

Original Article

Biological functions of Circular RNA_LARP4/ Upstream frameshift 1 in development of gastric cancer

Jing Zhang^{1, #, *}, Yaqing Yang^{2, #}, Xuzheng Song¹, Kuo Xing¹, Yu Chen^{3, *}

¹ Department of Gastroenterology, 983rd Hospital of Joint Logistics Support Forces, Tianjin, China

² Department of Neurology, 983rd Hospital of Joint Logistics Support Forces, Tianjin, China

³ Department of Gastroenterology, First Affiliated Hospital of Air Force Medical University, Xi'an, China

Article Info



Article history:

Received: November 24, 2023

Accepted: February 23, 2024

Published: April 30, 2024

Use your device to scan and read the article online



Abstract

Recently, the progression of gastric cancer (GC), as one of the most ordinary malignant tumors, has been reported to be associated with circular RNAs. This study aimed to identify the role of circular RNA_LARP4 in GC. We performed real-time quantitative polymerase chain reaction (RT-qPCR) in 46 paired GC patients and GC cell lines to detect the expression of circular RNA_LARP4. Moreover, the role of circular RNA_LARP4 in GC proliferation was identified through proliferation assay and colony formation assay, while the role of circular RNA_LARP4 in GC metastasis was measured through scratch wound assay and transwell assay. Furthermore, the potential targets of circular RNA_LARP4 were predicted through bioinformatics methods and further identified by western blot assay and RT-qPCR. Circular RNA_LARP4 expression was remarkably lower in GC tissues compared with that in adjacent samples. Besides, cell proliferation of GC was inhibited after overexpression of circular RNA_LARP4, while cell migration and invasion of GC was inhibited after overexpression of circular RNA_LARP4. Furthermore, Upstream frameshift 1 (UPF1) was predicted as the potential target of circular RNA_LARP4 and was upregulated *via* overexpression of circular RNA_LARP4 in GC. Circular RNA_LARP4 inhibits GC cell proliferation and metastasis *via* targeting UPF1 *in vitro*, which might provide a new tumor suppressor in GC development.

Keywords: Circular RNA_LARP4, Gastric cancer, UPF1.

1. Introduction

Gastric cancer (GC) stands as a prominent contributor to global cancer mortality, ranking as the fifth most prevalent malignant tumor worldwide, particularly pervasive in Eastern Asia where nearly half of all cases are reported, with China exhibiting the highest incidence rate [1,2]. Despite a decline in morbidity in recent decades, there is a concerning rise in the occurrence of gastric cancer and premalignant gastric lesions among young adults aged 35-44 years [3,4]. The diagnostic and treatment rates for early-stage gastric cancer in Japan and South Korea are notably higher at 70% and 50%, respectively, while in China, these rates remain below 10% [5]. This stark contrast underscores the imperative for early detection and treatment to address the challenging prognosis associated with advanced cases, emphasizing the urgent need to unravel the cellular and molecular intricacies of GC and advance the development of tailored and effective therapeutic strategies.

Despite significant strides in human genome sequencing technology, the molecular underpinnings of GC remain elusive. The spotlight on noncoding RNAs (ncR-

NAs), a majority of which originate from human DNA, has become increasingly pronounced. Circular RNAs (circRNAs), a subset of ncRNAs, have emerged as key players in the landscape of cancer progression. Notably, these circular molecules have garnered attention for their pivotal roles in various cancers. For instance, in hepatocellular carcinoma, miR-200b downregulates RhoA and circRNA_000839, thereby inhibiting cell invasion and migration [6]. In prostate cancer, androgen-responsive circ-SMARCA5 exhibits significant overexpression, fostering the proliferation of prostate cancer cells [7]. The knockdown of hsa_circ_0061140 in ovarian cancer impedes cell growth and metastasis by acting as a sponge for miR-370 [8]. Circ-ABCB10, through sponging miR-1271, contributes to the proliferation and progression of breast cancer cells [9]. In prostate cancer, circ MYLK functions as an oncogene by regulating mir-29a expression, thereby promoting cancer progression [10].

While considerable knowledge exists regarding the involvement of circular RNAs (circRNAs) in cancer, the precise role of Circular RNA_LARP4 in gastric cancer has remained largely uncharted. This study pioneers an explo-

* Corresponding author.

E-mail address: zj254983@163.com (J. Zhang); chenyu0226022@163.com (Y. Chen).

These authors contributed equally

Doi: <http://dx.doi.org/10.14715/cmb/2024.70.4.28>

ration into the specific functions of Circular RNA_LARP4 in gastric cancer (GC) through a meticulous blend of bioinformatics analysis and functional experiments. Our approach aims to uncover the hitherto unknown aspects of Circular RNA_LARP4 in GC pathogenesis. Moreover, we delved into its correlation with UPF1, offering a comprehensive elucidation of the intricate molecular mechanisms that underscore the participation of Circular RNA_LARP4 in the development of gastric cancer. Through this multifaceted investigation, we seek to contribute valuable insights that could potentially shape our understanding of GC biology and inform future therapeutic strategies.

2. Materials and Methods

2.1. Tissue Specimens

A total of 46 paired tumor tissues and adjacent non-tumor tissues were collected from gastric cancer (GC) patients. All diagnoses were confirmed by two independent pathologists. The study received approval from the Ethics Committee of the 983rd Hospital of Joint Logistics Support Forces, and participants provided written informed consent.

2.2. Cell Culture

Four GC cancer cell lines (BGC-823, SGC-7901, HGC-27, and MKN-45) and a human gastric epithelial cell line (GES) were obtained from the Chinese Academy of Science (Shanghai, China). Cells were cultured in 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA), Roswell Park Memorial Institute-1640 (RPIM-1640; Invitrogen, Carlsbad, CA, USA), and penicillin/streptomycin (Sigma, St. Louis, MO, USA) in a 5% CO₂ incubator at 37°C.

2.3. Cell Transfection

Lentivirus against circular RNA_LARP4 or an empty vector was provided by GenePharma (Shanghai, China). Transfection into HGC-27 GC cells was performed using Lipofectamine 2000 reagent (Invitrogen; Carlsbad, CA, USA) according to the manufacturer's protocol.

2.4. RNA Extraction and Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA from cells or tissues was extracted using TRIzol reagent (TaKaRa Bio, Inc., Otsu, Shiga, Japan) and reverse-transcribed to complementary deoxyribose nucleic acids (cDNAs). RT-qPCR primers: Circular RNA_LARP4: Forward 5'-GGGCATCAGGAGCAAACCTTA-3'; Reverse 5'-GTGTTGGGCTGGAAGCCATC-3'; GAPDH: Forward 5'-GCACCGTCAAGGCTGAGAAC-3'; Reverse 5'-TGGTGAAGACGCCAGTGGA-3'. PCR conditions: Pre-denaturation at 95°C for 1 min, followed by 40 cycles at 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s. Relative expression was calculated using the 2- $\Delta\Delta C_t$ method.

2.5. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide) Assay

Transfected cells (2×10^3) were seeded in 96-well plates, and cell proliferation was assessed using the MTT assay at 0 h, 24 h, 48 h, and 72 h. Absorbance at 490 nm was measured with an ELISA reader system (Multiskan Ascent, LabSystems, Helsinki, Finland).

2.6. Colony Formation Assay

H1299 cells were cultured for 10 days in a 6-well plate. Colonies were treated with 10% formaldehyde for 30 min and stained with 0.5% crystal violet for 5 min. Image-Pro Plus 6.0 (Media Cybernetics, Silver Springs, MD, USA) was used for data analysis.

2.7. Scratch Wound Assay

Cells in 6-well plates were cultured in RPIM-1640 medium overnight. After scratching with a plastic tip, cells were cultured in serum-free RPIM-1640. Wound closure was observed at 48 h. Each assay was independently repeated three times.

2.8. Transwell Assay

Transwell chambers with 8 μ m pores were used. Cells were seeded into the upper chambers, and 20% FBS-RPIM-1640 was added to the lower chamber. After 24 h, inserts were fixed with methanol and stained with hematoxylin. The number of migrated cells was counted using a light microscope.

2.9. Matrigel Assay

Transwell chambers with 8 μ m pores were used. The membrane was precoated with 50 μ L Matrigel. After 24 h, inserts were fixed with methanol and stained with hematoxylin. The number of migrated cells was counted using a light microscope.

2.10. Western Blot Analysis

Proteins were extracted using RIPA reagent (Beyotime, Shanghai, China) and quantified with a BCA protein assay kit (TaKaRa Bio, Inc., Shiga, Japan). SDS-PAGE separated target proteins, which were then incubated with antibodies (Cell Signaling Technology) for GAPDH and UPF1. Protein expression was assessed using Image J software (NIH, Bethesda, MD, USA).

2.11. Statistical Analysis

Statistical analyses were performed using SPSS 21.0. The independent-sample t-test was used to compare differences between the two groups, with $p < 0.05$ considered statistically significant.

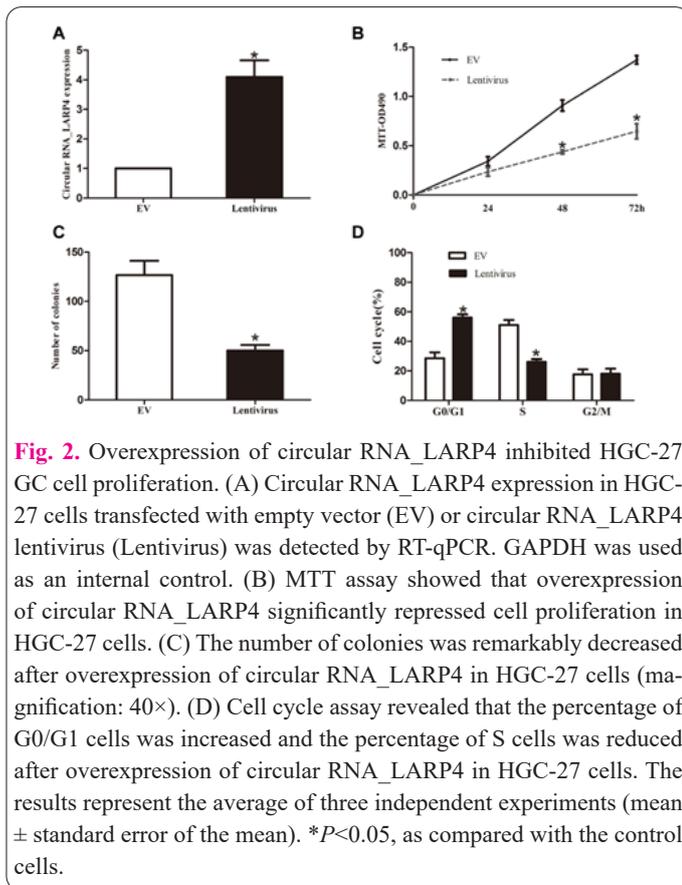
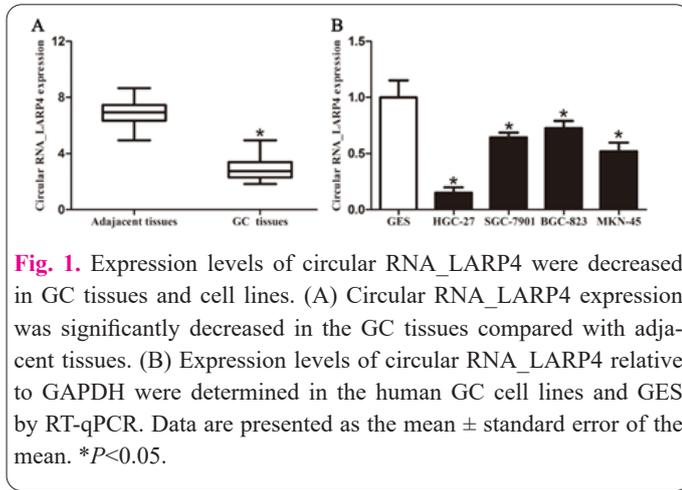
3. Results

3.1. Dysregulated Circular RNA_LARP4 in GC Patients and Cells

We performed RT-qPCR in 46 paired GC patients' tissue samples and GC cell lines. As was shown in Figure 1A, downregulated circular RNA_LARP4 was observed in tumor tissue samples compared with that in adjacent tissues. Compared with the expression in NP69, circular RNA_LARP4 level was significantly lower in GC cells, which is shown in Figure 1B.

3.2. Inhibition of Cell Proliferation by Circular RNA_LARP4 in HGC-27 GC Cells

Before we explored whether circular RNA_LARP4 could play a vital role in GC, circular RNA_LARP4 was transfected with lentivirus in HGC-27 cells. As shown in Figure 2A, circular RNA_LARP4 expression was remarkably upregulated in RNA level after transfection of lentivirus. Then cell proliferation assay and colony formation assay were carried out in HGC-27 cells. As shown



in Figure 2B, evident repression of cell proliferation *via* overexpression of circular RNA_LARP4 (transfection with lentivirus) compared with the EV (transfection with empty vector) was viewed in HGC-27 cells (*P*<0.05). Besides, the number of colonies was remarkably decreased after overexpression of circular RNA_LARP4 in GC cells, which was shown in Figure 2C. Moreover, we further explored whether the cell cycle of GC was regulated by circular RNA_LARP4 and found that the percentage of G0/G1 cells was increased and the percentage of S cells was reduced after overexpression of circular RNA_LARP4 in HGC-27 cells, which was shown in Figure 2D. Above results suggest circular RNA_LARP4 might act as a new suppressor in GC and influence cell proliferation and cell cycle of GC.

3.3. Inhibition of Cell Migration and Invasion by Circular RNA_LARP4 in HGC-27 GC Cells

We further explored the effect of circular RNA_LARP4

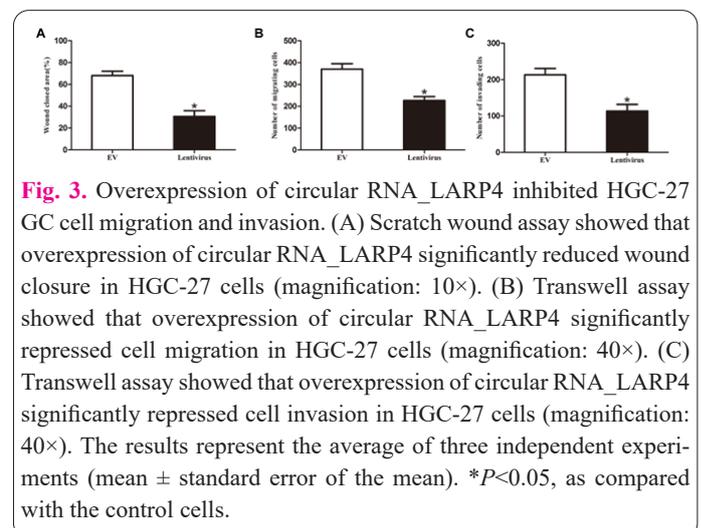
on the migrated and invaded abilities of GC through scratch wound assay and transwell assay. Scratch wound assay showed that an evident decrease was observed in the wound closure of HGC-27 cells transfected with circular RNA_LARP4 lentivirus which was shown in Figure 3A. Moreover, as was shown in Figure 3B and Figure 3C, an evident decrease in the number of migrated HGC-27 cells was observed after they were transfected with circular RNA_LARP4 lentivirus, while an evident decrease in the number of invaded HGC-27 cells was observed after they were transfected with circular RNA_LARP4 lentivirus. The above results suggest circular RNA_LARP4 might also influence the metastasis of GC.

3.4. UPF1 As the Direct Target of Circular RNA_LARP4 in GC

The target proteins of circular RNA_LARP4 were scanned through StarBase v2.0 (<http://starbase.sysu.edu.cn/starbase2/rbpCircRNA.php>), among which UPF1 was selected and its association with circular RNA_LARP4 was further explored. As was shown in Figure 4A, an upregulation of UPF1 was observed after overexpression of circular RNA_LARP4. Moreover, the western blot assay revealed that the expression of UPF1 was upregulated after overexpression of circular RNA_LARP4, which was shown in Figure 4B. Furthermore, as was shown in Figure 4C, UPF1 was observed downregulated in tumor tissue samples compared with that in adjacent tissues. Besides, the UPF1 level was significantly lower in GC cells compared with the expression in GES, as shown in Figure 4D. Meanwhile, the results of linear correlation analysis showed that the expression of UPF1 is positively correlated to circular RNA_LARP4 expression in GC tissues (Figure 4E).

4. Discussion

The majority of circRNAs, as a large class of noncoding RNAs that are tissue-specific, and ubiquitously expressed, are located in the cytoplasm and are more stable than linear RNA due to their resistance to exonucleolytic degradation [11]. CircRNAs, reported as potential prognostic biomarkers and therapeutic targets for many cancers, offer a clinical tool for predicting the treatment response, and assessing the disease state and clinical outcome, including the progression of GC. Serving as microRNA (miRNA) sponges is the vital function of cellular circRNAs. Of note,



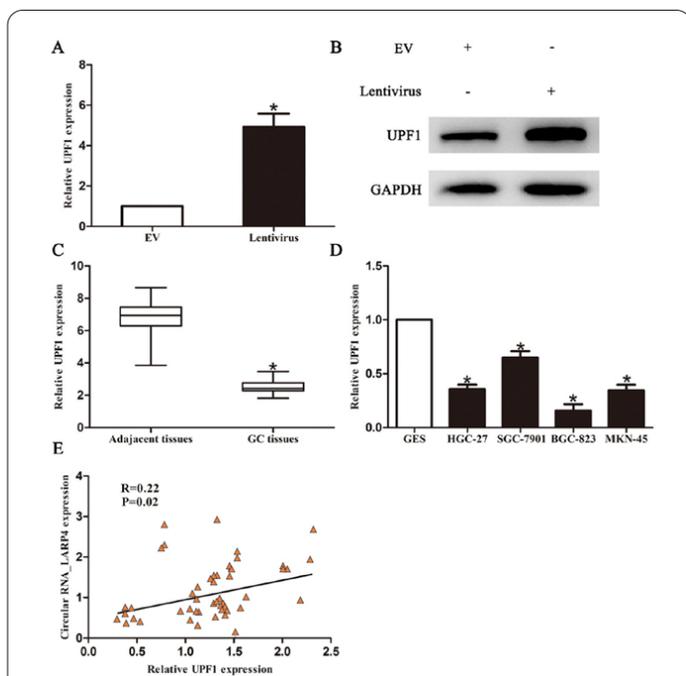


Fig. 4. The association between circular RNA_LARP4 and UPF1 in GC. (A) RT-qPCR results showed that the UPF1 expression was increased in circular RNA_LARP4 lentivirus (Lentivirus) group compared with empty vector (EV) group. (B) Western blot assay results showed that the UPF1 expression was increased in circular RNA_LARP4 lentivirus (Lentivirus) group compared with empty vector (EV) group. (C) Downregulated UPF1 was observed in tumor tissue samples compared with that in adjacent tissues. (D) Compared with the expression in GES, UPF1 level was significantly lower in GC cells. (E) The linear correlation between the expression level of UPF1 and circular RNA_LARP4 in GC tissues. The results represent the average of three independent experiments. Data are presented as the mean ± standard error of the mean. **P*<0.05.

overexpression of circ-PVT1 promotes cell proliferation in gastric cancer by serving as a sponge for the miR-125 family which may be a potential factor and prognostic biomarker in GC [12]. Circ-0003159 is remarkably negatively related to the progression of GC [13].

Circular RNA_LARP4, derived from LARP4 gene locus, has been reported to inhibit cancer metastasis by serving as a La-related RNA-binding protein and is significantly downregulated in ovarian cancer which may serve as a potential biomarker for prognosis of ovarian cancer patients [14]. Moreover, circ-LARP4 suppresses the proliferation and invasion of GC cells through regulating expression of LATS1 and sponging miR-424-5p [15]. In our study, circular RNA_LARP4 was found to be downregulated in both GC tissue and cells. The ability of GC cell growth, migration and invasion was suppressed after circular RNA_LARP4 was overexpressed, which indicated that circular RNA_LARP4 functioned as a tumor suppressor in the tumorigenesis of GC.

UPF1 (UPF1 RNA helicase and ATPase), predicted as the target proteins of DLX6-AS1 through StarBase v2.0, has been reported to play a crucial role in tumor growth and invasion of many cancers. For instance, UPF1 participates in development of gastric cancer, which is regulated by lncRNA MALAT1 [16]. Besides, SNHG6 acts as an oncogene in hepatocellular carcinoma by regulating miR-101-3p/UPF1 axis [17]. UPF1 also represses tumor development of hepatocellular carcinoma *via* targeting

Smad7 [18]. Our study showed that UPF1 expression was downregulated in GC cells and tissues. Furthermore, UPF1 could be upregulated after overexpression of circular RNA_LARP4 and was positively correlated to circular RNA_LARP4 expression in GC tissues. All these results showed that UPF1 was upregulated by overexpression of circular RNA_LARP4 in GC.

5. Conclusions

To sum up, circular RNA_LARP4 could inhibit GC cell proliferation and metastasis through upregulating UPF1, which provides a clinical tool for predicting the treatment response, and assessing the disease state and clinical outcome of GC.

Conflict of Interest

The authors declare that they have no conflict of interest.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the 983rd Hospital of Joint Logistics Support Forces.

Informed Consent

Participants in this study provided written informed consent.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Jing Zhang, Yaqing Yang: Conceptualization, methodology, writing original draft preparation. Xuzheng Song, Kuo Xing: Investigation, software, statistical analysis. Jing Zhang, Yu Chen: Reviewing and editing, funding acquisition, supervision. All authors read and approved the final manuscript.

Funding

None.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Ca-Cancer J Clin* 68:394-424. doi: 10.3322/caac.21492
2. Rahman R, Asombang AW, Ibdah JA (2014) Characteristics of gastric cancer in Asia. *World J Gastroentero* 20:4483-4490. doi: 10.3748/wjg.v20.i16.4483
3. Song H, Held M, Sandin S, Rautelin H, Eliasson M, Soderberg S et al (2015) Increase in the Prevalence of Atrophic Gastritis Among Adults Age 35 to 44 Years Old in Northern Sweden Between 1990 and 2009. *Clin Gastroenterol H* 13:1592-1600. doi: 10.1016/j.cgh.2015.04.001
4. Anderson WF, Camargo MC, Fraumeni JJ, Correa P, Rosenberg PS, Rabkin CS (2010) Age-specific trends in incidence of noncardia gastric cancer in US adults. *Jama-J Am Med Assoc* 303:1723-1728. doi: 10.1001/jama.2010.496
5. Ren W, Yu J, Zhang ZM, Song YK, Li YH, Wang L (2013) Missed

- diagnosis of early gastric cancer or high-grade intraepithelial neoplasia. *World J Gastroentero* 19:2092-2096. doi: 10.3748/wjg.v19.i13.2092
6. Wang BG, Li JS, Liu YF, Xu Q (2017) MicroRNA-200b suppresses the invasion and migration of hepatocellular carcinoma by downregulating RhoA and circRNA_000839. *Tumour Biol* 39:1393370087. doi: 10.1177/1010428317719577
 7. Kong Z, Wan X, Zhang Y, Zhang P, Zhang Y, Zhang X et al (2017) Androgen-responsive circular RNA circSMARCA5 is up-regulated and promotes cell proliferation in prostate cancer. *Biochem Bioph Res Co* 493:1217-1223. doi: 10.1016/j.bbrc.2017.07.162
 8. Chen Q, Zhang J, He Y, Wang Y (2018) hsa_circ_0061140 Knockdown Reverses FOXM1-Mediated Cell Growth and Metastasis in Ovarian Cancer through miR-370 Sponge Activity. *Mol Ther-Nucl Acids* 13:55-63. doi: 10.1016/j.omtn.2018.08.010
 9. Liang HF, Zhang XZ, Liu BG, Jia GT, Li WL (2017) Circular RNA circ-ABCB10 promotes breast cancer proliferation and progression through sponging miR-1271. *Am J Cancer Res* 7:1566-1576. doi:
 10. Dai Y, Li D, Chen X, Tan X, Gu J, Chen M et al (2018) Circular RNA Myosin Light Chain Kinase (MYLK) Promotes Prostate Cancer Progression through Modulating Mir-29a Expression. *Med Sci Monitor* 24:3462-3471. doi: 10.12659/MSM.908009
 11. Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, Liu J et al (2013) Circular RNAs are abundant, conserved, and associated with ALU repeats. *RNA* 19:141-157. doi: 10.1261/rna.035667.112
 12. Chen J, Li Y, Zheng Q, Bao C, He J, Chen B et al (2017) Circular RNA profile identifies circPVT1 as a proliferative factor and prognostic marker in gastric cancer. *Cancer Lett* 388:208-219. doi: 10.1016/j.canlet.2016.12.006
 13. Tian M, Chen R, Li T, Xiao B (2018) Reduced expression of circRNA hsa_circ_0003159 in gastric cancer and its clinical significance. *J Clin Lab Anal* 32:e22281. doi: 10.1002/jcla.22281
 14. Zou T, Wang PL, Gao Y, Liang WT (2018) Circular RNA_LARP4 is lower expressed and serves as a potential biomarker of ovarian cancer prognosis. *Eur Rev Med Pharmacol* 22:7178-7182. doi: 10.26355/eurrev_201811_16250
 15. Zhang J, Liu H, Hou L, Wang G, Zhang R, Huang Y et al (2017) Circular RNA_LARP4 inhibits cell proliferation and invasion of gastric cancer by sponging miR-424-5p and regulating LATS1 expression. *Mol Cancer* 16:151. doi: 10.1186/s12943-017-0719-3
 16. Li L, Geng Y, Feng R, Zhu Q, Miao B, Cao J et al (2017) The Human RNA Surveillance Factor UPF1 Modulates Gastric Cancer Progression by Targeting Long Non-Coding RNA MALAT1. *Cell Physiol Biochem* 42:2194-2206. doi: 10.1159/000479994
 17. Chang L, Yuan Y, Li C, Guo T, Qi H, Xiao Y et al (2016) Upregulation of SNHG6 regulates ZEB1 expression by competitively binding miR-101-3p and interacting with UPF1 in hepatocellular carcinoma. *Cancer Lett* 383:183-194. doi: 10.1016/j.canlet.2016.09.034
 18. Chang L, Li C, Guo T, Wang H, Ma W, Yuan Y et al (2016) The human RNA surveillance factor UPF1 regulates tumorigenesis by targeting Smad7 in hepatocellular carcinoma. *J Exp Clin Oncol* 35:8. doi: 10.1186/s13046-016-0286-2