ_0022340 was concentrated in the Bio-miR-382-5p-WT group rather than in the Bio-miR-382-5p-MUT group (Figure 4D). Next, the binding sites between circ_0022340 and miR-382-5p were obtained through the starbase website (http://starbase.sysu.edu.cn), and the luciferase reporter assay further confirmed that the luciferase activity was evidently declined in HCT116 and SW480 cells co-transfected with miR-382-5p mimics and circ_0022340 WT, while no significant difference was observed in HCT116 and SW480 cells co-transfected with miR-382-5p mimics and circ_0022340 MUT (Figure 4E and 4F). To investigate the role of miR-382-5p in CRC, gain-of-function assays were conducted. In EdU and colony formation assays, miR-382-5p overexpression significantly reduced the cell proliferation in CRC (Figure 4G and 4H). In transwell assays, the number of migrated cells was also suppressed by miR-382-5p mimics (Figure 4I). Collectively, miR-382-5p is the downstream gene of circ_0022340, and miR-382-5p suppresses CRC cell proliferation and migration.

ELK1 serves as a direct target gene of miR-382-5p and ELK1 knockdown inhibits CRC cell proliferation and migration

MiRNAs primarily function via negatively regulating the expression of protein-coding genes through binding to the 3′ untranslated regions (3′UTR) of mRNAs with imperfect base pairing, resulting in the regulation of gene expression in cells(35). To elucidate the target gene miR-382-5p in CRC cells, we used several bioinformatics programs including PITA, RNA22 and miRanda, the result of which is that nine mRNAs could combine with miR-382-5p (Figure 5A). Then we applied RT-qPCR to separately analyze the expression levels of miRNAs in HCT116 and SW480 cells transfected with sh-circ_0022340 or miR-382-5p mimics. As revealed in Figure 5B, only ELK1 and PDE8A expression were statistically significant, and RNA pull-down assay further confirmed that only ELK1 was evidently enriched in the bio-miR-382-5p WT group (Figure 5C). Moreover, RT-qPCR and western blot testified that the mRNA level and protein level of ELK1 were significantly down-regulated in HCT116 and SW480 cells transfected with miR-382-5p mimics (Figure 5D). Intriguingly, the mRNA level and protein level of ELK1 were decreased by the knockdown of circ_0022340, while enhanced by co-transfection of miR-382-5p inhibitor (Figure 5E). RIP assay further reflected that circ_0022340, miR-382-5p and ELK1 were all positively enriched in Ago2 pellets, indicating that these three RNAs belonged to the RISC (Figure 5F). Additionally, we identified the binding sites of miR-382-5p on ELK1 3′UTR through the starBase database (Figure 5G), and we found that the luciferase activity of ELK1 WT was significantly reduced by miR-382-5p mimics, which was different from that in ELK1 MUT groups (Figure 5H). Finally, we stably knocked down ELK1 expression (Figure 5I) and assessed the effect of ELK1 on CRC cell proliferation and migration. It was revealed in EdU and colony formation assays that silencing of ELK1 suppressed CRC cell proliferation (Figure 5J and 5K). Synchronously, the capacity of migration in CRC cells was also impeded by ELK1 deletion via transwell assays (Figure 5L). Taken together, ELK1 is the target gene of miR-382-5p and ELK1 promotes CRC cell proliferation and migration.

**Circ_0022340 facilitates CRC progression by targeting miR-382-5p to promote ELK1**

To explore the interaction among circ_0022340, miR-382-5p and ELK1 in CRC progression, rescue experiments were conducted. As shown in Figure 6A, the EdU-positive cells were reduced by circ_0022340 deletion, while co-transfection of miR-382-5p inhibitor or pcDNA3.1-ELK1 partly restored this effect. Similarly, the reduced number of colonies mediated by circ_0022340 silencing could be partially enhanced by co-transfection of miR-382-5p inhibitor or pcDNA3.1-ELK1 (Figure 6B). Furthermore, miR-382-5p inhibitor or ELK1 overexpression partly reversed the inhibitory effect of circ_0022340 silencing on.....
CRC cell migration (Figure 6C). All these data reveal that circ_0022340 facilitates CRC cell proliferation and migration by targeting the miR-382-5p/ELK1 axis.

**Circ_0022340 promotes the progression of CRC through regulating ELK1 and SYT7**

Finally, to elucidate the relationship among circ_0022340, ELK1 and SYT7 on CRC cell proliferation and migration, a series of rescue experiments were implemented. As shown in Figures 7A and 7B, the results from EdU and colony formation assays displayed that silencing of circ_0022340 inhibited the proliferation and migration in HCT116 cell, and this effect was partially rescued by co-transfection of pcDNA3.1-ELK1, while completely enhanced by co-transfection of pcDNA3.1-ELK1 and pcDNA3.1-SYT7. Besides, overexpression of ELK1 partly restored the inhibited number of migrated cells mediated by circ_0022340 deletion (Figure 7C). Moreover, we also explored the impact of circ_0022340 on the tumor growth of CRC in vivo. As displayed in Figures 7D and 7E, the tumor volume and tumor weight were largely reduced by circ_0022340 knockdown in the HCT116 cell. Meanwhile, RT-qPCR and western blot corroborated that the expression levels of circ_0022340, SYT7 and ELK1 as well as the protein levels of SYT7 and ELK1 were depleted by circ_0022340 reduction in HCT116 cell (Figure 7F and 7G). In addition, IHC results further showed that ELK1, SYT7, the proliferation index Ki67 and PCNA all presented lower positivity upon circ_0022340 knockdown (Figure 7H). All these results suggest that circ_0022340 promotes CRC cell proliferation and migration of CRC cells through regulating ELK1 and SYT7.

The scientific concept map describes the functional mechanism of circ_0022340 in CRC cells

We concluded that circ_0022340 promoted CRC cell proliferation and migration through recruiting HNRNPC to stabilize EBF1 mRNA and thereby up-regulate its host gene SYT7 as well as sponging miR-382-5p to promote ELK1 expression (Figure 8).

**Discussion**

Recently, circRNAs have been a hot topic in human CRC research. They exhibit important roles in regulating signaling molecules associated with the progression of CRC, thereby providing novel strategies to improve CRC therapy(36). Accumulating evidence has revealed that circRNAs take part in the occurrence and develop-
CRC via post-transcriptional regulation (45). For example, silencing could suppress the tumor growth in CRC. We performed in vivo experiments to find that circ_0022340 overexpression of ELK1 and STT7 could entirely restore the inhibitory effect mediated by circ_0022340 deletion. Moreover, we carried on a series of rescue experiments to validate that our research proved that ELK1 was the target gene of miR-382-5p. In addition, loss-of-function assays further verified that ELK1 played a tumor-promoting role in CRC. Similarly, previous studies have identified that ELK1 can function as an oncogenic role in breast cancer and prostate cancer (52, 53). Finally, rescue assays further revealed that circ_0022340 facilitated CRC cell proliferation and migration via modulating HNRNPC/EBF1/SYT7 axis. All these results supported the ceRNA hypothesis of circ_0022340 in CRC cells and suggested that circ_0022340 might be a potential target for CRC treatment. In conclusion, our study elucidated the functional role and potential mechanism of circ_0022340 in CRC. Our study confirmed that circ_0022340 promoted CRC cell proliferation and migration through modulating HNRNPC/EBF1/SYT7 or miR-382-5p/ELK1 axis which might contribute to the exploration of CRC treatment.

Conflict of interest

The authors declare that they have no competing interest in this study.

Acknowledgements

We sincerely appreciate all support from our laboratory.

References

8. Weng W, Wei Q, Toden S, Yoshida K, Nagasaka T, Fujiwara T, Cai S, Qin H, Ma Y, Goel A. Circular RNA circRS-7-A Promising Prognostic Biomarker and a Potential Therapeutic Target in Co-


