**Name of journal:** ***World Journal of Gastroenterology***

**Manuscript NO: 37130**

**Manuscript Type: ORIGINAL ARTICLE**

***Basic Study***

**Long noncoding RNA RP4 functions as a competing endogenous RNA through miR-7-5p sponge activity in colorectal cancer**

Liu ML *et al*. role of lncRNA RP4 in crc

Mu-Lin Liu, Qiao Zhang, Xiao Yuan, Long Jin, Li-Li Wang, Tao-Tao Fang, Wen-Bin Wang

**Mu-Lin Liu, Long Jin, Li-Li Wang, Tao-Tao Fang,** Department of Gastrointestinal Surgery, the First Affiliated Hospital of Bengbu Medical College; Bengbu 233004, Anhui Province, China

**Qiao Zhang,** Department of General Surgery, the First Affiliated Hospital of Xinxiang Medical University; Xinxiang 453100, Henan Province, China

**Xiao Yuan, Wen-Bin Wang,** Department of General Surgery, the Fourth Affiliated Hospital of Anhui Medical University; Hefei 230022, Anhui Province, China

**ORCID number:** Mu-Lin Liu(0000-0003-3930-678X); Qiao Zhang(0000-0001-5413-4547); Xiao Yuan(0000-0002-4299-7377); Long Jin (0000-0002-7765-4091); Li-Li Wang( 0000-0003-0439-8119); Tao-Tao Fang(0000-0002-1831-1937); Wen-Bin Wang(0000-0002-9023-6855).

**Author contributions:** Liu ML, Zhang Q and Yuan X contributed equally to this work; Liu ML and Zhang Q conceived the study and participated in its design and coordination; Yuan X drafted and revised the manuscript; Jin L helped in the statistical analysis; Wang LL and Fang TT performed the experiments; Wang WB conceived the study and revised the manuscript; all authors read and approved the final manuscript.

**supported by** Scientific Research Foundation of Anhui Education Department, No. KJ2017A219 to Liu ML; Scientific Research Foundation of Academic Leader of Anhui Province, No. 2016H105 to Liu ML; education talent Foundation of universities of Anhui Education Department, No. gxbjZD2016070 to Liu ML; National Natural Science Foundation of China, No. 81500373 to Wang WB; and Natural Science Foundation of Anhui Province, No. 1608085MH193 to Wang WB.

**Institutional review board statement:** The study was reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Bengbu Medical College.

**Institutional animal care and use committee statement:** All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of SHRM.

**Conflict-of-interest statement:** The authors declare that there is no conflict of interest related to this study.

**Data sharing statement:** The datasets supporting the conclusions of this article are included within the article.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Correspondence to: Wen-Bin Wang, PhD, Doctor, Professor,** Department of General Surgery, the Fourth Affiliated Hospital of Anhui Medical University, No. 372, Tunxi Road, Hefei 230022, Anhui Province, China. surdoctor@163.com

**Telephone:** +86-551-62879386

**Fax:** +86-552-3070260

**Received:** November 15, 2017

**Peer-review started:** November 16, 2017

**First decision:** December 20, 2017

**Revised:** December 26, 2017

**Accepted:** January 16, 2018

**Article in press:**

**Published online:**

**Abstract**

***AIM***

To investigate the role oflong noncoding (lnc)RNA RP4 in colorectal cancer.

***Methods***

Lentivirus-mediated lncRNA RP4 overexpression and knockdown were performed in the SW480 colorectal cancer cell line. Cell proliferation, tumor growth, and early apoptosis were evaluated by a cell counting kit-8 assay, an *in vivo* xenograft tumor model, and annexin V/propidium iodide staining, respectively. Analysis of the lncRNA RP4 mechanism involved assessment of its expression association with miR-7-5p, and the *SH3GLB1* gene. Western blotting analyses of the effects of lncRNA RP4 on the autophagy-mediated cell death pathway and phosphatidylinositol-3-kinase (PI3K)/Akt signaling were evaluated by western blotting.

***Results***

Cell proliferation, tumor growth, and early apoptosis in SW480 cells were negatively regulated bylncRNA RP4. Functional experiments indicated that lncRNA RP4 directly upregulates *SH3GLB1* expression by acting as a competing endogenous RNA (ceRNA) for miR-7-5p. This interaction led to activation of the autophagy-mediated cell death pathway and de-repression of PI3K and Akt phosphorylation in colorectal cancer cells in vitro.

***Conclusion***

Our results demonstrated that lncRNA RP4 is a ceRNA that plays an important role in the pathogenesis of colorectal cancer, and could be a potential therapeutic target for colorectal cancer treatment.

**Key words:** Colorectal cancer; long noncoding RNA RP4; SH3GLB1; miR-7-5p; competing endogenous RNA

**© The Author(s) 2018.** Published by Baishideng Publishing Group Inc. All rights reserved.

**C****ore tip:** In the present study, we investigated the role of long noncoding RNAs (lncRNAs) RP4 in colorectal cancer using an in vitro cell model and an in vivo xenograft model. Mechanistic analysis suggested that lncRNA RP4 functions in colorectal cancer pathogenesis as a competing endogenous RNA that regulates SH3GLB1 expression by acting as a sponge for miR-7-5p. It could also serve as a potential therapeutic target for colorectal cancer treatment.

Liu ML, Zhang Q, Yuan X, Jin L, Wang LL, Fang TT, Wang WB. Long noncoding RNA RP4 functions as a competing endogenous RNA through miR-7-5p sponge activity in colorectal cancer. *World J Gastroenterol* 2018; In press

**Introduction**

Colorectal cancer is the fourth most common cancer and the fifth most common cause of cancer-related death in China, with an estimated 331300 newly diagnosed patients and 159300 deaths in 2012[1]. Surgical resection followed by adjuvant chemotherapy is the most commonly used strategy for colorectal cancer management. However, although the overall 5-year survival rate of colorectal cancer has improved to 65%, the 5-year survival rate was only 15% in patients presenting with distant metastasis[2], reflecting the poor treatment response in some patients. Therefore, it is necessary to identify effective therapeutic targets to improve treatment and prognosis.

Long noncoding RNAs (lncRNAs), > 200 nucleotides in length, are a recently discovered novel class of genes with regulatory functions but lacking a protein-coding ability. Several studies have identified important roles for lncRNAs in a wide range of cellular processes, including X chromosome inactivation, splicing, imprinting, epigenetic control, and gene transcription regulation[3-5]. Moreover, the dysregulated expression of lncRNAs is present in various human diseases, especially in cancers including breast cancer, lung cancer, gastric cancer, and colorectal cancer[6-8]. Indeed, several recent pieces of evidence suggest that lncRNAs are involved in the development and progression of human colorectal cancer and may serve as novel therapeutic targets[9-11]. However, the role of lncRNAs in colorectal cancer is largely unknown.

The dysregulation of lncRNA RP4 has previously been shown by expression profile analyses of a transcriptome microarray, so the present study investigated the role oflncRNA RP4 in colorectal cancer using an *in vitro* cell model and an *in vivo* xenograft model. Mechanistic analysis suggested that lncRNA RP4 functions in colorectal cancer pathogenesis as a competing endogenous RNA (ceRNA) that regulates *SH3GLB1*expression by acting as a sponge for the micro (mi)RNA miR-7-5p. It could also serve as a potential therapeutic target for colorectal cancer treatment.

**Materials and methods**

***Ethics statement***

Investigation has been conducted in accordance with the ethical standards and according to the Declaration of Helsinki and according to national and international guidelines and has been approved by the authors' institutional review board, which adheres to generally accepted international guidelines for animal experimentation.

***Cell culture***

The human colorectal cancer cell line SW480 was obtained from American Type Culture Collection (ATCC). Cells were maintained as monolayers in cell culture flasks with RPMI1640 medium containing 10% (v/v) fetal bovine serum and 1% antibiotics. They were cultured at 37°C in a humidified atmosphere with 5% CO2. All cell culture media and additives were purchased from Invitrogen (CA, United States).

***Lentiviral short hairpin (sh)RNA particles***

Recombinant lentiviral particles expressing lncRNA RP4 or lncRNA RP4 small interfering (si)RNA were obtained from GenePharm Co., Ltd. (Shanghai, China). Cells were grown to approximately 40% confluency and infected with lentiviral particles in complete medium for 48 h. To increase the infection efficiency, cells were co-treated with the cationic polymer polybrene (8 μg/ml in water). Neither shRNA nor polybrene affected cell viability. siRNA and shRNA had no off-target effects, and did not affect cell adherence, shape, or viability at the indicated multiplicity of infection.

***Real-time quantitative reverse transcription***

Total cellular RNA was extracted from SW480 cells using TRIzol reagent (Invitrogen). RT-PCR was carried out using a One Step SYBR® PrimeScript™ RT-PCR kit (Takara, Dalian, China) and an iQ5 Real-time PCR Detection system (Bio-Rad, Hercules, CA, United States) for evaluation of the expression of lncRNA RP4. The miRNA miR-7-5p was obtained using the PureLink™ miRNA Isolation Kit (Invitrogen), and the quantification of miRNA expression was performed by a TaqMan MicroRNA Assay Kit (Applied Biosystems, Foster City, CA, United States). The expression of β-actin and U6 snRNA genes was assessed simultaneously in all samples as an internal control for lncRNA/mRNA and miRNA expression. Relative gene expression was determined by the 2–ΔΔCT method[12]. Oligonucleotide primers specific for lncRNA RP4, *SH3GLB1*, and β-actin are listed in Table 1.

***Western blotting***

Cells were lysed in RIPA buffer, centrifuged at high speed, then underwent protein quantification using a bicinchoninic acid assay. Cellular proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membranes. After blocking, the membranes were incubated with anti-total- or -phosphor-PI3K, phospho-Akt, LC3A/B, Bax, and caspase 3 monoclonal primary antibodies (Cell Signaling Technology, Cambridge, MA, United States). β-actin (Santa Cruz Biotechnology, Santa Cruz, CA, United States) was used as the loading control. Appropriate horseradish peroxidase-conjugated secondary antibodies were applied to detect labeled proteins. The protein bands were developed with SuperSignal Ultra Chemiluminescent Substrate (Pierce, Rockford, IL, United States) on X-ray films (Kodak, Tokyo, Japan).

***Cell proliferation***

SW480 cells (3 × 103 cells) were seeded in 96-well plates in complete medium and infected with lncRNA RP4 or lncRNA RP4 siRNA or control lentivirus particles. Two days later, cell proliferation was evaluated by the cell counting kit-8 method according to the manufacturer’s instructions using a microplate reader (Molecular Devices, Sunnyvale, CA, United States) to measure the absorbance.

***Nude mice model of ectopic tumors***

Athymic nude (nu/nu) mice at 6 weeks old were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. Tumors were generated by the subcutaneous injection of 2 × 106 SW480 cells infected with lncRNA RP4 or lncRNA RP4 siRNA or control lentivirus particles and suspended in 50 µl PBS into the dorsal region near the thigh. Mice were then weighed and assessed for tumor size every 7 wk by measuring the tumor length and width.

***Cell apoptosis analysis***

SW480 cells (3 × 105 cells) were seeded in 6-well plates in complete medium and infected with lncRNA RP4 or lncRNA RP4 siRNA or control lentivirus particles. Two days later, cell proliferation was evaluated by flow cytometry (FACScalibur; BD Biosciences, CA, United States) after annexin V/propidium iodide staining (Beyotime institution, Nantong, China).

***Statistical analysis***

All statistical analyses were carried out using SPSS v18 software (SPSS, Chicago, IL). Data are presented as the mean ± SD. The Student’s *t*-test or one-way analysis of variance were used to examine differences between two or multiple groups. Correlation analyses of the expression levels of lncRNA RP4, *SH3GLB1*, and miR-7-5p were performed using Pearson’s correlation coefficient. A *p*-value < 0.05 was considered statistically significant.

**Results**

***lncRNA RP4 regulates proliferation, tumor growth, and early apoptosis in colorectal cancer cells***

To investigate the role of lncRNA RP4 in the pathogenesis of colorectal cancer, we performed lentivirus-mediated overexpression and knockdown. As shown in Figure 1A, SW480 cell proliferation was negatively regulated by lncRNA RP4 overexpression and knockdown. While early apoptosis was positively regulated by lncRNA RP4 overexpression and knockdown (Figure 1C and D). These results suggested that lncRNA RP4 exerts a negative regulatory role in colorectal cancer proliferation and a positive regulatory role in early apoptosis in colorectal cancer.

***lncRNA RP4 inhibits the growth of colorectal cancer on mice***

Compared with control group, colorector cancer with lncRNA RP4 siRNA showed a biger volume. While there was a smaller volume in the group with lncRNA RP4 overexpression (Figure 1B). Consistent with the results in cell line, the results in vivo also suggested that lncRNA RP4 plays an inhibited role in colorectal cell growth.

***lncRNA RP4 inhibits the growth of colorectal cancer cells by the regulation of SH3GLB1***

To explore the mechanism of lncRNA RP4-mediated effects in colorectal cancer cells, we examined *SH3GLB1* expression in SW480 cells following lncRNA RP4 overexpression and knockdown. LncRNA RP4 was found to positively regulate *SH3GLB1* expression, and correlation analyses further confirmed the existence of a significant correlation between lncRNA RP4 and *SH3GLB1* expression (Figure 2).

***lncRNA RP4 functions as a miR-7-5p decoy in the regulation of SH3GLB1***

Because no direct interaction exists between lncRNA RP4 and *SH3GLB1*, we further analyzed the potential functional mechanism by the introduction of miRNA. lncRNAs were recently reported to act as decoys that sequester miRNAs and prevent them from binding to targets, hence modulating many functional mRNA targets through translation. Bioinformatics analysis (webserver lnCeDB <http://gyanxet-beta.com/lncedb/>), predicted potential interactions between lncRNA RP4 and miR-7-5p ([Figure 3A](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4873821/figure/f3/), which was confirmed by correlation analysis (Figure 3B and C). We also observed a positive regulatory effect of miR-7-5p on cell proliferation via the negative regulation of *SH3GLB1* (Figure 3D and E). These results suggested that lncRNA RP4 functions as an miR-7-5p decoy in colorectal cancer cells.

***Involvement of the autophagy-mediated cell death pathway and PI3K/Akt signaling pathway in lncRNA-RP4 mediated effects in colorectal cancer cells***

According to previous findings[13,14], autophagy-mediated cell death is involved in the early apoptosis of cancer, while the PI3K/Akt signaling pathway plays a role in cancer cell proliferation and growth[15,16]. Analysis of the effects of lncRNA-RP4 on intracellular signaling revealed that lncRNA-RP4 overexpression and knockdown respectively upregulated and downregulated expression levels of the autophagy marker LC3 and apoptosis-related molecules Bax and caspase 3 (Figure 4A). We also observed the negative regulation of PI3K and Akt phosphorylation by lncRNA-RP4 in colorectal cancer cells (Figure 4B). Taken together, we propose a schematic whereby lncRNA RP4 functions as a decoy that competitively binds miR-7-5p, upregulating the specific repressor *SH3GLB1*, activating autophagy-mediated cell death, and inhibiting PI3K/Akt signaling, thereby suppressing colorectal carcinogenesis (Figure 4C).

**Discussion**

Noncoding regions account for more than 90% of the entire human genome, and are thought to play a critical role in the regulation of physiological function given that only 9% of human genes are protein-coding. As a representative of noncoding regions, approximately 18% of lncRNAs are associated with human tumors and have been shown to act as major contributors in the development and progression of human cancers[17]. Multiple mechanisms have been suggested for the regulatory role of lncRNAs in physiological functions, including trans- and cis-regulatory mechanisms. In a trans-regulatory mechanism, lncRNAs (such as HOTAIR) could affect the transcription of specific genes through their interaction with chromatin-remodeling complexes and complex recruitment to genomic DNA sequences[18]. Some lncRNAs (such as lincRNA-21) also act as cis-regulators by exerting their function on nearby transcripts[19]. Growing evidence has shown that lncRNAs may act as ceRNAs *via* their miRNA response elements for specific miRNA targets, blocking the target binding ability of a single miRNA or multiple miRNAs[20,21]. Several lncRNAs have been suggested to function as ceRNAs, including PTENP1[22], H19[23], and CCAT1[24].

In the present study, we investigated the potential role of lncRNA RP4 as a ceRNA of *SH3GLB1* that competes for miRNA-7-5p binding sites, thereby regulating the expression of *SH3GLB1* mRNA targeted by miRNA-7-5p. The overexpression of lncRNA RP4 inhibited colorectal cancer cell proliferation and tumor growth both *in vitro* and *in vivo*, and increased early apoptosis. These findings suggest that lncRNA RP4 plays a critical role in the modulation of colorectal cancer progression.

To further elucidate the role of lncRNA RP4 in colorectal cancer, we analyzed its regulatory mechanism as a ceRNA by bioinformatics analysis and experimental verification. qRT-PCR analysis showed that lncRNA RP4 overexpression downregulated miR-7-5p expression in colorectal cancer cells, while an inverse correlation was detected between lncRNA RP4 and miR-7-5p expression. Additional functional experiments confirmed that miR-7-5p overexpression promoted cell proliferation, while an inverse correlation was detected between miR-7-5p and *SH3GLB1* expression. Consistent with these findings, miR-7-5p has been found to affect cell proliferation, anchorage-independent growth, migration and invasion, apoptosis, and chemosensitivity by targeting specific oncogenic genes in various types of tumor[25-27].

SH3GLB1, a membrane curvature-inducing protein, interacts with BECN1 though UVRAG and regulates the post-Golgi trafficking of membrane-integrated ATG9A during autophagy[28]. In the present study, we found that lncRNA RP4 overexpression upregulated autophagy. Recently, Takahashi *et al*[29] reported that SH3GLB1 is a haploinsufficient tumor suppressor that functions to prevent the acquisition of apoptosis resistance and malignant transformation during *Myc*-driven lymphomagenesis. Our data supported the tumor suppressor role of SH3GLB1 in colorectal cancer. During tumor development and progression, protein interactions between SH3GLB1 and BAX resulted in the activation of caspase 3, thereby inducing apoptosis[30]. Similarly, we showed that lncRP4-induced SH3GLB1 upregulation increased levels of BAX and caspase 3 in colorectal cancer cells.

Previous studies observed that dysregulated PI3K/Akt signaling in human colorectal cancer is associated with the growth and proliferation pattern of cancer cells[15,16], while the PI3K/Akt pathway negatively regulates autophagy[31,32]. Consistent with this, we detected reduced PI3K and Akt phosphorylation in lncRP4-overexpressing colorectal cancer cells.

The present study has a number of limitations. First, because of a lack of colorectal cancer tissue, we could not evaluate the expression pattern of lncRNA RP4, miR-7-5p, or *SH3GLB1* in carcinoma tissues, so were unable to elucidate the clinical significance of lncRP4 in colorectal cancer. The collection of more colorectal cancer tissue will be necessary to overcome this. Second, we did not use small inhibitors of different signaling pathways, yet it is conceivable that the mechanism of lncRNA RP4 involves multiple modalities.

Taken together, our results demonstrate that lncRNA RP4 plays an important role in the progression of human colorectal cancer by functioning as a ceRNA to regulate the expression of *SH3GLB1* through miR-7-5p sponge activity. The pleiotropic effects of lncRNA RP4 on colorectal cancer pathogenesis suggest that is has the potential to be a therapeutic target for colorectal cancer.

**Article Highlights**

***Research background***

Colorectal cancer is the fourth most common cancer and the fifth most common cause of cancer-related death in China. Surgical resection followed by adjuvant chemotherapy, the most commonly used strategy has poor treatment response in some patients. Therefore, it is necessary to identify effective therapeutic targets to improve treatment and prognosis.

***Research motivation***

Long noncoding RNAs (lncRNAs), which may serve as novel therapeutic targets, is involved in the development and progression of human colorectal cancer. In our previous study, lncRNA RP4 was found to be dysregulated in colorectal cancer via microarray analysis. This indicated that this lncRNA may play an important role in colorectal cancer. Thus, in the present study, lncRNA RP4 was investigated to find out its role in colorectal cancer progress through an *in vitro* cell model and an *in vivo* xenograft model. Besides, the possible mechanisms in the regulation of lncRNA RP4 had not been well described.

***Research objectives***

To investigate the role oflong noncoding (lnc)RNA RP4 in colorectal cancer, and to find out the possible mechanisms of the regulation.

***Research methods***

Cell counting kit-8 assay in vitro and xenograft tumor model in vivo were performed to evaluate the role of lncRNA RP4 in the regulation of proliferation. Annexin V/propidium iodide staining was performed to detect the role of lncRNA RP4 in apoptosis. QPCR and Western blot were performed to identify the relationship between lncRNA RP4 and SH3GLB1. And then Western blot was done to analyse PI3K/Akt signaling pathway and autophagy pathway in the regulation.

***Research results***

Both Cell counting kit-8 assay in vitro and xenograft tumor model in vivo were found that lncRNA RP4 could inhibit the proliferation and growth of colorectal cancer cell. lncRNA RP4 could promote early apoptosis. lncRNA RP4 was found to positively regulate SH3GLB1 expression, and correlation analyses further confirmed the existence of a significant correlation between lncRNA RP4 and SH3GLB1 expression. We also observed a positive regulatory effect of miR-7-5p on cell proliferation via the negative regulation of SH3GLB1.

***Research conclusions***

Our results demonstrate that lncRNA RP4 plays an important role in the progression of human colorectal cancer by functioning as a ceRNA to regulate the expression of SH3GLB1 through miR-7-5p sponge activity. The pleiotropic effects of lncRNA RP4 on colorectal cancer pathogenesis suggest that is has the potential to be a therapeutic target for colorectal cancer.

***Research perspectives***

This study suggests that the lncRNA intervention may be a promising treatment strategy for colorectal cancer. The future study might focus on the specific regulatory role of lncRNA RP4 in colorectal cancer *in vivo*, and the therapeutic effect of lncRNA RP4 needs to be validated in clinical practice.

**References**

1 **Chen W**, Zheng R, Zuo T, Zeng H, Zhang S, He J. National cancer incidence and mortality in China, 2012. *Chin J Cancer Res* 2016; **28**: 1-11 [PMID: 27041922 DOI: 10.3978/j.issn.1000-9604.2016.02.08]

2 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin* 2016; **66**: 7-30 [PMID: 26742998 DOI: 10.3322/caac.21332]

3 **Yue M**, Charles Richard JL, Ogawa Y. Dynamic interplay and function of multiple noncoding genes governing X chromosome inactivation. *Biochim Biophys Acta* 2016; **1859**: 112-120 [PMID: 26260844 DOI: 10.1016/j.bbagrm.2015.07.015]

4 **Butler AA**, Webb WM, Lubin FD. Regulatory RNAs and control of epigenetic mechanisms: expectations for cognition and cognitive dysfunction. *Epigenomics* 2016; **8**: 135-151 [PMID: 26366811 DOI: 10.2217/epi.15.79]

5 **Kanduri C**. Long noncoding RNAs: Lessons from genomic imprinting. *Biochim Biophys Acta* 2016; **1859**: 102-111 [PMID: 26004516 DOI: 10.1016/j.bbagrm.2015.05.006]

6 **Tordonato C**, Di Fiore PP, Nicassio F. The role of non-coding RNAs in the regulation of stem cells and progenitors in the normal mammary gland and in breast tumors. *Front Genet* 2015; **6**: 72 [PMID: 25774169 DOI: 10.3389/fgene.2015.00072]

7 **Prensner JR**, Iyer MK, Balbin OA, Dhanasekaran SM, Cao Q, Brenner JC, Laxman B, Asangani IA, Grasso CS, Kominsky HD, Cao X, Jing X, Wang X, Siddiqui J, Wei JT, Robinson D, Iyer HK, Palanisamy N, Maher CA, Chinnaiyan AM. Transcriptome sequencing across a prostate cancer cohort identifies PCAT-1, an unannotated lincRNA implicated in disease progression. *Nat Biotechnol* 2011; **29**: 742-749 [PMID: 21804560 DOI: 10.1038/nbt.1914]

8 **Li W**, Zheng J, Deng J, You Y, Wu H, Li N, Lu J, Zhou Y. Increased levels of the long intergenic non-protein coding RNA POU3F3 promote DNA methylation in esophageal squamous cell carcinoma cells. *Gastroenterology* 2014; **146**: 1714-26.e5 [PMID: 24631494 DOI: 10.1053/j.gastro.2014.03.002]

9 **Yin D**, He X, Zhang E, Kong R, De W, Zhang Z. Long noncoding RNA GAS5 affects cell proliferation and predicts a poor prognosis in patients with colorectal cancer. *Med Oncol* 2014; **31**: 253 [PMID: 25326054 DOI: 10.1007/s12032-014-0253-8]

10 **Han Y**, Yang YN, Yuan HH, Zhang TT, Sui H, Wei XL, Liu L, Huang P, Zhang WJ, Bai YX. UCA1, a long non-coding RNA up-regulated in colorectal cancer influences cell proliferation, apoptosis and cell cycle distribution. *Pathology* 2014; **46**: 396-401 [PMID: 24977734 DOI: 10.1097/PAT.0000000000000125]

11 **Ding J**, Lu B, Wang J, Wang J, Shi Y, Lian Y, Zhu Y, Wang J, Fan Y, Wang Z, De W, Wang K. Long non-coding RNA Loc554202 induces apoptosis in colorectal cancer cells via the caspase cleavage cascades. *J Exp Clin Cancer Res* 2015; **34**: 100 [PMID: 26362196 DOI: 10.1186/s13046-015-0217-7]

12 **Ji Y**, Strawn TL, Grunz EA, Stevenson MJ, Lohman AW, Lawrence DA, Fay WP. Multifaceted role of plasminogen activator inhibitor-1 in regulating early remodeling of vein bypass grafts. *Arterioscler Thromb Vasc Biol* 2011; **31**: 1781-1787 [PMID: 21571686 DOI: 10.1161/ATVBAHA.111.228767]

13 **Amantini C,** Morelli MB, Farfariello V, et al. Different effects of sunitinib, sorafenib, and pazopanib on inducing cancer cell death: The role of autophagy J]. Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 2013,31(6\_suppl):270. PMID: 28136738 DOI: 10.1200/jco.2013.31.6\_suppl.270]

14 **Fulda S**. Autophagy in Cancer Therapy. *Front Oncol* 2017; **7**: 128 [PMID: 28674677 DOI: 10.3389/fonc.2017.00128]

15 **Gomez-Pinillos A,** Shah P, Lavilla C, et al. Effect of inhibition of the pi3k/akt/mtor pathway on ar splicing and downstream targets J]. Journal of clinical oncology: official journal of the American Society of Clinical Oncology, 2013,31(6\_suppl):101. PMID: 28137014 DOI: 10.1200/jco.2013.31.6\_suppl.101]

16 **Jeon YW**, Ahn YE, Chung WS, Choi HJ, Suh YJ. Synergistic effect between celecoxib and luteolin is dependent on estrogen receptor in human breast cancer cells. *Tumour Biol* 2015; **36**: 6349-6359 [PMID: 25851346 DOI: 10.1007/s13277-015-3322-5]

17 **Khachane AN**, Harrison PM. Mining mammalian transcript data for functional long non-coding RNAs. *PLoS One* 2010; **5**: e10316 [PMID: 20428234 DOI: 10.1371/journal.pone.0010316]

18 **Bhan A**, Mandal SS. LncRNA HOTAIR: A master regulator of chromatin dynamics and cancer. *Biochim Biophys Acta* 2015; **1856**: 151-164 [PMID: 26208723 DOI: 10.1016/j.bbcan.2015.07.001]

19 **Dimitrova N**, Zamudio JR, Jong RM, Soukup D, Resnick R, Sarma K, Ward AJ, Raj A, Lee JT, Sharp PA, Jacks T. LincRNA-p21 activates p21 in cis to promote Polycomb target gene expression and to enforce the G1/S checkpoint. *Mol Cell* 2014; **54**: 777-790 [PMID: 24857549 DOI: 10.1016/j.molcel.2014.04.025]

20 **Wang K**, Long B, Zhou LY, Liu F, Zhou QY, Liu CY, Fan YY, Li PF. CARL lncRNA inhibits anoxia-induced mitochondrial fission and apoptosis in cardiomyocytes by impairing miR-539-dependent PHB2 downregulation. *Nat Commun* 2014; **5**: 3596 [PMID: 24710105 DOI: 10.1038/ncomms4596]

21 **Tay Y**, Rinn J, Pandolfi PP. The multilayered complexity of ceRNA crosstalk and competition. *Nature* 2014; **505**: 344-352 [PMID: 24429633 DOI: 10.1038/nature12986]

22 **Yu G**, Yao W, Gumireddy K, Li A, Wang J, Xiao W, Chen K, Xiao H, Li H, Tang K, Ye Z, Huang Q, Xu H. Pseudogene PTENP1 functions as a competing endogenous RNA to suppress clear-cell renal cell carcinoma progression. *Mol Cancer Ther* 2014; **13**: 3086-3097 [PMID: 25249556 DOI: 10.1158/1535-7163.MCT-14-0245]

23 **Kallen AN**, Zhou XB, Xu J, Qiao C, Ma J, Yan L, Lu L, Liu C, Yi JS, Zhang H, Min W, Bennett AM, Gregory RI, Ding Y, Huang Y. The imprinted H19 lncRNA antagonizes let-7 microRNAs. *Mol Cell* 2013; **52**: 101-112 [PMID: 24055342 DOI: 10.1016/j.molcel.2013.08.027]

24 **Ma MZ**, Chu BF, Zhang Y, Weng MZ, Qin YY, Gong W, Quan ZW. Long non-coding RNA CCAT1 promotes gallbladder cancer development via negative modulation of miRNA-218-5p. *Cell Death Dis* 2015; **6**: e1583 [PMID: 25569100 DOI: 10.1038/cddis.2014.541]

25 **Kalinowski FC**, Brown RA, Ganda C, Giles KM, Epis MR, Horsham J, Leedman PJ. microRNA-7: a tumor suppressor miRNA with therapeutic potential. *Int J Biochem Cell Biol* 2014; **54**: 312-317 [PMID: 24907395 DOI: 10.1016/j.biocel.2014.05.040]

26 **Kalinowski FC**, Giles KM, Candy PA, Ali A, Ganda C, Epis MR, Webster RJ, Leedman PJ. Regulation of epidermal growth factor receptor signaling and erlotinib sensitivity in head and neck cancer cells by miR-7. *PLoS One* 2012; **7**: e47067 [PMID: 23115635 DOI: 10.1371/journal.pone.0047067]

27 **Giles KM**, Brown RA, Ganda C, Podgorny MJ, Candy PA, Wintle LC, Richardson KL, Kalinowski FC, Stuart LM, Epis MR, Haass NK, Herlyn M, Leedman PJ. microRNA-7-5p inhibits melanoma cell proliferation and metastasis by suppressing RelA/NF-κB. *Oncotarget* 2016; **7**: 31663-31680 [PMID: 27203220 DOI: 10.18632/oncotarget.9421]

28 **Takahashi Y**, Young MM, Serfass JM, Hori T, Wang HG. Sh3glb1/Bif-1 and mitophagy: acquisition of apoptosis resistance during Myc-driven lymphomagenesis. *Autophagy* 2013; **9**: 1107-1109 [PMID: 23680845 DOI: 10.4161/auto.24817]

29 **Takahashi Y**, Hori T, Cooper TK, Liao J, Desai N, Serfass JM, Young MM, Park S, Izu Y, Wang HG. Bif-1 haploinsufficiency promotes chromosomal instability and accelerates Myc-driven lymphomagenesis via suppression of mitophagy. *Blood* 2013; **121**: 1622-1632 [PMID: 23287860 DOI: 10.1182/blood-2012-10-459826]

30 **Fino KK**, Yang L, Silveyra P, Hu S, Umstead TM, DiAngelo S, Halstead ES, Cooper TK, Abraham T, Takahashi Y, Zhou Z, Wang HG, Chroneos ZC. SH3GLB2/endophilin B2 regulates lung homeostasis and recovery from severe influenza A virus infection. *Sci Rep* 2017; **7**: 7262 [PMID: 28779131 DOI: 10.1038/s41598-017-07724-5]

31 **Mans LA**, Querol Cano L, van Pelt J, Giardoglou P, Keune WJ, Haramis AG. The tumor suppressor LKB1 regulates starvation-induced autophagy under systemic metabolic stress. *Sci Rep* 2017; **7**: 7327 [PMID: 28779098 DOI: 10.1038/s41598-017-07116-9]

32 **Wang S**, Li J, Du Y, Xu Y, Wang Y, Zhang Z, Xu Z, Zeng Y, Mao X, Cao B. The Class I PI3K inhibitor S14161 induces autophagy in malignant blood cells by modulating the Beclin 1/Vps34 complex. *J Pharmacol Sci* 2017; **134**: 197-202 [PMID: 28779993 DOI: 10.1016/j.jphs.2017.07.001]

**P-Reviewer:** Luchini C **S-Editor:** Gong ZM

**L-Editor:** **E-Editor:**

**Specialty type:** Gastroenterology and hepatology

**Country of origin:** China

**Peer-review report classification**

Grade A (Excellent): 0

Grade B (Very good): 0

Grade C (Good): C

Grade D (Fair): 0

Grade E (Poor): 0

C:\Users\USER\Desktop\lnc-RP4\投稿\final\Figure1 modified.tif

**Figure 1 LncRNA RP4 regulates proliferation, tumor growth, and early apoptosis in colorectal cancer cells.** Lentivirus-mediated lncRNA RP4 overexpression and knockdown were performed in the colorectal cancer line SW480, and cell proliferation, tumor growth, and early apoptosis were examined. A: Cell proliferation was examined by the CCK-8 assay, and lncRNA RP4 overexpression and knockdown were shown to decrease and increase cell proliferation, respectively. B: Tumor growth was evaluated by tumor volume change, and lncRNA RP4 overexpression and knockdown were shown to significantly decrease and increase tumor volume, respectively, at weeks 14, 21, and 28. C: Flow cytometry assessment of early apoptosis. LncRNA RP4 overexpression and knockdown increased and decreased early apoptosis, respectively, in colorectal cancer. D: Early apoptosis quantification, e*p* < 0.001 for the between-group comparison.

C:\Users\USER\Desktop\lnc-RP4\投稿\final\修回\Figure2 modified.tif

**Figure 2 lncRNA RP4 affects the expression of *SH3GLB1* in colorectal cancer cells.** Lentivirus-mediated lncRNA RP4 overexpression and knockdown were performed in SW480 cells, and *SH3GLB1* expression was evaluated by real-time quantitative PCR, followed by association analyses between *SH3GLB1* and lncRNA RP4 levels. A: LncRNA RP4 overexpression and knockdown respectively increased and decreased *SH3GLB1* expression in SW480 cells. B: Correlation analyses revealed a linear association between the expression of *SH3GLB1* and lncRNA RP4, with an *r2* value of 0.827. a*p* < 0.05 and c*p* < 0.001 for between-group comparisons.

**C:\Users\USER\Desktop\lnc-RP4\投稿\final\修回\Figure3  modified.tif**

**Figure 3 lncRNA RP4 functions as an miR-7-5p decoy in colorectal cancer cells.** A: The predicted positions of miR-7-5p binding sites on the *SH3GLB1* and lncRNA RP4 transcript. B: LncRNA RP4 overexpression and knockdown respectively decreased and increased the expression of miR-7-5p in SW480 cells. C: Correlation analyses revealed a linear association between the expression of lncRNA RP4 and miR-7-5p, with an *r2* value of 0.482. D: SW480 cells were transfected with an miR-7-5p mimic and inhibitor, and cell proliferation was evaluated by the CCK-8 assay. miR-7-5p overexpression and knockdown increased and decreased cell proliferation, respectively. E. Real-time quantitative PCR showed that miR-7-5p overexpression and knockdown respectively decreased and increased *SH3GLB1* expression level in SW480 colorectal cancer cells. a*p* < 0.05, b*p* < 0.01, and c*p* < 0.001 for between-group comparisons.

C:\Users\USER\Desktop\lnc-RP4\投稿\final\修回\Figure4 modified.tif

**Figure 4 Involvement of the autophagy-mediated cell death pathway and PI3K/Akt signaling pathway in lncRNA RP4-mediated effects in colorectal cancer cells.** A: LncRNA RP4 overexpression and knockdown respectively decreased and increased expression of the autophagy marker LC3, and apoptosis-related proteins Bax and caspase 3 in SW480 cells, suggesting that it positively regulates autophagy-mediated cell death in colorectal cancer cells. B: LncRNA RP4 overexpression and knockdown respectively decreased and increased PI3K and Akt phosphorylation in SW480 cells, indicating that it negatively regulates PI3K/Akt in colorectal cancer cells. C: Schematic of lncRNA RP4 functioning as a decoy by competitively binding miR-7-5p, upregulating the specific repressor SH3GLB1, activating autophagy-mediated cell death, and inhibiting the PI3K/Akt signaling pathway, thereby suppressing colorectal carcinogenesis. a*p* < 0.05, b*p* < 0.01, and c*p* < 0.001 for between-group comparisons.

**Table 1 Primer sequence of the genes**

|  |  |  |
| --- | --- | --- |
| Genes | Forward primer (5’-3’) | Reverse primer (5’-3’) |
| ENST00000565575 | ATCCGTTCCAAATCCTGTCGT | TTCAAGCAGAGGCTGTATCGTG |
| *SH3GLB1* | CGCTGTCTGAATGACTTTGT | CCTTTCTGCTGCCACTACAC |
| *β-actin* | GTGGCCGAGGACTTTGATTG | CCTGTAACAACGCATCTCATATT |