Research Article

Downregulated Expression of hsa_circ_0005556 in Gastric Cancer and Its Clinical Significance

Liangwei Yang,1,2 Yu Yu,1,2 Xiuchong Yu,1,2 Jiaming Zhou,1,2 Zhiping Zhang,2 Shibo Ying,3 Junming Guo,1 and Zhilong Yan2

1Medical School of Ningbo University, Ningbo No. 1 Hospital, Department of Biochemistry and Molecular Biology, Zhejiang Key Laboratory of Pathophysiology, Ningbo 315211, China
2Department of Gastrointestinal Surgery, Ningbo No. 1 Hospital, Ningbo 315010, China
3Institute of Occupational Diseases, Zhejiang Academy of Medical Sciences, Hangzhou 310013, China

Correspondence should be addressed to Junming Guo; guojunming@nbu.edu.cn and Zhilong Yan; yanzhilong@nbu.edu.cn

Received 15 May 2019; Revised 9 September 2019; Accepted 30 September 2019; Published 19 November 2019

Academic Editor: Stamatios E. Theocharis

Copyright © 2019 Liangwei Yang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. Gastric cancer (GC) has a poor prognosis due to the lack of ideal tumor markers. Circular RNAs (circRNAs) are a novel type of noncoding RNA related to the occurrence of GC. Among our research, we investigated the role of hsa_circ_0005556 in GC.

Materials and Methods. The expression of hsa_circ_0005556 of 100 paired GC tissues and adjacent normal tissues was detected using quantitative reverse transcription-polymerase chain reaction (qRT-PCR). A receiver operating characteristic (ROC) curve was established to evaluate the diagnostic value of hsa_circ_0005556. The correlation between the expression of hsa_circ_0005556 and corresponding clinicopathological characteristic was explored.

Results. hsa_circ_0005556 was significantly downregulated in GC tissues contrasted with adjacent normal tissues (n = 100, p < 0.001). The areas under the ROC curve (AUC) of hsa_circ_0005556 were up to 0.773, while 64% sensitivity and 82% specificity, respectively. Moreover, its expression levels were significantly associated with differentiation (p = 0.001), TNM stage (p = 0.013), and lymphatic metastasis (p = 0.039). GC patients of high hsa_circ_0005556 levels had a longer overall survival (OS) than those of the low group (p = 0.047). Conclusion. hsa_circ_0005556 is a potential biomarker for GC, which may guide judgment of the indication of endoscopic treatment for early gastric cancer (EGC).

1. Introduction

Gastric cancer (GC), a serious global health risks, ranks as the fifth most common cancer and the third primary cause of cancer-related death globally. Every year, beyond 1000000 new cases and 783000 new deaths are reported [1]. Incidence rates are markedly elevated in Eastern Asia [1]. The prognosis of GC is closely associated with the stage when diagnosed. The 5-year survival rate of stage I patients can reach 90%, while that of the stage IV patients who already show distant metastases is only approximately 14% [2]. Most patients with early gastric cancer (EGC) have no obvious specific symptoms [3]. The diagnosis of GC relies on upper gastrointestinal (GI) endoscopy. However, in most countries, upper GI endoscopy is not a routine medical examination [4]. Most GC patients are diagnosed at an advanced stage. On a global scale, the 5-year survival rate of GC is an average of 20-40% [5]. Therefore, to improve the diagnosis of EGC, it is critical to find potent, stable, and ideal diagnostic biomarkers of GC.

With the development of RNA high-throughput sequencing technology and advances in biophysical technology, an increasing number of noncoding RNAs have been discovered [6]. Some studies have found that noncoding RNA is involved in the pathogenesis of many cancers [7, 8]. Circular RNAs (circRNAs) are a novel type of endogenous noncoding RNAs produced by precursor mRNA backsplicing using exons, introns, or intergenic regions [9]. In contrast with linear RNAs, circRNAs are discriminated by a circle structure linked by a covalent bond...
and neither a 5’ cap structure nor a 3’ polyadenylated tail. As a result of the circular structure, circRNAs are more stable, conserved, and highly abundant than other noncoding RNAs, such as miRNAs and lncRNAs [10–13]. Circular RNA_ LARP4 was proved to downregulate in GC tissues and correlated to good survival [14]. It inhibited GC cell proliferation and invasion via sponging hsa-miR-424-5p and regulating the expression of tumor suppressor kinase 1 (LATS1). Another study indicated that the level of circPVT1 is elevated in GC samples and predicted favorable survival of patients with GC. circPVT1 acts as a sponge for members of the miR-125 family to promote the proliferation of GC cell [15]. Due to these biological properties, circRNAs may be important biomarkers for the occurrence and development of GC.

Based on our previous microarray analysis (GEO No. GSE89143, Guo, 2016: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE89143), the expression of hsa_circ_0005556 was quite different in GC tissue compared to paired normal tissue. So, we chose hsa_circ_0005556 as a research target to analyze its diagnostic values of GC. The gene of hsa_circ_0005556 is located at chr2:15693549-15698758. Its spliced sequence length is 218 nt, and the related gene symbol is the neuroblastoma-amplified sequence (NBAS). To our knowledge, few studies have identified the functions of hsa_circ_0005556. Here, quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was utilized to explore the expression of hsa_circ_0005556 in GC patients. And the relationship with clinicopathological factors was also analyzed. We identified hsa_circ_0005556 as a diagnostic biomarker in GC.

2. Materials and Methods

2.1. Samples and Clinical Information. GC tissue samples were obtained from Ningbo Yinzhou People’s Hospital from January 2010 to December 2015. During this time, all specimens were obtained from GC patients undergoing gastroenterology and collected by the surgeons. GC tissues were taken from the mucosa of the center of the tumor. The adjacent normal tissues were taken from the mucosa 5 cm beyond the edge of carcinoma. All fresh samples were instantly stored in RNA-fixing reagent (BioTeka, Beijing, China), then preserved at -80°C until RNA isolation.

The clinical information of all samples was collected. The GC diagnosis was confirmed by histopathology. The pathologic stage of the tumor was assigned basing on the tumor-node metastasis (TNM) staging system of the International Union Against Cancer (8th edition). The histological grade of the tumor was assigned basing on the American Joint Committee on Cancer (AJCC) cancer staging manual (8th edition). Written informed consent was acquired from all individuals. This research acquired the approval from the Human Research Ethics Committee of Ningbo University (IRB No. 20100303).

2.2. Total RNA Extraction and Quality Control. According to the manufacturer’s instructions, total RNA of tissues was extracted with TRIzol reagent (Invitrogen, Karlsruhe, Germany). Next, the DS-11 spectrophotometer machine (DeNovix, Wilmington, DE, USA) was used to verify purity and concentration of total RNA. The A260/A280 ratio was used to evaluate the RNA purity, which only ranged from 1.8 to 2.0 for the samples that were qualified and used.

2.3. Reverse Transcription. Total RNA was performed to synthesize cDNA using the GoScript RT System (Promega, Madison, WI, USA) with a random primer, following the manufacturer’s instructions.

2.4. Quantitative Reverse Transcription-Polymerase Chain Reaction. qRT-PCR analysis was implemented on an Mx3005P real-time PCR System (Stratagene, La Jolla, CA, USA) with GoTaq qPCR Master Mix (Promega). The sequences of divergent primers were designed and produced by BGI (Shenzhen, China) Co., Ltd. The primer of hsa_circ_0005556 was 5’-GTGTGTGGAAAATCAGCCTAG-3’ and 5’-AAACAAGCGAACCCTCAT-3’. The primer of glyceraldehyde 3-phosphate dehydrogenase (GAPDH, as control) primers was 5’-CTGCAAGTGTACGTGGTG-3’ and 5’-TCATGTAGCCAGGATGCC-3’. The conditions of thermal cycling were as follows: hot start at 95°C for 5 minutes, next 40 cycles at 95°C for 15 seconds, 56°C for 30 seconds, and 72°C for 30 seconds. The qRT-PCR products were confirmed by Sanger sequencing, which was completed by Geneseeed (Guangzhou, China). The relative quantification level of circRNA was calculated by the ΔCq (quantification cycle) method. A larger ΔCq indicates a lower level. Through three independent experiments, all of the data were expressed as the mean ± SD.

2.5. Statistical Analysis. Statistical data were analyzed by Statistical Product and Service Solutions (SPSS) 19.0 software (SPSS, Chicago, IL, USA). Figures and tables were produced by GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA). The data in this paper are in accordance with the normal distribution. The Shapiro-Wilk test, Student’s t-test, analysis of variance (ANOVA), receiver operating characteristic (ROC) curves, and Kaplan-Meier survival curves were properly used. A value of p < 0.05 (two sided) was considered to be statistically significant.

3. Results

3.1. The Expression of hsa_circ_0005556 in GC Tissues. To detect the expression of hsa_circ_0005556 in GC tissues, qRT-PCR was used in 100 pairs of GC and adjacent normal samples. As shown by Sanger sequencing, the sequencing of the PCR products of hsa_circ_0005556 contains the cyclization site (Figure 1). The difference in ΔCq between cancer and adjacent normal tissues was in accordance with the normal distribution. The results indicated that hsa_circ_0005556 was significantly downregulated in GC tissues contrast to adjacent normal tissues (n = 100, p < 0.001, Figures 2(a) and 2(b)). Among all samples, samples with decreased expression accounted for 81.4% (Figure 2(c)).

3.2. Prediction of hsa_circ_0005556 Binding to miRNAs by Sequence Analysis. Many circRNAs have complementary...
sites with microRNA (miRNAs) called microRNA response elements. Given that miRNAs function as downstream regulatory elements of circRNAs to affect the progression of GC, identification of the signal axis from circRNAs to miRNAs is important. We predicted the potential binding miRNAs for hsa_circ_0005556 using sequence analysis. The results showed there were 6 potential binding miRNAs (Table 1). According to these predicted circRNA-miRNA sponging sites, hsa_circ_0005556 may bind certain miRNAs, and their verified functions indicated a potential role involved in the adjustment of GC progression.

3.3. Diagnostic and Prognostic Value of hsa_circ_0005556 in GC. Since we discovered hsa_circ_0005556 was significantly downregulated in GC tissues, we further analyzed the connection between the expression of hsa_circ_0005556 and some clinicopathological characteristics of patients with GC. As described in Table 2, the hsa_circ_0005556 level in tissues were significantly correlated with differentiation (p = 0.001), TNM stage (p = 0.013), and lymphatic metastasis (p = 0.039). Besides, no relationship was detected between its level and the rest of clinicopathological factors, like age, gender, tumor diameter, invasion, distal metastasis, carbohydrate antigen 19-9 (CA19-9), and carcinoembryonic antigen (CEA).

Next, the Kaplan-Meier survival analysis was conducted to analyze the overall survival (OS) of GC patients based on hsa_circ_0005556 expression. GC patients were divided into two groups, the low or high group according to the mean value of hsa_circ_0005556 in GC tissues. The results showed that patients in the low group had shorter OS compared to those in the high group (p = 0.047, Figure 3).
A ROC curve was established to test the potential diagnostic value of hsa_circ_0005556 (Figure 2(d)). The area under the ROC curve (AUC) reached 0.773, while 64% sensitivity and 82% specificity, respectively. The likelihood ratio of hsa_circ_0005556 was up to 3.56. Taken together, our research forecasted that hsa_circ_0005556 might act as an ideal biomarker for detecting GC.

4. Discussion

circRNAs were mistakenly considered to be aberrant splicing byproducts when they were discovered in viruses 40 years ago [29, 30]. Due to the revolutionary breakthrough in RNA-sequencing technique and biophysics techniques, many circRNAs have been discovered and were shown to be stable, conserved, and highly abundant [31, 32]. Due to these characteristics, circRNAs have certain advantages over other noncoding RNAs in the diagnosis and treatment of diseases.

The prognosis of GC is tightly associated with disease stage. Later stages implied a poor 5-year survival rate [33]. Thus, establishment of an early cancer screening system is important. circRNAs are acting in the regulation of cancer progression. In our research, contrast to adjacent normal tissues, hsa_circ_0005556 was downregulated in GC samples (Figure 2). Moreover, GC patients of a high hsa_circ_0005556 level in GC tissues showed better outcomes than those of a low level. These results indicated that hsa_circ_0005556 act a valuable role in both diagnosis and prognosis, which may be a potential biomarker that contributes to GC screening.

In recent years, a number of studies have focused on finding ideal biomarkers. Compared with another study on hsa_circ_0006633, whose specificity and sensitivity are 81% and 60%, respectively [34], hsa_circ_0005556 has a more precise diagnostic value (Figure 2(d)). hsa_circ_0006633 is associated with distant metastasis of GC (Table 1), suggesting long-term monitoring of GC recurrence. Different circRNAs play different roles in the management of GC.

Table 1: The predicted miRNAs targeting hsa_circ_0005556 and their potential functions in tumors.

| MicroRNA | Target | Tumor type | Function | Ref. |
|----------|--------|------------|----------|-----|
| HIF-1α, VEGF | Breast cancer | Proliferation, invasion, chemotherapy resistance | [16, 17] |
| miR-548c-3p | c-Myb | Glioma | Proliferation, migration | [18] |
| —— | ITGA1 | Osteosarcoma | Proliferation | [19] |
| —— | Gastric cancer | Differential expression in HP(+) and HP(-) | [20] |
| miR-587 | PPP2R1B | Colorectal cancer | Chemotherapy resistance | [21] |
| —— | Glioblastoma multiforme | Prognosis | [22] |
| miR-4739 | β-Catenin | Gastric cancer | Proliferation, invasion, chemosensitivity | [23] |
| miR-125a-3p | TIM-3 | Acute myeloid leukemia | —— | [24] |
| —— | Fyn | Pancreatic ductal adenocarcinoma | Chemosensitivity | [25] |
| miR-125a-3p | MTA1 | Lung cancer | Proliferation, migration, invasion | [26] |
| —— | CDK3 | Lung cancer | —— | [27] |
| miR-297 | VEGFA | Oral squamous cell carcinoma | Proliferation | [28] |

HIF-1α: hypoxia-inducible factor-1 alpha, VEGF: vascular endothelial growth factor, c-Myb: protooncogene c-Myb, ITGA1: integrin αv, TIM-3: T cell immunoglobulin and mucin-3, MTA1: metastasis-associated gene 1, CDK3: cyclin-dependent kinase 3, VEGFA: vascular endothelial growth factor A.

The functions of circRNAs have not been fully revealed. Jeck et al. proposed the theory of miRNA sponges [11], in which circRNAs have miRNA response elements to serve on competing endogenous RNAs. We predicted 6 potential miRNA-binding sites of hsa_circ_0005556 using a bioinformation database [35, 36]. Based on the PubMed database, the regulated targets of these miRNAs and their possible functions in GC, breast cancer, lung cancer, and other cancers are listed in Table 1. Based on the sponge theory, hsa_circ_0005556 may act as a ceRNA to bind these miRNAs, which may take part in the regulation of GC progression. However, these predictions have not yet been verified. Next, we need to carry out a corresponding experiment to explore whether hsa_circ_0005556 regulates the function of GC by binding these sites.

The association between circRNAs and some clinicopathological features has a strong clinical value. Sufficient tumor resection is the key therapeutic factor for resectable GC [37, 38]. The main treatments for resectable GC include surgical resection and endoscopic resection. Surgical resection has been regarded as the gold standard because gastrectomy plus D2 lymphadenectomy has contributed to raise survival rates [3]. However, conventional surgery is associated with many complications, high costs, and extensive trauma. Despite the widespread use of laparoscopic techniques, they do not completely address these shortcomings. Comparatively, endoscopic treatment is a minimally invasive method that is safer and less costly in patients with EGC. A series of studies have proven that endoscopic resection is as effective as conventional surgery in EGC [39, 40]. However, the scope of endoscopic treatment is extremely strict, indicating that preoperative evaluation is crucial. Guidelines from Europe, Japan, and America all note that differentiation, invasion, and lymphatic metastasis are the key factors to assess the indication [41–43]. Current examination methods, including upper GI endoscopy and biopsy, endoscopic ultrasound, and CT, are not completely accurate. Particularly in judging lymph node metastasis, the CT method shows a high level of missed
diagnoses because the detection rate of micrometastatic lymph node smaller than 10 mm in diameter is low [44]. Once a mistaken assessment is made, the incorrect choice in patient treatment will lead to an unavoidable poor prognosis. In our research, there was a significant relationship between decreased hsa_circ_0005556 expression in GC and some clinicopathological factors, such as differentiation, lymphatic metastasis, and TNM stage (Table 1). The patients with a lower hsa_circ_0005556 level had worse degree of tumor differentiation and later TNM stage. Although there was no linear relationship between the hsa_circ_0005556 level and the number of lymph node metastases, the data showed that it can significantly distinguish between N(-) and N(+) (p = 0.01). These characteristics may help determine the diagnosis of EGC. As a result, hsa_circ_0005556 may contribute to the choice of treatment for GC, especially to determine whether the patient is suitable for endoscopic treatment instead of conventional surgery.

The developing standard of care in GC is evaluation of human epidermal growth factor receptor 2 (Her2), mismatch repair (MMR), and programmed cell death ligand 1 (PD-L1) status in tumors, with related targeted therapies informing medical and surgical management and studies for these proteins also being done on preoperative specimens [45, 46]. To advocate for the utility of circRNA in this current paradigm, we also tested whether the expression levels of these other markers are associated with hsa_circ_0005556 in the set of tumors. In our medical center, no tests were conducted for MMR and PD-L1 before 2015, and the test for Her2/neu was only carried out in 45 specimens. Patients were divided into two groups, Her2-negative and Her2-positive. We analyzed the hsa_circ_0005556 expression in the tissues of the two groups. The data were in accordance with the normal distribution. The results showed the hsa_circ_0005556 expression of the Her2-positive group was shorter than those of the high expression group (p = 0.047).

5. Conclusion

In summary, our data indicated that hsa_circ_0005556 expression is significantly downregulated in GC tissues.
The downregulated expression level was correlated with differentiation, TNM stage, and lymphatic metastasis. Our research suggests that hsa_circ_0005556 may become a biomarker for GC. hsa_circ_0005556 data may help to decide the method of treatment for EGC by judging the indications of endoscopic treatment.

**Data Availability**

(1) The microarray analysis data used to support the findings of this study have been deposited in the GEO dataset (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE89143).

(2) The quantitative reverse transcription-polymerase chain reaction (qRT-PCR) of hsa_circ_0005556 data used to support the findings of this study are included within the article.

**Conflicts of Interest**

The authors declare no conflict of interest.

**Acknowledgments**

This research was supported by the Applied Research Innovation Team Project of Ningbo (No. 2017C110019).

**References**

[1] F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, and A. Jemal, "Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries," *CA: A Cancer Journal for Clinicians*, vol. 68, no. 6, pp. 394–424, 2018.

[2] T. Sano, D. G. Coit, H. H. Kim et al., "Proposal of a new stage grouping of gastric cancer for TNM classification: International Gastric Cancer Association staging project," *Gastric Cancer*, vol. 20, no. 2, pp. 217–225, 2017.

[3] E. Van Cutsem, X. Sagar, B. Topal, K. Haustermans, and H. Prenen, "Gastric cancer," *The Lancet*, vol. 388, no. 10060, pp. 2654–2664, 2016.

[4] N. S. Chuks, "Challenges of Gastrointestinal Endoscopy in Resource-Poor Countries," in *Gastrointestinal Endoscopy*, Oliviu Pascu, IntechOpen, 2011.

[5] C. Allemani, T. Matsuda, V. di Carlo et al., "Global surveillance of trends in cancer survival 2000–14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries," *The Lancet*, vol. 391, no. 10125, pp. 1023–1075, 2018.

[6] J. Salzman, C. Gawad, P. L. Wang, N. Lacayo, and P. O. Brown, "Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types," *PLoS One*, vol. 7, no. 2, article e30733, 2012.

[7] S. J. Conn, K. A. Pillman, J. Toubia et al., "The RNA binding protein quaking regulates formation of circRNAs," *Cell*, vol. 160, no. 6, pp. 1125–1134, 2015.

[8] A. Bachmayr-Heyda, A. T. Reiner, K. Auer et al., "Correlation of circular RNA abundance with proliferation – exemplified with colorectal and ovarian cancer, idiopathic lung fibrosis, and normal human tissues," *Scientific Reports*, vol. 5, no. 1, article 8057, 2015.

[9] X. Li, L. Yang, and L. L. Chen, "The biogenesis, functions, and challenges of circular RNAs," *Molecular Cell*, vol. 71, no. 3, pp. 428–442, 2018.

[10] Q. Zheng, C. Bao, W. Guo et al., "Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs," *Nature Communications*, vol. 7, no. 1, article 11215, 2016.

[11] W. R. Jeck, J. A. Sorrentino, K. Wang et al., "Circular RNAs are abundant, conserved, and associated with ALU repeats," *RNA*, vol. 19, no. 2, pp. 141–157, 2013.

[12] A. Rybak-Wolf, C. Stottmeister, P. Glaz et al., "Circular RNAs in the mammalian brain are highly abundant, conserved, and dynamically expressed," *Molecular Cell*, vol. 58, no. 5, pp. 870–885, 2015.

[13] C. Zhang, H. Wu, Y. H. Wang et al., "Circular RNA of cattle casein genes are highly expressed in bovine mammary gland," *Journal of Dairy Science*, vol. 99, no. 6, pp. 4750–4760, 2016.

[14] J. Zhang, H. Liu, L. Hou et al., "Circular RNA_LARP4 inhibits cell proliferation and invasion of gastric cancer by sponging miR-424-5p and regulating LAT51 expression," *Molecular Cancer*, vol. 16, no. 1, p. 151, 2017.

[15] J. Chen, Y. Li, Q. Zheng et al., "Circular RNA profile identifies circPVT1 as a proliferative factor and prognostic marker in gastric cancer," *Cancer Letters*, vol. 388, pp. 208–219, 2017.

[16] X. Guo, S. Lee, and P. Cao, "The inhibitive effect of sh-HIF1A-AS2 on the proliferation, invasion, and pathological damage of breast cancer via targeting miR-548c-3p through regulating HIF-1α/VEGF pathway in vitro and vivo," *OncoTargets and Therapy*, vol. 12, pp. 825–834, 2019.

[17] S. Srikanth, K. Abdelmohsen, E. K. Lee et al., "Translational control of TOP2A influences doxorubicin efficacy," *Molecular and Cellular Biology*, vol. 31, no. 18, pp. 3790–3801, 2011.

[18] J. Lu, M. Zhang, X. Yang, T. Cui, and J. Dai, "MicroRNA-548c-3p inhibits T98G glioma cell proliferation and migration by downregulating c-Myb," *Oncology Letters*, vol. 13, no. 5, pp. 3866–3872, 2017.

[19] Z. Luo, D. Li, X. Luo et al., "Decreased expression of miR-548c-3p in osteosarcoma contributes to cell proliferation via targeting ITGAV," *Cancer Biotherapy & Radiopharmaceuticals*, vol. 31, no. 5, pp. 153–158, 2016.

[20] H. Chang, N. Kim, J. H. Park et al., "Different microRNA expression levels in gastric cancer depending on *Helicobacter pylori* infection," *Gut and Liver*, vol. 9, no. 2, pp. 188–196, 2015.

[21] Y. Zhang, G. Talmon, and J. Wang, "MicroRNA-587 antagonizes F-UV induced apoptosis and confers drug resistance by regulating PPP2R1B expression in colorectal cancer," *Cell Death & Disease*, vol. 6, no. 8, article e1845, 2015.

[22] S. Yerukala Sathipati, H. L. Huang, and S. Y. Ho, "Estimating survival time of patients with glioblastoma multiforme and characterization of the identified microRNA signatures," *BMC Genomics*, vol. 17, article 1022, Supplement 13, 2016.

[23] L. Dong, J. Deng, Z. M. Sun et al., "Interference with the β-catenin gene in gastric cancer induces changes to the miRNA expression profile," *Tumor Biology*, vol. 36, no. 9, pp. 6973–6983, 2015.
Disease Markers

[24] F. Emamdoost, H. Khanahmad, M. Ganjalikhani-Hakemi, and A. Dooost, “The miR-125a-3p inhibits TIM-3 expression in AML cell line HL-60 in vitro,” Indian Journal of Hematology and Blood Transfusion, vol. 33, no. 3, pp. 342–347, 2017.

[25] G. Liu, L. Ji, M. Ke, Z. Ou, N. Tang, and Y. Li, “miR-125a-3p is responsible for chemosensitivity in PDAC by inhibiting epithelial-mesenchymal transition via Fyn,” Biomedicine & Pharmacotherapy, vol. 106, pp. 523–531, 2018.

[26] H. Zhang, X. Zhu, N. Li et al., “miR-125a-3p targets MTA1 to suppress NSCLC cell proliferation, migration, and invasion,” Acta Biochimica et Biophysica Sinica, vol. 47, no. 7, pp. 496–503, 2015.

[27] Y. Zhang, L. Yang, C. Ling, and W. Heng, “HuR facilitates cancer stemness of lung cancer cells via regulating miR-873/CDK3 and miR-125a-3p/CDK3 axis,” Biotechnology Letters, vol. 40, no. 4, pp. 623–631, 2018.

[28] C. Z. Zhang, “Long intergenic non-coding RNA 668 regulates VEGFA signaling through inhibition of miR-297 in oral squamous cell carcinoma,” Biochemical and Biophysical Research Communications, vol. 489, no. 4, pp. 404–412, 2017.

[29] J. E. Wilusz and P. A. Sharp, “A circuitous route to noncoding RNA,” Science, vol. 340, no. 6131, pp. 440–441, 2013.

[30] Q. Wu, Y. Wang, M. Cao et al., “Homology-independent discovery of replicating pathogenic circular RNAs by deep sequencing and a new computational algorithm,” Proceedings of the National Academy of Sciences of the United States of America, vol. 109, no. 10, pp. 3938–3943, 2012.

[31] E. Lasda and R. Parker, “Circular RNAs: diversity of form and function,” RNA, vol. 20, no. 12, pp. 1829–1842, 2014.

[32] H. Suzuki and T. Tsukahara, “A view of pre-mRNA splicing from RNase R resistant RNAs,” International Journal of Molecular Sciences, vol. 15, no. 6, pp. 9331–9342, 2014.

[33] R. A. Tessler, M. Dellinger, M. K. Richards et al., “Pediatric gastric adenocarcinoma: a National Cancer Data Base review,” Journal of Pediatric Surgery, vol. 54, no. 5, pp. 1029–1034, 2019.

[34] R. Lu, Y. Shao, G. Ye, B. Xiao, and J. Guo, “Low expression of hsa_circ_0006633 in human gastric cancer and its clinical significances,” Tumor Biology, vol. 39, no. 6, 2017.

[35] “RegRNA 2.0,” http://regrna2.mbc.nctu.edu.tw/.

[36] “CircInteractome,” https://circinteractome.nia.nih.gov/.

[37] E. Van Cutsem, M. Dicato, R. Geva et al., “The diagnosis and management of gastric cancer: expert discussion and recommendations from the 12th ESMO/World Congress on Gastrointestinal Cancer, Barcelona, 2010,” Annals of Oncology, vol. 22, Supplement 5, pp. v1–v9, 2011.

[38] M. P. Lutz, J. R. Zalberg, M. Duceux et al., “Highlights of the EORTC St. Gallen International Expert Consensus on the primary therapy of gastric, gastroesophageal and oesophageal cancer – Differential treatment strategies for subtypes of early gastroesophageal cancer,” European Journal of Cancer, vol. 48, no. 16, pp. 2941–2953, 2012.

[39] I. J. Choi, J. H. Lee, Y. I. Kim et al., “Long-term outcome comparison of endoscopic resection and surgery in early gastric cancer meeting the absolute indication for endoscopic resection,” Gastrointestinal Endoscopy, vol. 81, no. 2, pp. 333–41.e1, 2015.

[40] Y. I. Kim, Y. W. Kim, I. Choi et al., “Long-term survival after endoscopic resection versus surgery in early gastric cancers,” Endoscopy, vol. 47, no. 4, pp. 293–302, 2015.

[41] Japanese Gastric Cancer Association, “Japanese gastric cancer treatment guidelines 2014 (ver. 4),” Gastric Cancer, vol. 20, no. 1, pp. 1–19, 2017.

[42] E. C. Smyth, M. Verheij, W. Allum et al., “Gastric cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up,” Annals of Oncology, vol. 27, Supplement_5, pp. v38–v49, 2016.

[43] J. A. Ajani, T. A. D’Amico, K. Almhanna et al., “Gastric cancer, version 3.2016, NCCN Clinical Practice Guidelines in Oncology,” Journal of the National Comprehensive Cancer Network, vol. 14, no. 10, pp. 1286–1312, 2016.

[44] T. Yoshikawa, K. Tanabe, N. Nishikawa et al., “Accuracy of CT staging of locally advanced gastric cancer after neoadjuvant chemotherapy: cohort evaluation within a randomized phase II study,” Annals of Surgical Oncology, vol. 21, Supplement 3, pp. 385–389, 2014.

[45] K. Muro, H. C. Chung, V. Shankaran et al., “Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial,” The Lancet Oncology, vol. 17, no. 6, pp. 717–726, 2016.

[46] C. Gomez-Martin, J. C. Plaza, R. Pazo-Cid et al., “Level of HER2 gene amplification predicts response and overall survival in HER2-positive advanced gastric cancer treated with trastuzumab,” Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology, vol. 31, no. 35, pp. 4445–4452, 2013.