Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

MYC-activated CERS6-AS1 sponges miR-6838-5p and regulates the expression of RUBCNL in colorectal cancer

Dong Chen[1], Huiming You[1], Xiaoping Xu[1], Daocheng Liu[1], Minmin Shen[1], Yuan Pan[1], Zhaohui Liu[1], Lei Zhang[1], Huijuan Wang[2*]

[1] Department of Colorectal Surgery, First People's Hospital of Linping District, Hangzhou, 311100, Zhejiang Province, China
[2] Department of Intensive Care Unit (ICU), First People's Hospital of Linping District, Hangzhou, 311100, Zhejiang Province, China

ARTICLE INFO

Original paper

Article history:
Received: August 17, 2022
Accepted: December 15, 2022
Published: December 31, 2022

Keywords:

Colorectal cancer, CERS6-AS1, miR-6838-5p, RUBCNL

ABSTRACT

As a leading gastrointestinal malignancy, colorectal cancer (CRC) is a serious threat to people's health. A great amount of researches have elaborated that long non-coding RNAs (lncRNAs) play a key role in all kinds of tumors. In the current study, we mainly probed into the mechanisms of CERS6 antisense RNA 1 (CERS6-AS1) underlying CRC. For this purpose, the CERS6-AS1 expression level in CRC cells was disclosed by quantitative real-time PCR (qRT-PCR). In vitro and in vivo assays have validated the functional role of CERS6-AS1 in CRC. Mechanism assays were carried out to confirm the potential mechanism of CERS6-AS1 in CRC. Results showed that through experiments, we identified that the CERS6-AS1 expression level was up-regulated in CRC and the depletion of CERS6-AS1 hindered cell proliferative and migratory abilities and stimulated cell apoptotic levels in CRC. In addition, silencing of CERS6-AS1 repressed tumor growth. Moreover, CERS6-AS1 activated by MYC could sequestermiR-6838-5p, and then regulate rubicon-like autophagy enhancer (RUBCNL) expression level to influence the CRC cell proliferation, migration and apoptosis. In conclusion, The study focused on the MYC/CERS6-AS1/miR-6838-5p/RUBCNL axis was helpful for the potential diagnosis and standardized treatment of CRC.

Doi: http://dx.doi.org/10.14715/cmb/2022.68.12.9

Introduction

Colorectal cancer (CRC) is a prevalent malignant tumor that occurs in the gastrointestinal tract. According to the statistics on cancer incidence and mortality, CRC ranks fifth among all malignant tumors(1). In addition, there are no obvious abnormal symptoms in the early stage (2). Therefore, most patients have entered into middle and later stages when they are diagnosed (3). Conventional methods include surgery, radiotherapy, chemotherapy, physical therapy, etc. (4, 5). However, the effect is not obvious, and the disease is easy to relapse. Hence, it is urgently needed for us to investigate new strategies for CRC treatment.

Long non-coding RNAs (lncRNAs) have become a hot topic in recent years, mainly because of their very important regulatory functions (6, 7). The regulatory function of lncRNA in gene expression and cancer development opens up a new path for the comprehension of the complexity of life forms from the perspective of the gene expression regulatory network(8, 9). For example, lncRNA-RMRP sponges miR-206 and affects the malignant properties of bladder cancer (10). Aberrant expression of lncRNA-ATB is related to papillary thyroid carcinoma(11). LncRNA FBXL19-AS1 facilitates breast cancer (BC) cell proliferation and invasive abilitiesbysequesteringmiR-718 (12). With the development of various biotechnologies, the involvement of lncRNAs acting as a tumor promoter or tumor suppressor in CRC progression has been also widely studied. Bian Z et al. have concluded that lncRNA-FEZF1-AS1 binds with PKM2 to activate STAT3 signaling, thereby accelerating proliferation and metastasis in CRC (13). Xu Y et al. also proved lncRNA MALAT1 affects the malignant behaviors in CRC cells by regulating SOX9 (14). Despite advanced investigations, the function of numerous lncRNA has not been covered in CRC and deserves further study. As reported previously, lncRNA CERS6 antisense RNA 1 (CERS6-AS1) can act as a malignancy promoter in BC (16). We aimed to further explore its role in CRC in this paper.

Based on former reports, competing endogenous RNA (ceRNA) activity can influence multiple human diseases, including cancers(15). A number of studies have proved that lncRNA-mediated ceRNA network could participate in CRC initiation and process, thus providing new sight for the discovery of potential therapeutic targets and biomarkers of CRC (20)(16). Zhou et al. have revealed the regulatory role of the HAND2-AS1/miR-1275/KLF14 axis in CRC (17). Wang et al. have verified that lncRNA SLCO4A1-AS1 is related to CRC progression via sequestering miR-508-3p and modulating PARD3 expression (18). In our study, we tried to discover the ceRNA mechanism involving CERS6-AS1 in CRC.

Materials and Methods

Cell lines and culture

In this study, the CRC cell lines we used included HTC116, SW620, DLD1 and HT29 while the FHC cell line was a normal human colorectal mucous membrane cell line and was used as a negative control. All cells were commercially acquired from ATCC(Manassas, VA, USA). Among these cell lines, the FHC cell line was kept

* Corresponding author. Email: wuyoufei84856554@163. com

in DMEM: F12 Medium; SW620 cell line was cultivated in L-12 Medium; DLD1 cell line was cultivated in RPMI-1640; and HTC116 and HT29 cell lines were placed in McCoy's 5a Medium. All mediums were added with 10% fetal bovine serum. All cell lines were kept in these complete mediums at 37℃ with 5% CO_2.

Cell transfection

The interference with CERS6-AS1 in CRC was carried out by transfecting cells with sh-CERS6-AS1. In addition, the miR-6838-5p expression level was also reduced by transfecting cells with a miR-6838-5p inhibitor. The sh-CERS6-AS1, sh-NC, mimic-NC and mimic-miR-6838-5 were all commercially attained from Genepharma. Additionally, pcDNA3. 1-RUBCNL for overexpression of RUBCNL was obtained from Realgene. As per the instruction of the supplier, Lipofectamine 2000 was applied to transfect CRC cell lines.

RNA isolation and quantitative real-time PCR (qRT-PCR)

Trizol reagent was adopted for the extraction of the total RNA from cells. M-MLVRT was utilized to obtain cDNA via reverse transcription of two micrograms of total RNA. SYBR green real-time Master Mix was adopted to performqRT-PCR. The method of $2^{-\triangle\triangle Ct}$ was applied to the examine relative RNA level.

Cell Counting Kit-8 (CCK-8)

CRC cells were inoculated in 96-well plates and kept in an incubator with 5% CO_2 for 24 h at 37℃. After 5 days of culture, each well was added with 10 mol/L CCK-8 solutions. The absorbance value at the wavelength of 450 nm was calculated.

Colony formation assay

Simply, all the cells were kept in a 6-well plate for 24 h, followed by 2 weeks of incubation. Afterward, cells were fixed utilizing methanol, followed by being dyed with 0. 1% crystal violet. Lastly, the colonies were calculated to determine cell proliferation.

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

Cells were taken at the logarithmic growth stage, followed by inoculation in 96-well plates with 4×10^3~1×10^5 cells in each well. Prior to the assay, the cells were cultivated to the normal growth stage. After that, the EdU solution (reagent A) was subjected to dilution at the ratio of 1:1000 in order to prepare50μM the EdU culture medium. Subsequent to the fixation, the cells weredyedutilizingApollo.

Transwell assay

CRC cells were placed in a transwell chamber system, followed by inoculation in the upper compartment. Subsequent to 48 h of incubation, methanol was utilized to fix the filter. After that,0. 1% crystal violet was adopted to dye the cells. The migrated cells in the lower part were calculated through microscopy.

Transferase-mediated dUTP nick end labeling (TUNEL) assay

CRC cell apoptotic level was examined by Cell Death Detection Kits. The cells were kept in 6-well plates. DAPI was used to stain the nuclei and fluorescence microscopy was used to capture the images.

Chromatin immunoprecipitation (ChIP)

All cells were subjected to fixation in 1% formaldehyde at room temperature for half of 1 h, and the DNA was cut to an average fragment size of 500 ~ 1000bp by ultrasound. Subsequently, the biotin-labeled antibody was used for the experiment, with anti-IgG utilized as a negative control.

Luciferase reporter assay

CERS6-AS1 promoter binding sequences containing MYC binding sites were obtained and mutated to establish wild-type and mutant-type reporter vectors. Similarly, CERS6-AS1 or RUBCNL binding sequences containing miR-6838-5p binding sites and mutant binding sites were sub-cloned into pmirGLOreporter vectors. The above operations and experiments were implemented three times according to the supplier's protocols.

Cytoplasmic and nuclear RNA isolation

In accordance with suppliers' instructions, Thermo Fisher BioReagents were applied to separate cytoplasmic and nuclear parts of CRC cells. The localization of CERS6-AS1 was determined by qRT-PCR.

Fluorescent in situ hybridization (FISH)

CRC cells were fixed utilizing 4% PFA for 30min. After that,0. 5% TritonX-100 was applied to permeabilize the cells at 4℃ for 15min. CERS6-AS1 probe or control probe was labeled with Digoxi-genin (DIG) and then subjected to 4 h of incubation with the cells at 55℃. Subsequent to three-time washing in PBS, the cells were next subjected to treatment with a pre-hybridization buffer.

Western blot analysis

BCA protein Assay Kit was employed to determine the proteins isolated from the cells. 12% sodium lauryl sulfate-polyacrylamide gels (SDS-PAGE) were applied for the separation of the proteins, which were then transferred to the polyvinylidene difluoride (PVDF) membranes. All the proteins were cultured at 37℃ for 2 h after being blocked with 5% defatted milk. Subsequent to the three-time washing with TBST buffer solution at room temperature, the membranes and secondary antibody were incubated for 2 h. The antibodies against RUBCNL and GAPDH were commercially attained from Abcam. Lastly, an enhanced chemiluminescence detection system was employed to display the strip.

RNA-binding protein immunoprecipitation (RIP)

As per the supplier's protocol, the EZMagna RIP Kit was used to conduct RIP. Complete RIP lysis buffer was utilized to lyse CRC cells. Next, the cell lysates were incubated with coupled magnetic beads with anti-Argonaute 2 (anti-Ago2) or control anti-IgG.

RNA pull-down

The 3'end biotinylated miR-6838-5p or miR-6838-5p-Mut at a final concentration of 20 nmol/L was transfected to CRC cells. After that, the transfected cells were lysed, and then the cell lysates were incubated with magnetic beads coated by streptavidin. The biotin-linked RNA complexes pulled down by beads were subjected to qRT-PCR

for examination of the abundance of CERS6-AS1.

Animal experiments

According to the guidelines of Liaocheng People's Hospital, the experiments on the animal were performed. The BALB/c nude mice (female, 5-week-old)were commercially attained from Vital River Co. Ltd., and the HTC116 cells transfected with sh-CERS6-AS1 and sh-NC were subjected to injection into the mice. The weight of the tumor was calculated every 7 days. All procedures and animal experiments were permitted by Liaocheng People's Hospital.

Bioinformatics analysis

GEPIA (http://gepia. cancer-pku. cn/index. html), TCGA (https://www. cancer. gov/about-nci/organization/ccg/research/structural-genomics/tcga) were used to predict CERS6-AS1 expression level. UCSC (http://genome. ucsc. edu/index. html), HumanTFDB (http://bioinfo. life. hust. edu. cn/HumanTFDB#!/) and Jaspar (http://jaspar. genereg. net/analysis) were retrieved to forecast transcription factors and binding sites.

Statistical analysis

In the current study, all the assays were independently carried out in triplicate. The data was recorded and analyzed utilizing SPSS 22. 0, and were displayed as the mean±SD. The experiments that only one of the factors considered were evaluated using one-way ANOVA while experiments concerning two factors were testified using two-way ANOVA. Student's t-test was utilized for different comparisons between the two groups. As for the P-value, $P < 0.05$ was regarded to be the threshold for statistical significance.

Results

CERS6-AS1 was overexpressed in CRC and promotes tumor growth

Bioinformatics software was applied for the analysis ofCERS6-AS1expression level in CRC tissues. GEPIA and TCGA databases unclosed that CERS6-AS1 expression in CRC tissues was dramatically up-regulated as compared to normal tissues (Fig. 1A-B). Subsequently, CERS6-AS1 expression levels in normal FHCcellline and CRC cell lines (HTC116, SW620, DLD1, HT29) were examined by qRT-PCR. It was presented that CERS6-AS1 expression was notably higher in these CRC cells relative to the control group (Fig. 1C). To further probe into the influence of CERS6-AS1 expression level on CRC, we first knocked down CERS6-AS1 expression in CRC cells (Fig. 1D). Animal experiments were conducted by injecting CERS6-AS1-depleted HTC116 cells into mice, and the tumors in mice were measured. It was found that depletion of CERS6-AS1 noticeably hindered tumor growth (Fig. 1E-F). The above-mentioned results indicated that CERS6-AS1 might facilitate CRC cell progression.

CERS6-AS1inhibition suppressed cell proliferation and migration while accelerating cell apoptosis in CRC

Next, functional assays were implemented to assess CERS6-AS1 functions in vitro. CCK-8 assay was first carried out to measure cell viability. From the experimental results, it was unearthed that the viability of CRC cells was significantly inhibited uponCERS6-AS1 deficiency (Fig. 2A). Meanwhile, as demonstrated by the results of colony formation and EdU assays,afterCERS6-AS1 was silenced, proliferative capacity in CRC cells was greatly weakened (Fig. 2B-C). Afterward, we carried out a transwell assay to unearth the migratory capacity of CRC cells. The outcomes disclosed that when CERS6-AS1 expression was suppressed, CRC cell migration was also significantly inhibited(Fig. 2D). In order to detect the apoptotic rate of the CRC cells, we conducted the TUNEL experiment. On the contrary, cell apoptosis was dramatically stimulated due to CERS6-AS1 depletion (Fig. 2E). Meanwhile, we detected the cleaved PARP and cleaved caspases (caspase-3 and caspase-9) expression after CERS1-AS1 knockdown. The result analyzed via western blot uncovered that cleaved PARP and cleaved caspases protein levels were enhanced after CERS1-AS1 knockdown (Fig. S1A). In summary, CERS6-AS1 promoted cell proliferation and migration but hindered cell apoptosis of CRC.

MYC activated the transcription of CERS6-AS1 in CRC cells

Four potential transcription factors (MYC, TRIM24, LMNB1 and CBX3) ofCERS6-AS1were obtained by the intersection of predicted transcription factors in HumanTFDB whose score was more than or equal to 20 and the transcription factors in GEPIA whose log2 fold change was more than or equal to 1 (Fig. 3A). Many reports have confirmed that MYC is a common transcription factor, so we chose MYC for further investigation. It was uncovered

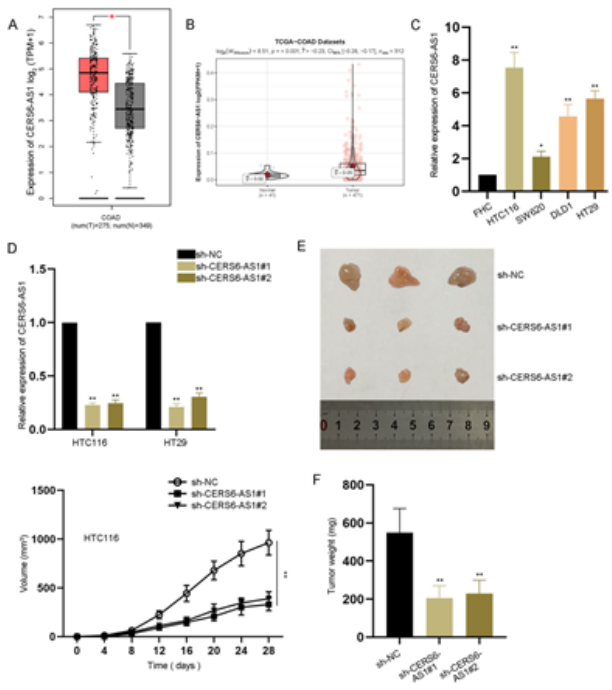

Figure 1. The expression of CERS6-AS1 was high in CRC and promoted tumor growth. (A) The expression level of CERS6-AS1 in normal and tumor tissues was shown in the GEPIA database. (B) The expression level of CERS6-AS1 in normal and tumor tissues was shown in the TCGA database. (C) Expression of CERS6-AS1 in CRC cell lines and normal human colorectal mucosal cell lines was detected by qRT-PCR. (D) qRT-PCR was used to detect the knockdown efficiency of sh-CERS6-AS1#1 and sh-CERS6-AS1#2. (E) The detection of tumor volume in mice and corresponding pictures. (F) The detection of tumor weight in mice. * P<0. 05, ** P<0. 01.

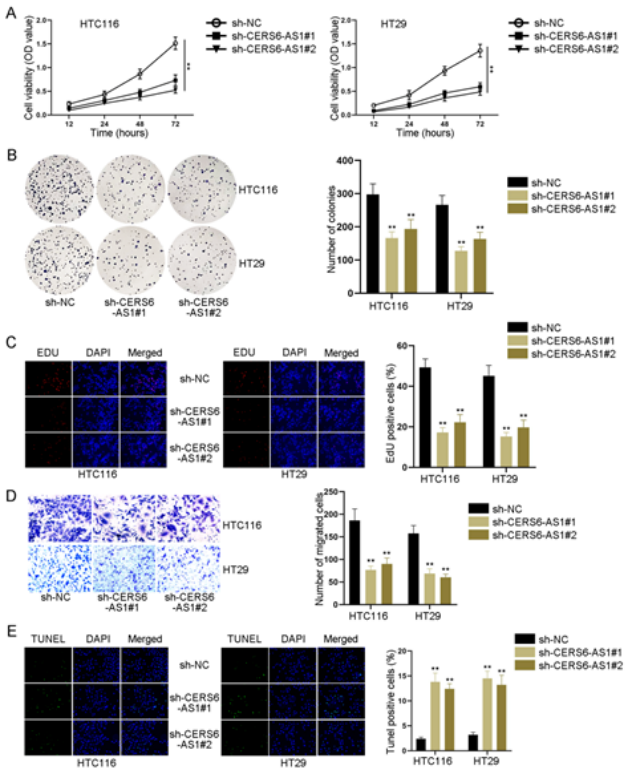

Figure 2. CERS6-AS1 inhibition suppressed cell proliferation and migration while accelerating cell apoptosis in CRC. (A)CCK-8 was used to detect the effect of CERS6-AS1 deficiency on cell viability. (B)Colony formation was used to detect the number of colonies caused by the inhibition of CERS6-AS1. (C)EdU assay detected cell proliferation when CERS6-AS1 was silenced. (D)Transwell assay was implemented to detect the migration ability of CRC cells. (E) TUNEL assay detected the effect of CERS6-AS1 silencing on cell apoptosis. ** P<0. 01.

by qRT-PCR outcomes that the CERS6-AS1 expression level was evidently up-regulated in CRC cells subsequent to transfection of pcDNA3. 1-MYC (Fig. 3B). Also, we knocked down MYC and measured the expression of CERS1-AS1, and the result revealed that MYC depletion would cause a CERS1-AS1 decrease (Fig. S1B). We used related bioinformatics software HumanTFDB and JASPAR to forecast the binding sites between MYC and CERS6-AS1 promoters (Fig. 3C). It was unearthed by the outcomes of ChIPthat MYC could interact with the CERS6-AS1promoter (Fig. 3D). The luciferase report showed that when the binding sites of MYC on CERS6-AS1promoter were not mutated, the luciferase activity was increased; while the luciferase activity did not change subsequent to the mutation of binding sites (Fig. 3E). The results in Fig. 3E reflected that MYC could bind to the CERS6-AS1promoter through this binding site. To conclude, MYC could act as a transcription factor to activate CERS6-AS1 in CRC cells.

CERS6-AS1 sponged miR-6838-5p in CRC cells

Subsequently, we studied the distribution of CERS6-AS1 in CRC cells. As indicated by both subcellular fractionation and FISH assays, CERS6-AS1 was predominantly distributed in CRC cell cytoplasm (Fig. 4A-B). Through starBase, we predicted6 potential miRNAs. Among them, we selected miR-6838-5p and focused on the relationship between CERS6-AS1 and miR-6838-5p in CRC for that only miR-6838-5p had not been researched in CRC. Additionally, the detection of qRT-PCRunmaskedthat miR-6838-5p expression level in CRC cells was low in relation to normal cells (Fig. 4C). It was uncovered byAgo2 RIP that CERS6-AS1 and miR-6838-5p had a strong binding affinity(Fig. 4D). StarBase demonstrated the binding sites between CERS6-AS1 and miR-6838-5p (Fig. 4E). It was confirmed via RNA pull-down assay that biotinylated CERS6-AS1-WT probe, instead of biotinylated CERS6-AS1-Mut probe, had the ability to pull downmiR-6838-5p (Fig. 4F). Luciferase reporter assay validated that mimic-miR-6838-5plessened the luciferase activity in the CERS6-AS1-WT group while failing to influence that in the CERS6-AS1-Mut group (Fig. 4G). Collectively, miR-6838-5p was sequestered by CERS6-AS1 in CRC cells.

MiR-6838-5p could target RUBCNL

In this section, we further studied the downstream target genes of miR-6838-5p. We intersected all the mRNAs predicted by starBase that could combine withmiR-6838-5p with the mRNAs that were up-regulated in TCGA database, and obtained seven common mRNAs (Fig. 5A). Among them, VMA21 and RUBCNL have not been studied in CRC, and then their expression levels were unclosed by qRT-PCR. It was uncovered that when miR-6838-5p was overexpressed, only RUBCNL expression was observably down-regulated in the HTC116 cell line (Fig. 5B). The results of western blot also showed that RUBCNL had higher protein levels in CRC cells(Fig. 5C).

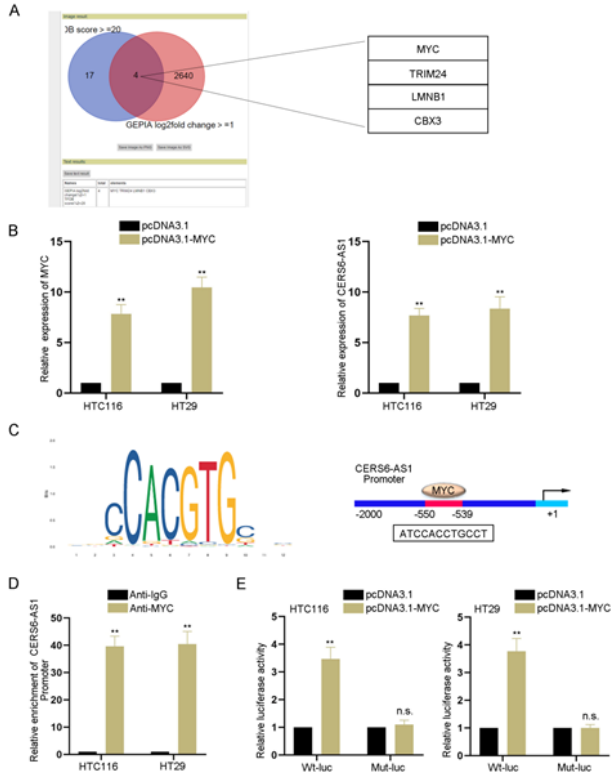

Figure 3. MYC activated the transcription of CERS6-AS1 in CRC cells. (A)Bioinformatics software was used to select transcription factors. (B)qRT-PCR was used to detect the efficiency of pcDNA3. 1 vector transfected with MYC and the expression of CERS6-AS1 in CRC cells transfected with pcDNA3. 1-MYC. (C) The binding sites between MYC and CERS6-AS1 promoter were predicted. (D)ChIP assay was used to detect the interaction between MYC and CERS6-AS1 promoter. (E)The binding sites were detected by luciferase reporter assay. ** P<0. 01.

The interacting relationship between RUBCNL and miR-6838-5p was confirmed by Ago2 RIP (Fig. 5D). Luciferase reporter assay outcomes unveiled that when the binding sites of RUBCNL and miR-6838-5p were not mutated, the binding affinity between them was strong(Fig. 5E). Then, we implemented qRT-PCR to determine miR-6838-5p and RUBCNL expression levels in HTC116 and HT29 cells subsequent to transfection with sh-MYC. It was uncovered that miR-6838-5p expression level was not influenced subsequent to MYC depletion, while RUBCNL expression was down-regulated (Fig. S1C). In conclusion, miR-6838-5p could target RUBCNL to modulate its expression.

InterferencewithmiR-6838-5p or up-regulation of RUBCNL rescued the impacts of CERS6-AS1depletion on the malignant progression of CRC

Herein, we carried out a range of rescue experiments, intending to further validate whether CERS6-AS1 influenced the malignant process of CRC cells by targeting the miR-6838-5p/RUBCNL axis. It was unclosed by the outcomes of CCK-8 and colony formation experiments that depletion of CERS6-AS1 reduced the proliferative ability of CRC cells, while impairment of miR-6838-5p or up-regulation of RUBCNL could reverse this phenomenon(Fig. 6A-B). Similarly, the results of EdU also unveiled that miR-6838-5p inhibitor or pcDNA3. 1-RUBCNL could restore the inhibition of sh-CERS6-AS1 on cell proliferation(Fig. 6C). In addition, the suppression of miR-6838-5p or enhancement of RUBCNL could also reduce the inhibition of CERS6-AS1 depletion on CRC cell migration(Fig. 6D). Apoptosis induced by CERS6-AS1 silencing could be repaired by miR-6838-5p inhibitor or pcDNA3. 1-RUBCNL as well(Fig. 6E). At last, we probed into miR-6838-5p and RUBCNL expression levels in tumor tissues excised from sacrificed mice. The qRT-PCR results verified that tumor tissues from mice injected with HTC116 cells transfected with sh-CERS6-AS1 were with lower RUBCNL expression while miR-6838-5p expression remained stable (Fig. S1D). All the above results demonstrated that CERS6-AS1 could exacerbate the malignancy of CRC cells by sequestering miR-6838-5p and eliminating the miRNA-induced suppression of RUBCNL expression.

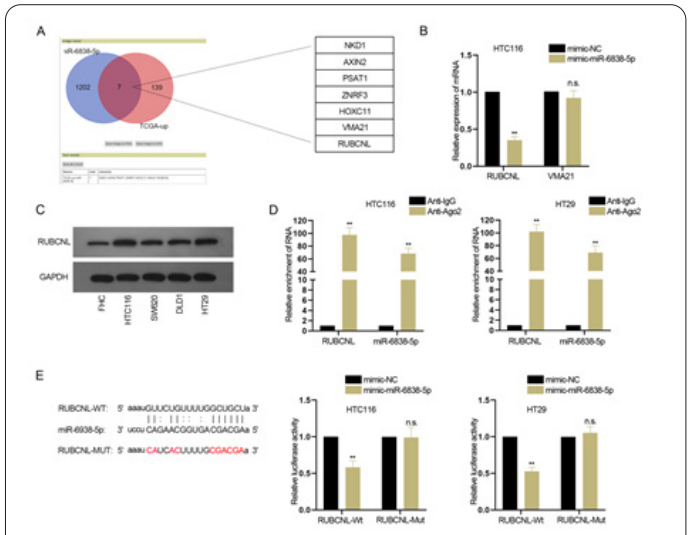

Figure 5. RUBCNL was the target gene of miR-6838-5p. (A) The mRNAs were selected by the Venn diagram. (B)The expression levels of two mRNAs in the HTC116 cell line were detected by qRT-PCR. (C)Western blotting was used to analyze the protein level of RUBCNL. (D) Ago2 RIP detected the binding force between RUBCNL and miR-6838-5p. (E) A luciferase reporter assay was conducted to verify the binding sites. ** P<0. 01.

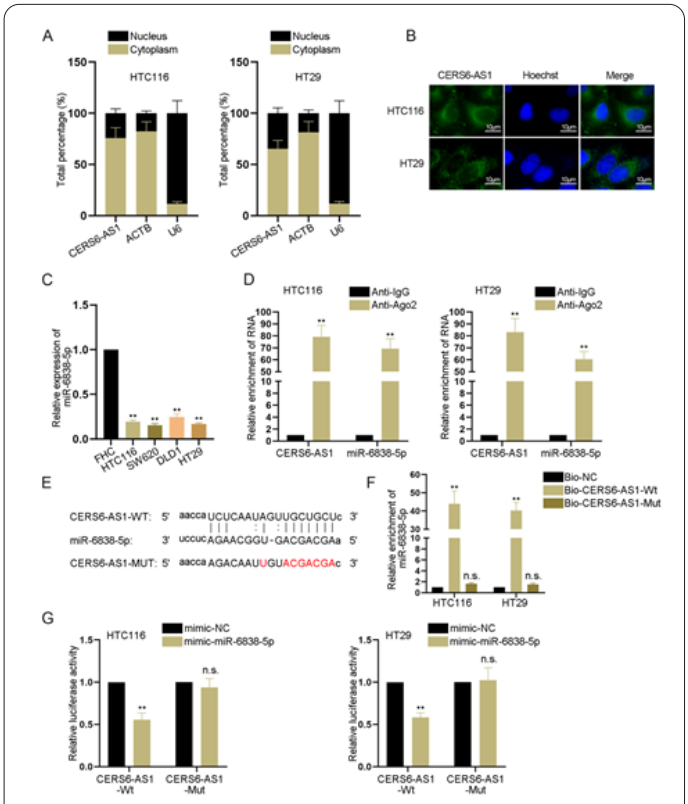

Figure 4. CERS6-AS1 sponged miR-6838-5p in CRC cells. (A-B) The localization of CERS6-AS1 in CRC was determined by subcellular fractionation and FISH assays. (C)qRT-PCR was used to detect the expression level of miR-6838-5p in CRC cell lines and normal human colorectal mucous membrane cell lines. (D)Ago2 RIP detected whether there was a binding force between CERS6-AS1 and miR-6838-5p. (E)StarBase predicted the binding sites. (F)RNA pull-down was conducted to verify the binding relationship. (G)The binding sites between CERS6-AS1 and miR-6838-5p were detected by luciferase reporter assay. ** P<0. 01.

Discussion

Since a great deal of evidence has uncovered that lncRNAs have an impact on the evolution and development of cancers, more and more studies have been conducted on lncRNAs and CRC. For instance, Xu et al. have proposed that by targeting the miR-145/SOX9 axis, lncRNA MALAT1 influences CRC progression (14). As indicated by former studies, CERS6-AS1 can play a promoting role in regulating malignancyofBC(19, 20). Moreover, several reports have determined that CERS6-AS1 has the possibility to serve as a biomarker in Alzheimer's disease and hepatocellular carcinoma (21, 22). In this study, we first used bioinformatics software to predict CERS6-AS1 expression level in CRC and identified that CERS6-AS1 was high-expressed in CRC tissues. In addition, CERS6-AS1 down-regulation dramatically repressed cell proliferative and migratory abilities and promoted apoptotic levels of CRC cells. These results suggested the oncogenic property of CERS6-AS1 in CRC.

MYC is a common transcription factor that can induce aberrant expression of most lncRNAs in cancers. As a transcription factor, MYC plays important role in the pathogenesis of cancer(23). Down-regulation of MYC was

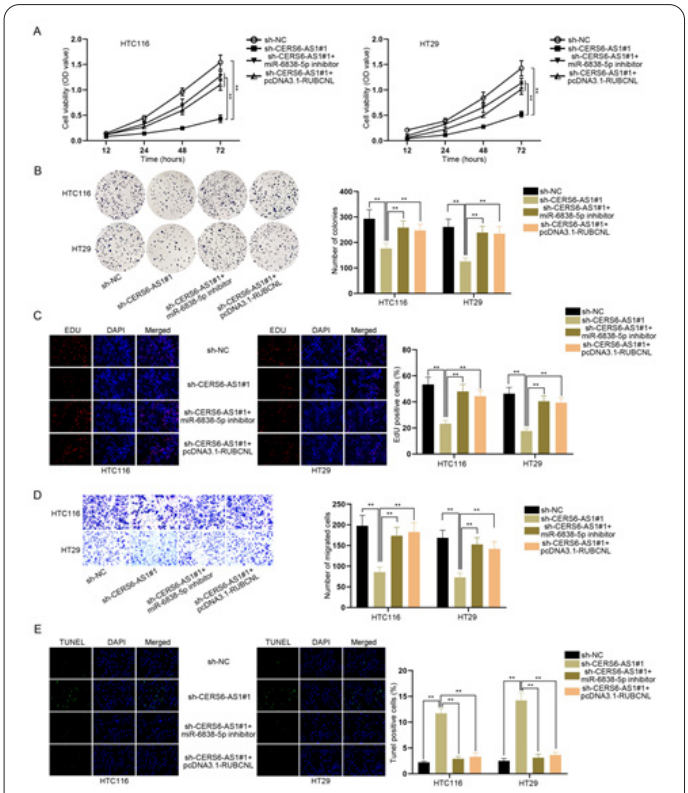

Figure 6. Inhibition of miR-6838-5p or overexpression of RUBCNL rescued the influences of CERS6-AS1 knockdown on malignant behaviors of CRC. (A)The results of CCK-8 showed cell viability in sh-NC, sh-CERS6-AS1#1, sh-CERS6-AS1#1+miR-6838-5p inhibitor and sh-CERS6-AS1#1+pcDNA3. 1-RUBCNL groups. (B-C)Colony formation and EdU assays were performed to observe cell proliferation. (D) Transwell assay was utilized to examine the migratory ability of CRC cells in different groups. (E) Cell apoptosis was assessed by TUNEL assay. ** P<0. 01.

proven to reset the altered metabolism and repressed cell growth in CRC (24). We also found that in CRC, MYC could serve as a transcription factor to activate the transcription of CERS6-AS1 and mediate the up-regulation of CERS6-AS1 in CRC.

The CeRNA network has been reported to participate in CRC initiation and process (25). It has been reported that ceRNA networks featuring lncRNA/miRNA/mRNA interactions are implicated in a wide range of biological progression in CRC(25). In general, there are several reports related to miRNA and cancer that need to be considered (26-31).

In the current research, we demonstrated that CERS6-AS1sequesteredmiR-6838-5p and modulated RUBCNL expression. Finally, a series of rescue experiments have unclosed that CERS6-AS1 affected CRC progression by targeting the miR-6838-5p/ RUBCNL axis.

We have identified that lncRNA CERS6-AS1 expression level in CRC cell lines was evidently up-regulated. Moreover, CERS6-AS1 was activated by MYC to sponge miR-6838-5p and then modulated RUBCNL expression level, thus affecting the biological function of CRC cells.

To our best knowledge, it is the first time to reveal the biological functions and potential mechanisms of CERS6-AS1 in CRC. Hence, our study provides help for the research of the potential mechanism of CRC. Though we have studied CERS6-AS1 functions in vivo and in vitro, there are still some limitations. For example, other potential mechanisms of CERS6-AS1 in CRC have not been studied. Therefore, the mechanisms of CERS6-AS1 underlying modulation of CRC cell phenotype need to be further explored.

Conflicts of interests

The authors state no conflicts of interest in this study.

Acknowledgements

We appreciate all the participants who provide support for the study.

Abbreviation

Colorectal cancer (CRC); long non-coding RNAs (lncRNAs); CERS6 antisense RNA 1 (CERS6-AS1); rubicon-like autophagy enhancer (RUBCNL); American Type Culture Collection (ATCC); Quantitative real-time PCR (qRT-PCR); Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLVRT); Cell Counting Kit-8 (CCK-8); 5-ethynyl-20-deoxyuridine (EdU); Transferase-mediated dUTP nick end labeling (TUNEL); Fluorescent in situ hybridization (FISH); RNA binding protein immunoprecipitation (RIP); Chromatin immunoprecipitation (ChIP); Digoxi-genin (DIG); sodium lauryl sulfate-polyacrylamide gels (SDS-PAGE); polyvinylidene difluoride (PVDF); Anti-Argonaute 2 (Ago 2); competing endogenous RNA (ceRNA).

References

1. Weitz J, Koch M, Debus J, Höhler T, Galle PR, Büchler MW. Colorectal cancer. Lancet. 2005 Jan 8-14;365(9454):153-65. doi: 10.1016/S0140-6736(05)17706-X. PMID: 15639298.
2. Švestka T, Krechler T. Preventing colorectal cancer. Casopis lekaru ceskych 2016; 155(2): 27-9.
3. Marković BB. Prevention and early detection of colorectal cancer. Acta Med Croatica. 2015 Nov;69(4):365-71. Croatian. PMID: 29083907.
4. Vatandoost N, Ghanbari J, Mojaver M, Avan A, Ghayour-Mobarhan M, Nedaeinia R, Salehi R. Early detection of colorectal cancer: from conventional methods to novel biomarkers. J Cancer Res Clin Oncol. 2016 Feb;142(2):341-51. doi: 10.1007/s00432-015-1928-z. Epub 2015 Feb 17. PMID: 25687380.
5. Haraldsdottir S, Einarsdottir HM, Smaradottir A, Gunnlaugsson A, Halfdanarson TR. Colorectal cancer - review. Laeknabladid 2014; 100(2): 75-82.
6. Jathar S, Kumar V, Srivastava J, Tripathi V. Technological Developments in lncRNA Biology. Adv Exp Med Biol. 2017;1008:283-323. doi: 10.1007/978-981-10-5203-3_10. PMID: 28815544.
7. Chan JJ, Tay Y. Noncoding RNA: RNA Regulatory Networks in Cancer. Int J Mol Sci 2018; 19(5).
8. Sun Q, Hao Q, Prasanth KV. Nuclear Long Noncoding RNAs: Key Regulators of Gene Expression. Trends in genetics: TIG 2018; 34(2): 142-57.
9. Wang J, Zhang X, Chen W, Hu X, Li J, Liu C. Regulatory roles of long noncoding RNAs implicated in cancer hallmarks. Int J Cancer 2020; 146(4): 906-16.
10. Cao HL, Liu ZJ, Huang PL, Yue YL, Xi JN. lncRNA-RMRP promotes proliferation, migration and invasion of bladder cancer via miR-206. Eur Rev Med Pharmacol Sci. 2019 Feb;23(3):1012-1021. doi: 10.26355/eurrev_201902_16988. PMID: 30779067.
11. Fu XM, Guo W, Li N, Liu HZ, Liu J, Qiu SQ, Zhang Q, Wang LC, Li F, Li CL. The expression and function of long noncoding RNA lncRNA-ATB in papillary thyroid cancer. Eur Rev Med Pharmacol Sci. 2017 Jul;21(14):3239-3246. PMID: 28770959.

12. Ding Z, Ye P, Yang X, Cai H. LncRNA FBXL19-AS1 promotes breast cancer cells proliferation and invasion via acting as a molecular sponge to miR-718. Biosci Rep 2019; 39(4).
13. Bian Z, Zhang J, Li M, Feng Y, Wang X, Zhang J, Yao S, Jin G, Du J, Han W, Yin Y, Huang S, Fei B, Zou J, Huang Z. LncRNA-FEZF1-AS1 Promotes Tumor Proliferation and Metastasis in Colorectal Cancer by Regulating PKM2 Signaling. Clin Cancer Res. 2018 Oct 1;24(19):4808-4819. doi: 10.1158/1078-0432.CCR-17-2967. Epub 2018 Jun 18. PMID: 29914894.
14. Xu Y, Zhang X, Hu X, Zhou W, Zhang P, Zhang J, et al. The effects of lncRNA MALAT1 on proliferation, invasion and migration in colorectal cancer through regulating SOX9. Mol Med (Cambridge, Mass) 2018; 24(1): 52.
15. Qi X, Zhang DH, Wu N, Xiao JH, Wang X, Ma W. ceRNA in cancer: possible functions and clinical implications. J Med Genet. 2015 Oct;52(10):710-8. doi: 10.1136/jmedgenet-2015-103334. Epub 2015 Sep 10. PMID: 26358722.
16. Wang X, Zhou J, Xu M, Yan Y, Huang L, Kuang Y, Liu Y, Li P, Zheng W, Liu H, Jia B. A 15-lncRNA signature predicts survival and functions as a ceRNA in patients with colorectal cancer. Cancer Manag Res. 2018 Nov 16;10:5799-5806. doi: 10.2147/CMAR.S178732. PMID: 30510449; PMCID: PMC6248371.
17. Zhou J, Lin J, Zhang H, Zhu F, Xie R. LncRNA HAND2-AS1 sponging miR-1275 suppresses colorectal cancer progression by upregulating KLF14. Biochem Biophys Res Commun. 2018 Sep 10;503(3):1848-1853. doi: 10.1016/j.bbrc.2018.07.125. Epub 2018 Aug 1. PMID: 30078677.
18. Wang Z, Jin J. LncRNA SLCO4A1-AS1 promotes colorectal cancer cell proliferation by enhancing autophagy via miR-508-3p/PARD3 axis. Aging 2019; 11(14): 4876-89.
19. Bao G, Huang J, Pan W, Li X, Zhou T. Long noncoding RNA CERS6-AS1 functions as a malignancy promoter in breast cancer by binding to IGF2BP3 to enhance the stability of CERS6 mRNA. Cancer Med 2020; 9(1): 278-89.
20. Yan L, Li K, Feng Z, Zhang Y, Han R, Ma J, Zhang J, Wu X, Liu H, Jiang Y, Zhang Y, Zhu Y. lncRNA CERS6-AS1 as ceRNA promote cell proliferation of breast cancer by sponging miR-125a-5p to upregulate BAP1 expression. Mol Carcinog. 2020 Oct;59(10):1199-1208. doi: 10.1002/mc.23249. Epub 2020 Aug 18. PMID: 32808708.
21. Zhou Y, Xu Z, Yu Y, Cao J, Qiao Y, Qiao H, Suo G. Comprehensive analysis of the lncRNA-associated ceRNA network identifies neuroinflammation biomarkers for Alzheimer's disease. Mol Omics. 2019 Dec 2;15(6):459-469. doi: 10.1039/c9mo00129h. PMID: 31755891.
22. Zhang J, Lou W. A Key mRNA-miRNA-lncRNA Competing Endogenous RNA Triple Sub-network Linked to Diagnosis and Prognosis of Hepatocellular Carcinoma. Front Oncol. 2020 Mar 17;10:340. doi: 10.3389/fonc.2020.00340. PMID: 32257949; PMCID: PMC7092636.
23. Fallah Y, Brundage J, Allegakoen P, Shajahan-Haq AN. MYC-Driven Pathways in Breast Cancer Subtypes. Biomolecules. 2017 Jul 11;7(3):53. doi: 10.3390/biom7030053. PMID: 28696357; PMCID: PMC5618234.
24. Satoh K, Yachida S, Sugimoto M, Oshima M, Nakagawa T, Akamoto S, Tabata S, Saitoh K, Kato K, Sato S, Igarashi K, Aizawa Y, Kajino-Sakamoto R, Kojima Y, Fujishita T, Enomoto A, Hirayama A, Ishikawa T, Taketo MM, Kushida Y, Haba R, Okano K, Tomita M, Suzuki Y, Fukuda S, Aoki M, Soga T. Global metabolic reprogramming of colorectal cancer occurs at adenoma stage and is induced by MYC. Proc Natl Acad Sci U S A. 2017 Sep 12;114(37): E7697-E7706. doi: 10.1073/pnas.1710366114. Epub 2017 Aug 28. PMID: 28847964; PMCID: PMC5604037.
25. Wang L, Cho KB, Li Y, Tao G, Xie Z, Guo B. Long Noncoding RNA (lncRNA)-Mediated Competing Endogenous RNA Networks Provide Novel Potential Biomarkers and Therapeutic Targets for Colorectal Cancer. Int J Mol Sci. 2019 Nov 16;20(22):5758. doi: 10.3390/ijms20225758. PMID: 31744051; PMCID: PMC6888455.
26. Azizi Dargahlou, S., Iriti, M., Pouresmaeil, M., Goh, L. P. W. MicroRNAs and their therapeutic and biomarker properties. Cell Mol Biomed Rep 2022; (): -. doi: 10.55705/cmbr.2022.365396.1085.
27. Ismaili A, Yari K, Moradi MT, Sohrabi M, Kahrizi D, Kazemi E, Souri Z. IL-1B (C+3954T) gene polymorphism and susceptibility to gastric cancer in the Iranian population. Asian Pac J Cancer Prev. 2015;16(2):841-4. doi: 10.7314/apjcp.2015.16.2.841. PMID: 25684535.
28. Kazemi E, Zargooshi J, Kaboudi M, Heidari P, Kahrizi D, Mahaki B, Mohammadian Y, Khazaei H, Ahmed K. A genome-wide association study to identify candidate genes for erectile dysfunction. Brief Bioinform. 2021 Jul 20;22(4):bbaa338. doi: 10.1093/bib/bbaa338. PMID: 33316063.
29. Kazemi E, Zargooshi J, Kaboudi M, Izadi F, Mohammadi Motlagh HR, Kahrizi D, Khazaie H, Mahaki B, Mohammadian Y. Investigation of gene expression and genetic simultaneous control associated with erectile dysfunction and diabetes. Cell Mol Biol (Noisy-le-grand). 2021 Nov 25;67(3):195-200. doi: 10.14715/cmb/2021.67.3.31. PMID: 34933709.
30. Kazemi E, Kahrizi D, Moradi MT, Sohrabi M, Yari K. Gastric Cancer and Helicobacter pylori: Impact of hopQII Gene. Cell Mol Biol (Noisy-le-grand). 2016 Feb 29;62(2):107-10. PMID: 26950460.
31. Kazemi E, Kahrizi D, Moradi MT, Sohrabi M, Amini S, Mousavi SA, Yari K. Association between Helicobacter pylori hopQI genotypes and human gastric cancer risk. Cell Mol Biol (Noisy-le-grand). 2016 Jan 11;62(1):6-9. PMID: 26828979.
32. Zhang BL, Fang T, Li S, Xiang La. Salmonella Shows Anti-Cancer Influences in Human Colon Cancer Cells by Promoting Apoptosis and G0/G1 Phase Cell Cycle Arrest via PI3K/AKT/mTOR Pathway Inhibition. J Biol Regul Homeost Agents. 2022; 36(6): 2203–2209.
33. Li Y, Zhang L, Li Y, Shi By, Zhang Yj. Limonin Exerts an Anti-Inflammatory Effect through the ERK/MEK Signaling Pathway in Colorectal Cancer. J Biol Regul Homeost Agents. 2022; 36(4): 973–984.