



# Bioinformatic and integrated analysis identifies an lncRNA–miRNA–mRNA interaction mechanism in gastric adenocarcinoma

Yong Liao<sup>1</sup> · Wen Cao<sup>2</sup> · Kunpeng Zhang<sup>1</sup> · Yang Zhou<sup>1</sup> · Xin Xu<sup>1</sup> · Xiaoling Zhao<sup>1</sup> · Xu Yang<sup>1</sup> · Jitao Wang<sup>1</sup> · Shouwen Zhao<sup>1</sup> · Shiyu Zhang<sup>1</sup> · Longfei Yang<sup>1</sup> · Dengxiang Liu<sup>1</sup> · Yanpeng Tian<sup>4</sup> · Weizhong Wu<sup>3</sup>

Received: 12 October 2020 / Accepted: 16 March 2021 / Published online: 29 March 2021

© The Genetics Society of Korea 2021

## Abstract

**Background** lncRNAs–miRNAs–mRNAs networks play an important role in Gastric adenocarcinoma (GA). Identification of these networks provide new insight into the role of these RNAs in gastric cancer.

**Objectives** Biological information databases were screened to characterize and examine the regulatory networks and to further investigate the potential prognostic relationship this regulation has in GA.

**Methods** By mining The Cancer Genome Atlas (TCGA) database, we gathered information on GA-related lncRNAs, miRNAs, and mRNAs. We identified differentially expressed (DE) lncRNAs, miRNAs, and mRNAs using R software. The lncRNA–miRNA–mRNA interaction network was constructed and subsequent survival examination was performed. Representative genes were selected out using The Biological Networks Gene Ontology plug-in tool on Cytoscape. Additional analysis of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) terms were used to screen representative genes for functional enrichment. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) were used to identify the expression of five candidate differential expressed RNAs.

**Results** Information of samples from 375 cases of gastric cancer and 32 healthy cases (normal tissues) were downloaded from the TCGA database. A total of 1632 DE-mRNAs, 1008 DE-lncRNAs and 104 DE-miRNAs were identified and screened. Among them, 65 DE-lncRNAs, 10 DE-miRNAs, and 10 DE-mRNAs form lncRNAs–miRNAs–mRNAs regulatory network. Additionally, 10 lncRNAs and 2 mRNAs were associated with the prognosis of GA. Multivariable COX analysis revealed that AC018781.1 and VCAN-AS1 were independent risk factors for GA. GO functional enrichment analysis found DE-mRNA was significantly enriched TERM ( $P < 0.05$ ). The KEGG signal regulatory network analysis found 11 significantly enrichment networks, the most prevailing was for the AGE-RAGE signaling pathway associated with Diabetic complications. Results of RT-qPCR was consistent with the in silico results.

**Conclusions** The results of the present study represent a view of GA from a analysis of lncRNA, miRNA and mRNA. The network of lncRNA–miRNA–mRNA interactions revealed here may potentially further experimental studies and may help biomarker development for GA.

**Keywords** Gastric adenocarcinoma · DE-RNA · LncRNAs · MiRNAs · MRNAs

## Introduction

Gastric cancer is one of the highest-incidence and highest-mortality tumors and poses a major challenge to human health worldwide (Ferlay et al. 2013; Jemal et al. 2011; Torre et al. 2015; Znaor et al. 2013). In China, gastric cancer has been revealed as the most common malignant tumor of the digestive tract. Data further suggests gastric cancer is becoming more prevalent, with males being more susceptible than females, and most cases were detected in adults

Yong Liao, Wen Cao and Kunpeng Zhang contributed equally to this work.

✉ Yanpeng Tian  
tianyanyanp2020@163.com

✉ Weizhong Wu  
wwzdh@sohu.com

Extended author information available on the last page of the article

aged between 55 and 70 (Catalano et al. 2009). Additionally, inflammation caused by *Helicobacter pylori* (HP) is closely related to gastric cancer. Inflammatory-related malignant transformation is a biological process involving multiple cells, genes, and non-coding RNAs (Migita et al. 2018; Senol et al. 2014; Wang et al. 2017a, b). Gastric adenocarcinoma is a type of gastric cancer that results from the deterioration of gastric gland cells, accounting for 95% of all gastric cancers (Blank et al. 2014; Dixon et al. 2016). Early diagnosis can significantly improve treatment prognosis of this cancer type, but the lack of effective early diagnosis approaches means most patients are often detected in late-stage or already have developed metastasis, reducing the effectiveness of treatment. As a result, elucidating the underlying mechanisms during the development and progression of GA is critical for the advancement of new tumor biomarkers and therapeutic targets.

Between 80 and 90% of human RNAs are non-coding RNAs. In the last decade, researches have described how many non-coding RNAs play important roles in various cellular events (Song et al. 2013). The mature miRNA is composed of approximately 22 nucleotides (nt), which are recognized by the ribonucleoprotein in the cell and assembled into an RNA-induced silencing complex (RISC). RISC can result in mRNA degradation or inhibition of mRNA translation by pairing with the 3' region of mRNA completely or incompletely (Kim et al. 2009), which can significantly modulate gene expression. Long non-coding RNAs (lncRNAs) are > 200 nt sized transcripts that are not translated. The abnormal expression of lncRNAs has been considered to be involved in various tumorigenic processes. It has been reported that changes in the expression level of lncRNA are closely related to the occurrence of gastric cancer (Fang et al. 2015). Work has shown that lncRNAs have microRNA responsible elements (MRE), the binding site that can sponge miRNAs, so that miRNA-mediated post-transcriptional regulation of target mRNAs can be impaired. In 2011, after clarifying the interaction between PTEN and its pseudogene PTENP1, these transcripts were named competitive endogenous RNA (ceRNA) (Poliseno et al. 2010). Recently, various reports have clarified the presence of ceRNA networks in a variety of cancers including gastric cancer (Xia et al. 2014). Identification of gastric cancer-associated ceRNA regulatory networks has been suggested to provide insight into the role of these RNAs in tumorigenesis and treatment outcomes in gastric cancer.

In this study, we constructed a regulatory network between lncRNAs–miRNAs–mRNAs. Here 10 lncRNAs and 2 mRNAs were identified to be associated with the prognosis of GA. Analysis further revealed that AC018781.1 and VCAN-AS1 are independent risk factors for GA. Through Gene Ontology (GO) enrichment analysis of DE(differentially expressed)-mRNA, it was found that it was

significantly enriched in TERM ( $P < 0.05$ ), and the Kyoto Encyclopedia of Genes and Genomes (KEGG) signal regulatory network analysis found that DE-mRNA was enriched in AGE-RAGE signaling pathway in the diabetic complications pathway. The differential expression profile analysis of three candidate lncRNAs and two mRNAs were confirmed by RT-qPCR. Ultimately, a promising ceRNA regulatory network related to the progression of GC was successfully identified. This novel approach of predicting GA related lncRNA and lncRNA–miRNA–mRNA networks could help to understand the underlying mechanism of GA.

## Materials and methods

### Database screening

The GA-associated gene (miRNA, lncRNA, mRNA) expression data was collected from the TCGA database (<https://gdc-portal.nci.nih.gov/>). This included 375 GA samples and 32 samples from normal tissues. Data was merged and gene ID conversion was performed by Perl script allow the development of Gene expression matrix.

### Identifying differentially expressed genes

The expression matrix of lncRNAs, miRNAs and mRNAs were analyzed by R language edge R package (version 3.5.1) to obtain the expression matrix of differentially expressed RNAs (cut-off: false discovery rate (FDR) < 0.01 and  $|\text{fold change}| > 2$ ). Correlated pairs of DE-lncRNAs and DE-miRNAs, as well as DE-miRNAs and DE-mRNA were evaluated by using Perl scripts. The lncRNAs–miRNAs–mRNAs regulation loops were obtained based on lncRNAs–miRNAs and miRNAs–mRNAs regulation pairs. The regulatory relationship between DE-lncRNAs and DE-miRNAs pairs were predicted by miRcode (<http://www.mircode.org>). The regulatory relationship between DE-miRNAs and DE-gene (DEG) pairs were predicted by miRDB, miRTarBase, TargetScan. Using the R language Venn diagram the regulatory pairs of DE-miRNAs and DEGs as well as DE-lncRNAs and DE-miRNAs (that have opposite expression trends with each other) were selected to construct a Wayne map of regulatory networks.

### Single factor, multi-factor COX regression

Univariate analysis was used to assess DE-lncRNA, DE-mRNA, DE-miRNA in combination with clinical pathological data. DE-RNAs with P value less than 0.05 were selected for multivariate COX regression analysis. This analysis was used to develop a prognostic model of gastric cancer and ROC curve was made to verify the model.

## ceRNAs regulation network map construction and survival analysis

The integrated co-expression network of DE-lncRNAs, DE-miRNAs and DE-mRNAs were visualized by Cytoscape software. Further, prognostic DEGs, DE-lncRNAs, and DE-miRNAs in the ceRNAs network were identified, and Kaplan–Meier survival plots of representative miRNAs, lncRNAs and mRNAs were drawn by the R survival package.

## Functional enrichment analysis

GO analysis was performed using the BINGO plugin of Cytoscape software. KEGG analysis was performed using KOBAS (<http://kobas.cbi.pku.edu.cn/>).

## RNA extraction and reverse transcription quantitative polymerase chain reaction (RT-qPCR) assay

Total RNA of GA samples and matched adjacent normal samples in 6 patients were obtained TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) and then was reversely transcribed into complementary DNA (cDNA) (PrimeScript™RT reagent Kit with gDNA Eraser, Takara, Otsu, Shiga, Japan). Real Time-PCR was performed using TaKaRa TB Green™ Premix Ex Taq™ II (Takara, Otsu, Shiga, Japan). GAPDH was used as reference genes to normalized the expression of candidate genes. Sequence or primers was showed in Table 1. The study was approved by Ethics Committee of Xingtai People's Hospital of Hebei Medical University and informed consents from all patients.

**Table 1** PCR primers used for qRT-PCR

Primer	Sequence (5'–3')
VCAN-AS1	5'-AGAGCATGTTTCCTTGGCTTT-3' 5'-TATGTCAGCTGTGATGTGGCA-3'
POU6F2-AS2	5'-ACAGCAGTGCCAGAAGGAGTATTG-3' 5'-GCAGACCTGAGCTTGTGAGTGAC-3'
IGF2-AS	5'-TCCACACCAGACAGCACAGACC-3' 5'-TCCGTGGTTGGCTCCAGGTG-3'
ATAD2	5'-GGAATCCCAAACCACTGGACA-3' 5'-GGTAGCGTCGTCGTAAAGCACA-3'
SERPINE1	5'-TCTCTTTGTGGTTCGGCACA-3' 5'-TTCGTCCCAAATGAAGGCGT-3'
GAPGH	5'-GCTCTCTGCTCCTCCTGTTC-3' 5'-ACGACCAAATCCGTTGACTC-3'

## Results

### Identification of DE-lncRNAs, DE-miRNAs and DE-mRNAs associated with GA

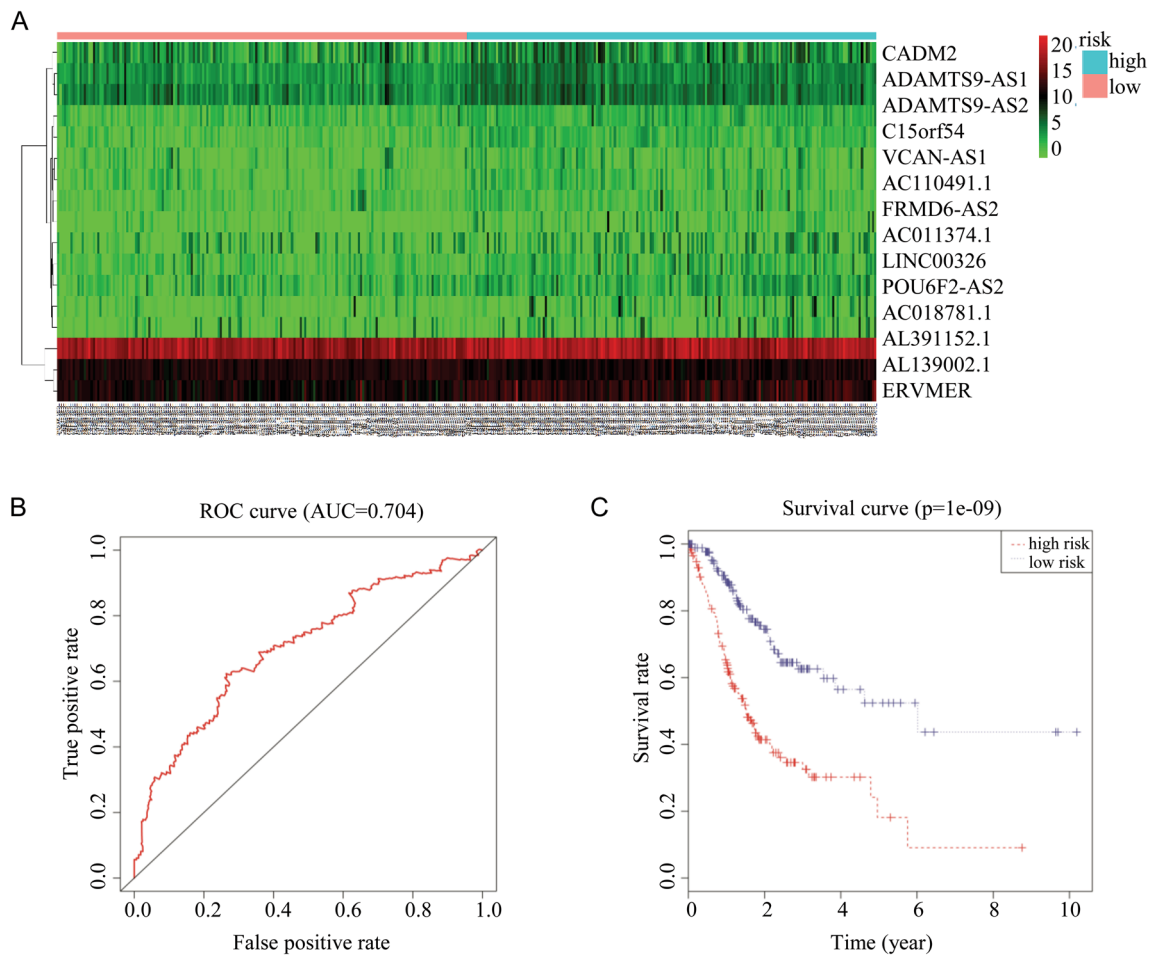
After analysis of RNA-seq and miRNA-seq data from TCGA, a total of 1632 mRNAs, 1008 lncRNAs, and 104 miRNAs were identified and screened for differential expression. Targeted predictive analysis using miRDB, miRTarBase and TargetScan indicated that mRNA expressed in GA contains potential targets for lncRNA and miRNA, of which 10 mRNA, 65 lncRNA and 10 miRNA are differentially expressed RNA (DE-RNA) (supplement 1).

### Identification of DE-lncRNAs, DE-miRNAs and DE-mRNAs that are associated with GA survival

Single-factor COX analysis of DE-RNA ( $P < 0.001$ ) suggested that 17 DE-RNAs (CADM2, ADAMTS9-AS1, ADAMTS9-AS2, C15orf54, VCAN-AS1, AC110491.1, FRMD6-AS2, AC011374.1, LINC00326, POU6F2-AS2, AC018781.1, AL391152.1, AL139002.1, ERVMER, COL1A1, ATAD2, SERPINE1) are risk factors for GA (supplement 2). A heat map was constructed as shown in (Fig. 1a). Multivariate Cox regression analysis showed that 10 combined DE-RNAs can predict GA prognostic, while AC018781.1 and VCAN-AS1 were predicted independent prognostic factors of GA (Table 2). Using these 10 DE-RNAs to construct a prognostic model, the survival time of low-risk patients was significantly higher than that of high-risk patients (Fig. 1b). In addition, ROC analysis indicated good predictability as area under the curve (AUC) was reported as 0.704, 95%CI (0.639–0.749) (Fig. 1c).

### Kaplan–Meier Survival analysis of DE-lncRNAs, DE-miRNA, DE-mRNA for GA

A total of 10 DE-lncRNAs (AC010145.1, AC018781.1, ADAMTS9-AS1, ADAMTS9-AS2, AL139002.1, AL391152.1, IGF2-AS, LINC00326, POU6F2-AS2, VCAN-AS1) (Fig. 2a–j) and two DE-mRNAs (ATAD2, SERPINE1) (Fig. 2k, l) were found to be associated with survival rates of GA ( $P < 0.05$ ) by Kaplan–Meier analyses. Additionally, no DE-miRNAs were found to be related to the prognosis of GA.

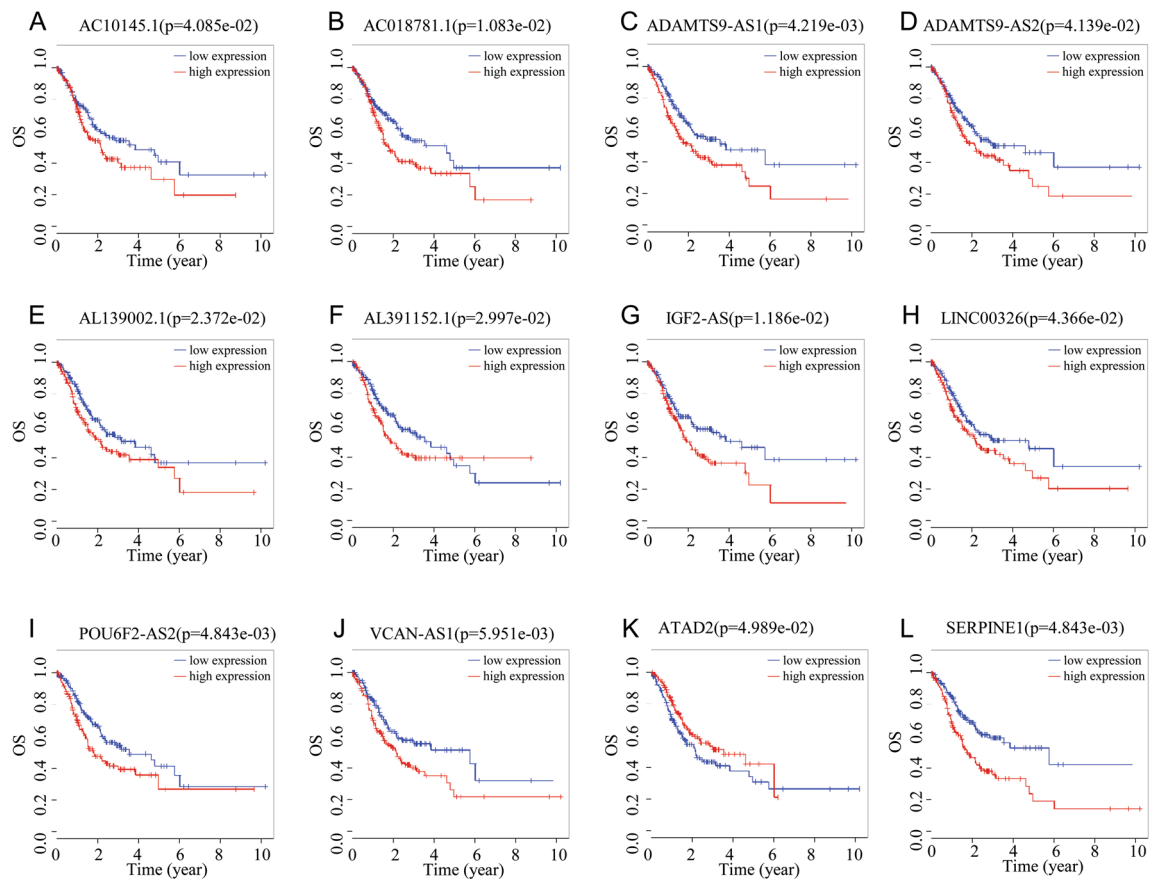


**Fig. 1** The identification of DE-lncRNAs, DE-miRNAs and DE-mRNAs that are associated with GA. **a** Construction of a DE-RNA heat map that affects the development of GA. **b** ROC analysis of DE-RNA predictability. **c** Survival analysis of DE-RNA and prognosis of GA

**Table 2** Multivariate Cox regression analysis for significant DE-RNAs

Type		Coef	Exp(coef)	SE(coef)	z	P
LncRNA	AC018781.1	0.1240	1.1320	0.0607	2.04	0.041*
LncRNA	ADAMTS9-	0.1242	1.1323	0.0785	1.58	0.113
LncRNA	AS1	0.0749	1.0778	0.0467	1.61	0.108
LncRNA	AL139002.1	0.0917	1.0960	0.0542	1.69	0.091
LncRNA	AL391152.1	0.1247	1.1328	0.0677	1.84	0.065
LncRNA	C15orf54	0.0658	1.0681	0.0423	1.56	0.120
LncRNA	ERVMER61-1	0.0812	1.0846	0.0542	1.50	0.134
LncRNA	LINC00326	0.1456	1.1567	0.0736	1.98	0.048*
mRNA	VCAN-AS1	-0.1983	0.8201	0.1118	-1.77	0.076
mRNA	ATAD2	0.0989	1.1039	0.0685	1.44	0.149
	SERPINE1					

\* $P < 0.05$ , the independent influence factors of GC. Cox regression equation for prognosis:  $y = 0.1240 \times AC018781.1 + 0.1242 \times ADAMTS9-AS1 + 0.0749 \times AL139002.1 + 0.0917 \times AL391152.1 + 0.1247 \times C15orf54 + 0.0658 \times ERVMER61-1 + 0.0812 \times LINC00326 + 0.1456 \times VCAN-AS1 - 0.1983 \times ATAD2 + 0.0989 \times SERPINE1$



**Fig. 2** Kaplan–Meier Survival analysis of DE-lncRNAs (**a** AC10145.1, **b** AC018781.1, **c** ADAMTS9-AS1, **d** ADAMTS9-AS2, **e** AL139002.1, **f** AL391152.1, **g** IGF2-AS, **h** LINC00326, **i**

**j** VCAN-AS1), DE-mRNA for GA (**k** ATAD2, **l** SERPINE1). Blue lines represent low expression and red lines represent high expression

### Construction of a ceRNA regulatory network seen in GA

DE-lncRNA, DE-miRNA, and DE-mRNA were used to construct a ceRNA network to further analyze the regulatory relationship. A total of 169 pairs of lncRNA-miRNA and 16 pairs of DE-miRNAs-DE mRNAs were involved in the construction of ceRNA network map (Fig. 3). The lncRNAs-miRNAs-mRNAs regulatory network includes 85 nodes and 184 edges.

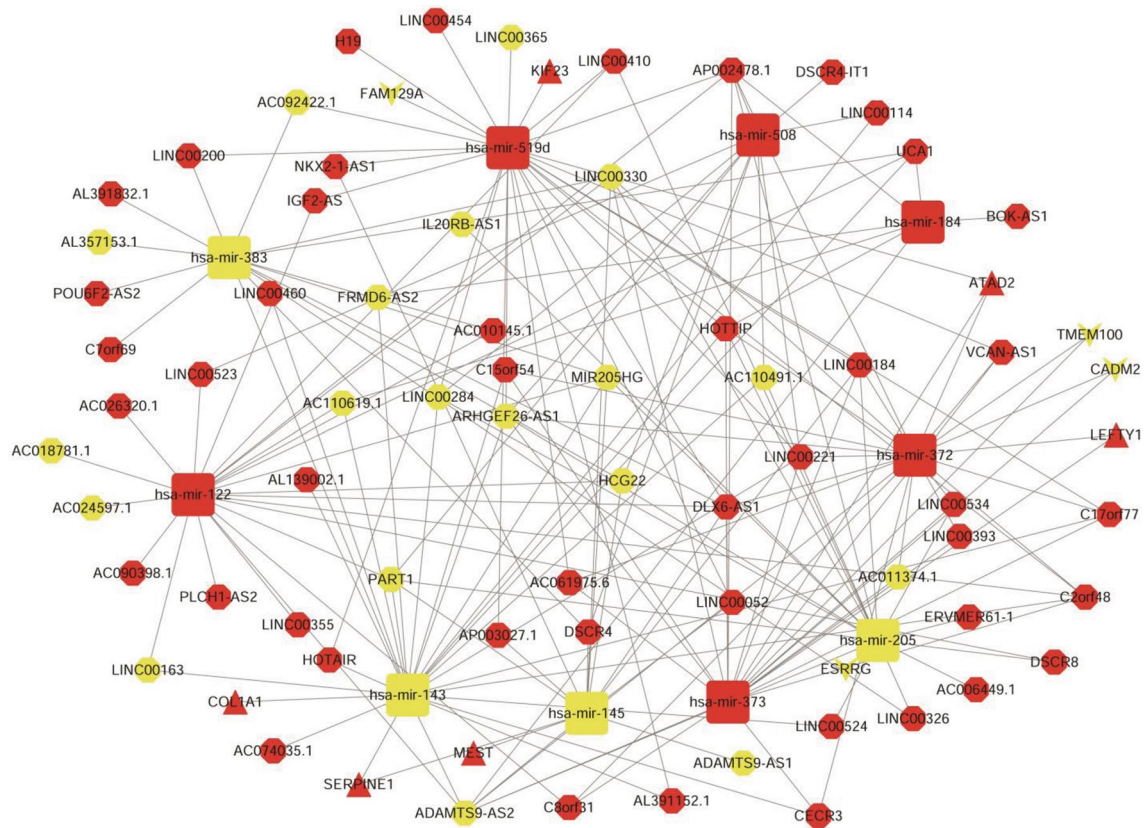
### Functional analysis of GO and KEGG pathways that are regulated in GA

GO analysis of 10 DE-mRNAs revealed that the three genes ESRRG, ATAD2, and COL1A1 were enriched in the "positive regulation of transcription, DNA-templated"

function on biologic processes (Table 3). As shown in Fig. 4, a total of 108 nodes and 166 edges constitute a functional regulatory network. KEGG analysis revealed 11 signaling pathways ( $P < 0.05$ ) the most significant among them was the AGE-RAGE signaling pathway associated with diabetic complications (Fig. 5).

### Expression of candidate DE-RNAs in patients

To further verify our findings, three DE-lncRNAs (VCAN-AS1, IGF2-AS, IPOU6F2-AS2) and two DE-mRNAs (ATAD2, SERPINE1) were further explored by RT-qPCR. The expression of VCAN-AS1, IGF2-AS, IPOU6F2-AS2 and SERPINE1 was higher in GA than that in matched adjacent normal samples. The expression of ATAD2 was lower in GA than that in matched adjacent normal samples (Fig. 6). These results were consistent with bioinformatic analysis.



**Fig. 3** Construction of a ceRNAs regulatory network for GA. Triangles represent mRNA, squares represent miRNAs, and circles represent lncRNA. Red represents high expression and yellow represents low expression

**Table 3** Representative DE-mRNAs were selected for gene ontology analysis with DAVID

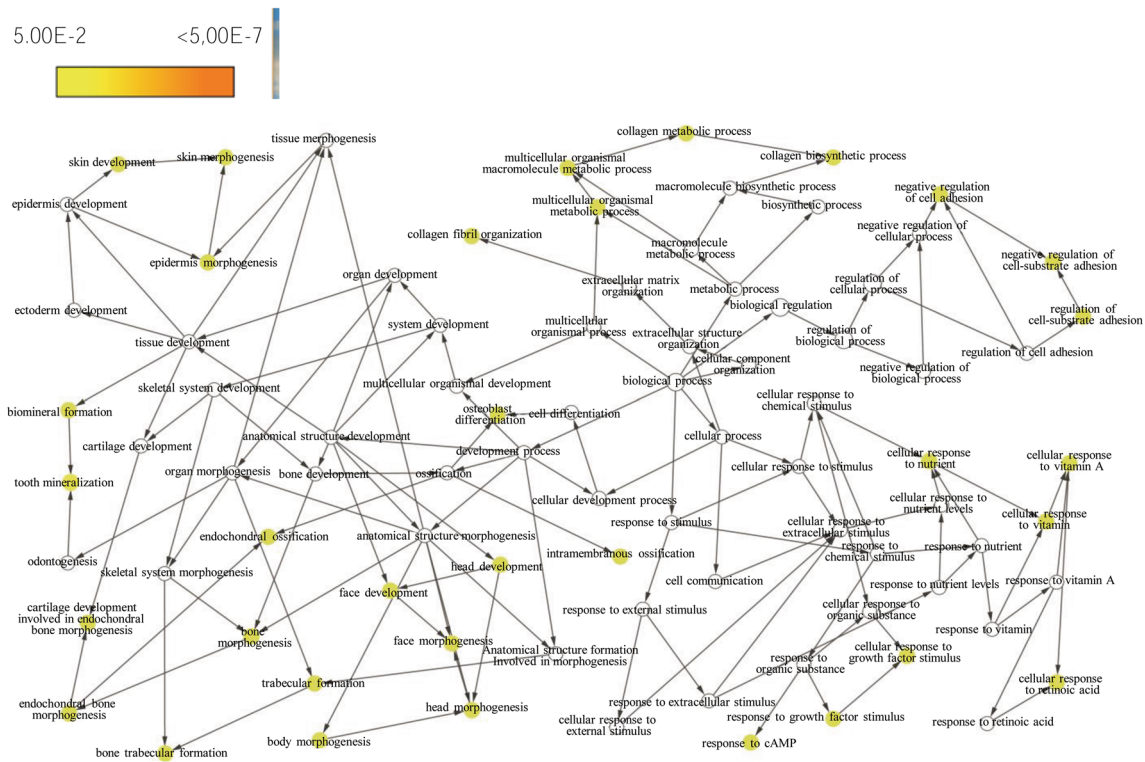
Category	Term	Count	%	P value	Genes
GOTERM_BP_DIRECT	GO:0045893 ~ positive regulation of transcription, DNA-templated	3	30	0.029296692*	ESRRG, ATAD2, COL1A1
GOTERM_BP_DIRECT	GO:0045944 ~ positive regulation of transcription from RNA polymerase II promoter	3	30	0.093402401	SERPINE1, ESRRG, ATAD2
GOTERM_MF_DIRECT	GO:0016887 ~ ATPase activity	2	20	0.093461135	KIF23, ATAD2

\*P < 0.05

## Discussion

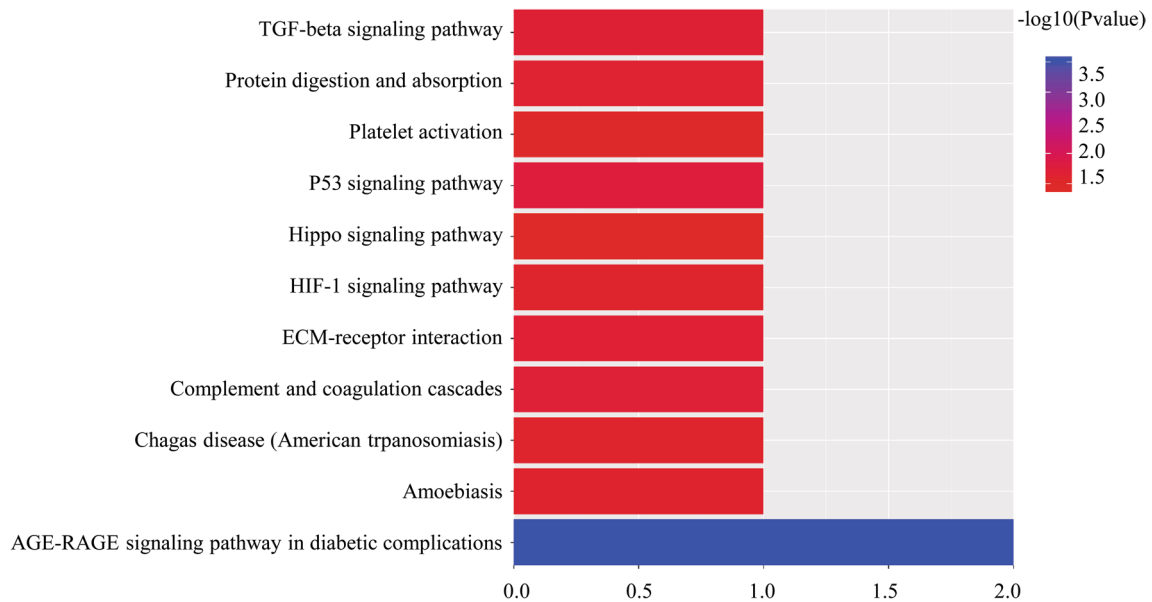
Epidemiological investigations have suggested the main contributing factors to GA development are *Helicobacter pylori* infection, diet, lifestyle, host gene type, and smoking (Kato and Asaka 2012; Uemura et al. 2001). The pathological process of GA is complex involving multiple genetic and phenotypic factors. GA is not just the result of the expression of specific prognostic genes, but also the complement of miRNA and lncRNAs (Chong et al. 2018), several post-transcriptional events like methylation level (Maeda et al.

2018) and other translational events. The development of GA entails the formation of a complex regulatory network involving RNAs. However, many studies are currently limited to specific genes or specific gene regulatory pathways. Few studies have examined the regulation network involving lncRNAs–miRNAs–mRNAs in GA. Here we explore the ceRNA regulatory network through bioinformatics and integrated analysis, with the aim to identify key genes involved in the development of GA and provide data that may be used in the development of molecular biomarkers and targeted drug screen for GA.



**Fig. 4** Functional analysis of GO in GA. Each node stands for a certain process and a larger size indicates a larger number of genes involved in the process. The colored nodes indicate statistical differ-

ence ( $P < 0.05$ ). White-colored nodes were only used to connect the biologic processes without statistical difference

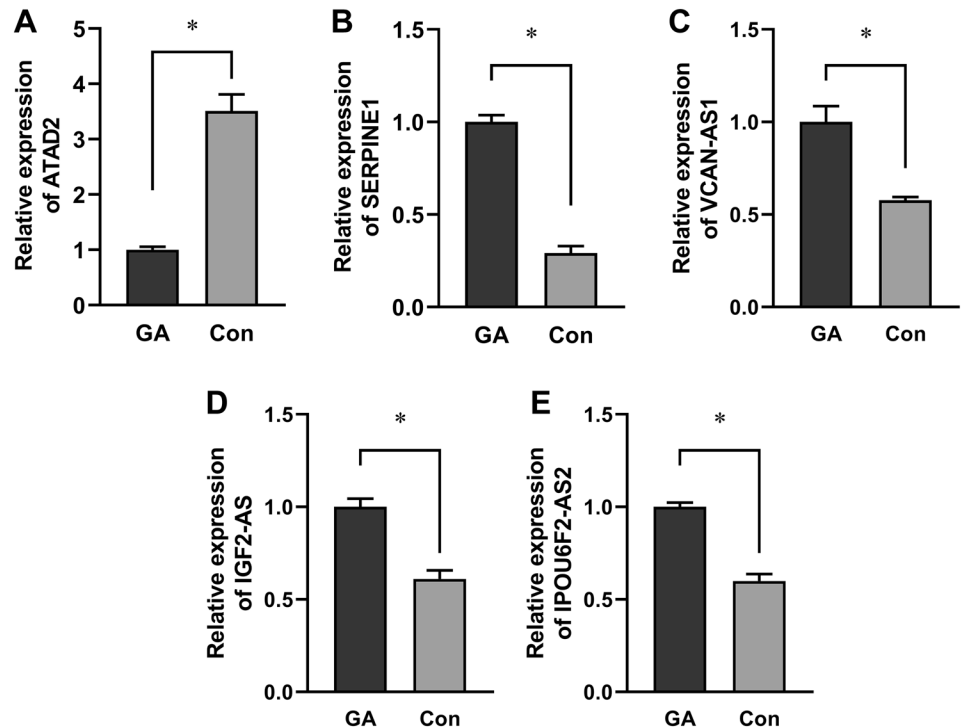


**Fig. 5** KEGG pathways in GA. The color represents the P value and the length represents the number of enriched genes

In this study, we investigated the factors affecting the development of GA by constructing lncRNAs–miRNAs–mRNAs regulatory network based on the ceRNA

hypothesis. To achieve this goal, we collected the expression data of GA-related genes (miRNAs, lncRNAs and mRNAs) from the TCGA database, and selected 1632

**Fig. 6** Relative expression of ATAD2 (a), SERPINE1 (b), VCAN-AS1 (c), IGF2-AS (d), IPOU6F2-AS2 (e). *GA* gastric adenocarcinoma, *Con* adjacent normal samples



DE-mRNAs, 1008 DE-lncRNAs, and 104 DE-miRNA. From this, a ceRNA regulatory network was developed from 65 DE-lncRNAs, 10 DE-miRNAs and 10 DE-mRNAs. At the same time, we identified 10 DE-lncRNAs (AC010145.1, AC018781.1, ADAMTS9-AS1, ADAMTS9-AS2, AL139002.1, AL391152.1, IGF2-AS, LINC00326, POU6F2-AS2, VCAN-AS1) and 2 DE-mRNAs (ATAD2, SERPINE1) that were associated with survival time of GA. Studies have shown that ADAMTS9-AS2 can participate in gastric cancer cell proliferation, apoptosis, migration and invasion. Furthermore, ADAMTS9-AS2 has been found to play a key role in the development of gastric cancer by regulating PI3K/Akt pathway (Cao et al. 2018). In addition, the SERPINE1 gene is involved in tumor gene activation (Rivas-Ortiz et al. 2017), and is currently studied in esophageal cancer (Klimczak-Bitner et al. 2016), rectal cancer (Wang et al. 2017a, b), endometrial cancer (Yildirim et al. 2017), head and neck cancer (Pavon et al. 2016) and other cancers. High expression of SERPINE1 is associated with poor prognosis in these cancer patients. These research results confirm the analysis results of our study, which proves confidence in the data generated. Additionally, for the COX regression analysis, we included AC018781.1, ADAMTS9-AS1, AL139002.1, AL391152.1, C15orf54, ERVMER61-1, LINC00326, VCAN-AS1, ATAD2 and SERPINE1 genes in the model. This analysis revealed that AC018781.1 and VCAN-AS1 are independent risk factors for GA. To identify these results, RT-qPCR were performed. VCAN-AS1, IGF2-AS, IPOU6F2-AS2 and

SERPINE1 was higher expressed but ATAD2 was lower expressed in GA than that in matched adjacent normal samples. These data indicate that VCAN-AS1, IGF2-AS, IPOU6F2-AS2 and SERPINE1 may play an oncogene role in GA, while ATAD2 may play a role of tumor suppressor gene in GA, and further studies are needed to confirm that they are potential tumor biomarkers.

The lncRNAs appear to play an important regulatory role in the modulation of gene expression. LncRNAs can bind endogenous miRNA to play a part in the ceRNA network (Cesana et al. 2011; Kallen et al. 2013; Wang et al. 2013). Previous studies have constructed a gastric cancer-related lncRNA-mRNA network showing that lncRNA RP11-363E7.4 was a key regulator both in the topology and random walk with a restart analysis. This new method which predicts gastric cancer-related lncRNA and lncRNA-miRNA-mRNA networks helps to understand the underlying mechanisms of gastric cancer (Wang et al. 2018).

Another study using bioinformatics conducted on gastric cancer in patients from India, predicted 19 lncRNA-regulated miRNAs and mRNAs related to gastric cancer (Arun et al. 2018). This study also conducted a comprehensive network analysis of lncRNA, miRNA and mRNA to determine the presence of ceRNA networks (Arun et al. 2018). In addition, studies on specific lncRNA expression patterns and ceRNA networks in gastric cancer have revealed that gastric cancer-specific lncRNAs are associated with clinical features, and these lncRNAs can be used as new candidate biomarkers and potential prognostic indicators for clinical diagnosis of gastric



cancer (Li et al. 2016). In this study, we constructed a GA-related ceRNA network map to further analyze the regulatory relationship between lncRNA, miRNA and mRNA. A total of 169 pairs of lncRNA-miRNA and 16 pairs of DE-miRNAs-DE mRNAs were involved in the construction of ceRNA network map. The lncRNAs-miRNAs-mRNAs regulatory network included 85 nodes and 184 edges, and such networks are important for further study of biomarkers and potential prognostic indicators of GA.

Further, we performed functional enrichment and pathway analysis of DE-RNAs. GO analysis of 10 DE-mRNAs revealed that three genes *ESRRG*, *ATAD2* and *COL1A1* were enriched on the "positive regulation of transcription, DNA-templated" biologic processes. In this case, a total of 108 nodes, and 166 edges constituted a functional regulation network. Additionally, KEGG analysis revealed 11 signaling pathways, of which the AGE-RAGE signaling pathway associated with diabetic complications was the most significant. Interestingly, recent studies have shown that the AGE-RAGE axis composed of AGE and its receptor RAGE plays an important role in the development of various tumors (Abe and Yamagishi 2008; Taguchi et al. 2000). RAGE is highly expressed in prostate cancer (Allmen et al. 2008; Ishiguro et al. 2005), liver cancer (Yaser et al. 2012), Pancreatic cancer (DiNorcia et al. 2012) and associated with tumor growth and progression. These results suggest that the prognosis of GA can be improved by further evaluation of key regulatory genes.

## Conclusions

In summary, we established a ceRNA regulatory network for lncRNAs-miRNAs-mRNAs in GA. The current findings provide new insights into the role of ceRNA networks in GA and identify potential diagnostic and prognostic biomarkers. Furthermore, the analysis provides a new reference for a better understanding of the pathogenesis of GA. Although our findings have important clinical implications, they still have certain limitations. First of all, the analysis uses a straightforward TCGA database which lacks any diversity as all the participants are Caucasian. Whether it is suitable to extrapolate findings to other ethnic groups is not clear yet, and further research is needed. Secondly, this study only examines microarray data and does not carry out any functional tests to verify the analysis. It will be important for further studies to verify these results.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s13258-021-01086-z>.

**Acknowledgements** This work was supported in part by the Key Research and Development Planning Project of Hebei Province of China (Grant no. 18277717D), the Scientific Research Foundation of

Hebei Municipal Commission of Health and Family Planning (Grant no. 20181612), the Science and Technology Project of Xingtai (Grant no. 2017ZC106), the Science and Technology Project of Xingtai (Grant no. 2017ZC113), the Subsidy Project of Introduction of Overseas Talents in Hebei Province (Grant no. C201861).

**Author contributions** (I) Conception and design: WW, YT and YL; (II) Administrative support: None; (III) Provision of study materials or patients: WC, KZ, YZ; (IV) Collection and assembly of data: WC, XX, XZ, SZ; (V) Data analysis and interpretation: SZ, LY, DL; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors; (VIII) Funding of sources: XY, JW.

## Declarartions

**Conflict of interest** Yong Liao, Wen Cao, Kunpeng Zhang, Yang Zhou, Xin Xu, Xiaoling Zhao, Xu Yang, Jitao Wang, Shouwen Zhao, Shiyu Zhang, Longfei Yang, Dengxiang Liu, Yanpeng Tian, Weizhong Wu declare that they have no conflict of interest.


## References

- Abe R, Yamagishi S (2008) AGE-RAGE system and carcinogenesis. *Curr Pharm Des* 14:940–945. <https://doi.org/10.2174/138161208784139765>
- Allmen EU, Koch M, Fritz G, Legler DF (2008) V domain of RAGE interacts with AGEs on prostate carcinoma cells. *Prostate* 68:748–758. <https://doi.org/10.1002/proc.20736>
- Arun K, Arunkumar G, Bennet D, Chandramohan SM, Murugan AK, Munirajan AK (2018) Comprehensive analysis of aberrantly expressed lncRNAs and construction of ceRNA network in gastric cancer. *Oncotarget* 9:18386–18399. <https://doi.org/10.18632/oncotarget.24841>
- Blank S et al (2014) A retrospective comparative exploratory study on two methyltetrahydrofolatereductase (MTHFR) polymorphisms in esophagogastric cancer: the A1298C MTHFR polymorphism is an independent prognostic factor only in neoadjuvantly treated gastric cancer patients. *BMC Cancer* 14:58. <https://doi.org/10.1186/1471-2407-14-58>
- Cao B, Liu C, Yang G (2018) Down-regulation of lncRNA ADAMTS9-AS2 contributes to gastric cancer development via activation of PI3K/Akt pathway. *Biomed Pharmacother* 107:185–193. <https://doi.org/10.1016/j.biopha.2018.06.146>
- Catalano V, Labianca R, Beretta GD, Gatta G, de Braud F, Van Cutsem E (2009) Gastric cancer. *Crit Rev OncolHematol* 71:127–164. <https://doi.org/10.1016/j.critrevonc.2009.01.004>
- Cesana M et al (2011) A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. *Cell* 147:358–369. <https://doi.org/10.1016/j.cell.2011.09.028>
- Chong DQ, Shan JL, Yang CS, Wang R, Du ZM (2018) Clinical prognostic value of A FOXM1 related long non-coding RNA expression in gastric cancer. *Eur Rev Med PharmacolSci* 22:417–421. [https://doi.org/10.26355/eurrev\\_201801\\_14190](https://doi.org/10.26355/eurrev_201801_14190)
- DiNorcia J et al (2012) RAGE gene deletion inhibits the development and progression of ductal neoplasia and prolongs survival in a murine model of pancreatic cancer. *J GastrointestSurg* 16(104–112):112. <https://doi.org/10.1007/s11605-011-1754-9>
- Dixon M, Mahar AL, Helyer LK, Vasilevska-Ristovska J, Law C, Coburn NG (2016) Prognostic factors in metastatic gastric cancer: results of a population-based, retrospective cohort study in Ontario. *Gastric Cancer* 19:150–159. <https://doi.org/10.1007/s10120-014-0442-3>

- Fang XY, Pan HF, Leng RX, Ye DQ (2015) Long noncoding RNAs: novel insights into gastric cancer. *Cancer Lett* 356:357–366. <https://doi.org/10.1016/j.canlet.2014.11.005>
- Ferlay J et al (2013) Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. *Eur J Cancer* 49:1374–1403. <https://doi.org/10.1016/j.ejca.2012.12.027>
- Ishiguro H, Nakaigawa N, Miyoshi Y, Fujinami K, Kubota Y, Uemura H (2005) Receptor for advanced glycation end products (RAGE) and its ligand, amphoterin are overexpressed and associated with prostate cancer development. *Prostate* 64:92–100. <https://doi.org/10.1002/pros.20219>
- Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. *CA Cancer J Clin* 61:69–90. <https://doi.org/10.3322/caac.20107>
- Kallen AN et al (2013) The imprinted H19 lncRNA antagonizes let-7 microRNAs. *Mol Cell* 52:101–112. <https://doi.org/10.1016/j.molcel.2013.08.027>
- Kato M, Asaka M (2012) Recent development of gastric cancer prevention. *Jpn J Clin Oncol* 42:987–994. <https://doi.org/10.1093/jco/hys151>
- Kim VN, Han J, Siomi MC (2009) Biogenesis of small RNAs in animals. *Nat Rev Mol Cell Biol* 10:126–139. <https://doi.org/10.1038/nrm2632>
- Klimczak-Bitner AA, Kordek R, Bitner J, Musial J, Szemraj J (2016) Expression of MMP9, SERPINE1 and miR-134 as prognostic factors in esophageal cancer. *Oncol Lett* 12:4133–4138. <https://doi.org/10.3892/ol.2016.5211>
- Li CY et al (2016) Integrated analysis of long non-coding RNA competing interactions reveals the potential role in progression of human gastric cancer. *Int J Oncol* 48:1965–1976. <https://doi.org/10.3892/ijo.2016.3407>
- Maeda M et al (2018) Novel epigenetic markers for gastric cancer risk stratification in individuals after *Helicobacter pylori* eradication. *Gastric Cancer* 21:745–755. <https://doi.org/10.1007/s10120-018-0803-4>
- Migita K et al (2018) The prognostic significance of inflammation-based markers in patients with recurrent gastric cancer. *Surg Today* 48:282–291. <https://doi.org/10.1007/s00595-017-1582-y>
- Pavon MA, Arroyo-Solera I, Cespedes MV, Casanova I, Leon X, Mangues R (2016) uPA/uPAR and SERPINE1 in head and neck cancer: role in tumor resistance, metastasis, prognosis and therapy. *Oncotarget* 7:57351–57366. <https://doi.org/10.18632/oncotarget.10344>
- Poliseno L, Salmena L, Zhang J, Carver B, Haveman WJ, Pandolfi PP (2010) A coding-independent function of gene and pseudogene mRNAs regulates tumour biology. *Nature* 465:1033–1038. <https://doi.org/10.1038/nature09144>
- Rivas-Ortiz CI, Lopez-Vidal Y, Arredondo-Hernandez L, Castillo-Rojas G (2017) Genetic alterations in gastric cancer associated with *Helicobacter pylori* infection. *Front Med (Lausanne)* 4:47. <https://doi.org/10.3389/fmed.2017.00047>
- Senol K, Ozkan MB, Vural S, Tez M (2014) The role of inflammation in gastric cancer. *Adv Exp Med Biol* 816:235–257. [https://doi.org/10.1007/978-3-0348-0837-8\\_10](https://doi.org/10.1007/978-3-0348-0837-8_10)
- Song H et al (2013) Long non-coding RNA expression profile in human gastric cancer and its clinical significances. *J Transl Med* 11:225. <https://doi.org/10.1186/1479-5876-11-225>
- Taguchi A et al (2000) Blockade of RAGE-amphoterin signalling suppresses tumour growth and metastases. *Nature* 405:354–360. <https://doi.org/10.1038/35012626>
- Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A (2015) Global cancer statistics, 2012. *CA Cancer J Clin* 65:87–108. <https://doi.org/10.3322/caac.21262>
- Uemura N et al (2001) *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med* 345:784–789. <https://doi.org/10.1056/NEJMoa001999>
- Wang Y et al (2013) Endogenous miRNA sponge lincRNA–RoR regulates Oct4, Nanog, and Sox2 in human embryonic stem cell self-renewal. *Dev Cell* 25:69–80. <https://doi.org/10.1016/j.devcel.2013.03.002>
- Wang K et al (2017a) Prognostic value of systemic immune-inflammation index in patients with gastric cancer. *Chin J Cancer* 36:75. <https://doi.org/10.1186/s40880-017-0243-2>
- Wang T, Xu H, Liu X, Chen S, Zhou Y, Zhang X (2017b) Identification of key genes in colorectal cancer regulated by miR-34a. *Med Sci Monit* 23:5735–5743. <https://doi.org/10.12659/msm.904937>
- Wang P et al (2018) A novel lncRNA–miRNA–mRNA triple network identifies lncRNA RP11–363E7.4 as an important regulator of miRNA and gene expression in gastric cancer. *Cell Physiol Biochem* 47:1025–1041. <https://doi.org/10.1159/000490168>
- Xia T, Liao Q, Jiang X, Shao Y, Xiao B, Xi Y, Guo J (2014) Long non-coding RNA associated-competing endogenous RNAs in gastric cancer. *Sci Rep* 4:6088. <https://doi.org/10.1038/srep06088>
- Yaser AM et al (2012) The role of receptor for advanced glycation end products (RAGE) in the proliferation of hepatocellular carcinoma. *Int J Mol Sci* 13:5982–5997. <https://doi.org/10.3390/ijms13055982>
- Yildirim ME, Karakus S, Kurtulgan HK, Kilicgun H, Ersan S, Bakir S (2017) The association of plasminogen activator inhibitor type 1 (PAI-1) level and PAI-1 4G/5G gene polymorphism with the formation and the grade of endometrial cancer. *Biochem Genet* 55:314–321. <https://doi.org/10.1007/s10528-017-9796-7>
- Znaor A et al (2013) Cancer incidence and mortality patterns in South Eastern Europe in the last decade: gaps persist compared with the rest of Europe. *Eur J Cancer* 49:1683–1691. <https://doi.org/10.1016/j.ejca.2012.11.030>

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

## Authors and Affiliations

Yong Liao<sup>1</sup> · Wen Cao<sup>2</sup> · Kunpeng Zhang<sup>1</sup> · Yang Zhou<sup>1</sup> · Xin Xu<sup>1</sup> · Xiaoling Zhao<sup>1</sup> · Xu Yang<sup>1</sup> · Jitao Wang<sup>1</sup> · Shouwen Zhao<sup>1</sup> · Shiyu Zhang<sup>1</sup> · Longfei Yang<sup>1</sup> · Dengxiang Liu<sup>1</sup> · Yanpeng Tian<sup>4</sup>  · Weizhong Wu<sup>3</sup>

<sup>1</sup> Department of Hepatobiliary Surgery, Xingtai People's Hospital of Hebei Medical University, Xingtai 054001, Hebei, People's Republic of China

<sup>2</sup> Department of Neurology, The Second Hospital of Hebei Medical University, Shijiazhuang 050000, Hebei, People's Republic of China

<sup>3</sup> Department of General Surgery, The First Hospital of Hebei Medical University, No. 89 Donggang Road, Shijiazhuang 050000, Hebei, People's Republic of China

<sup>4</sup> Department of Obstetrics and Gynecology, The Second Hospital of Hebei Medical University, No. 215 West Heping Road, Shijiazhuang 050000, Hebei, People's Republic of China