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

**Manuscript NO: 37473**

**Manuscript type: ORIGINAL ARTICLE**

***Basic Study***

three-miRNA indicator predicts prognosis of gastric cancer patients with bioinformatics analysis

Zhang C *et al.* Prognosis prediction of gastric cancer patients

Cheng Zhang, Chun-dong Zhang, Ming-hui Ma, Dong-qiu Dai

Cheng Zhang, Chun-dong Zhang, Ming-hui Ma, Dong-qiu Dai, Department of Gastroenterological Surgery, the Fourth Affiliated Hospital of China Medical University, Shenyang 110032, Liaoning province, China

**ORCID number:** Cheng Zhang (0000-0001-5317-8775); Chun-Dong Zhang (0000-0002-5274-5210); Ming-Hui Ma (0000-0002-2566-0932); Dong-Qiu Dai (0000-0002-1154-3276).

**Author contributions:** Dai DQ designed this study; Zhang C, Zhang CD and Ma MH conducted the data analysis; Zhang C wrote the article.

**Supported by** National Natural Science Foundation of China, No. 30572162; and Natural Science Foundation of Liaoning Province, No. 201602817.

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

**Data sharing statement:** No additional data are available.

**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: Dong-Qiu Dai, PhD, Chief Doctor, Professor, Surgical Oncologist**, Department of Gastroenterological Surgery, the Fourth Affiliated Hospital of China Medical University, 4 Chongshan Road, Shenyang 110032, Liaoning province, China. daidq63@163.com

**Telephone:** +86-24-62043110

**Fax:** +86-24-62043110

**Received:** December 9, 2017

**Peer-review started:** December 10, 2017

**First decision:** December 21, 2017

**Revised:** December 25, 2017

**Accepted:** January 17, 2018

**Article in press:**

**Published online:**

**Abstract**

***AIM***

To identify multiple miRNAs for predicting the prognosis of gastric cancer (GC) patients with bioinformatics analysis.

***METHODS***

The original microarray datasets GSE93415, which included 20 GC and 20 adjacent normal gastric mucosae, was downloaded from the Gene Expression Omnibus database and used for screening the differentially expressed miRNAs (DEMs). The cut-off criteria were *P* < 0.05 and fold change > 2.0. In addition, we acquired the miRNAs expression profiles and clinical information of 361 GC patients from The Cancer Genome Atlas database to assess the prognostic role of the DEMs. The targets genes of miRNAs were predicted by TargetScan, miRDB, miRWalk and DIANA websites, then the common target genes were selected for functional enrichment analysis.

***RESULTS***

A total of 110 DEMs including 19 up-regulated and 91 down-regulated miRNAs were identified between 20 pairs of GC and adjacent normal tissues, the Kaplan-Meier survival analysis found that a three-miRNA indicator (miR-145-3p, miR-125b-5p and miR-99a-5p) had an obvious correlation with the survival of GC patients. Furthermore, Univariate and multivariate Cox regression analysis indicated that the three-miRNA indicator could be a significant prognostic marker of GC patients. The common target genes of the three-miRNA are added up to 108 and used for Gene Functional Enrichment analysis. Biological Process and Molecular Function showed that the target genes are involved in cell recognition, gene silencing and nucleic acid bingding, transcription factor activity, transmembrane receptor activity. Cellular Component revealed that the genes are portion of nucleus, chromatin silencing complex and TORC1/2 complex. Biological Pathway indicated that the genes participate in several cancer-related pathways, such as focal adhesion, PI3K and mTOR signaling pathway.

***CONCLUSION***

This study justified that a three-miRNA indicator could play a role in predicting the survival of GC patients.

**Key words:** Gastric cancer; Differentially expressed miRNAs; Prognosis; Gene functional enrichment; Bioinformatic analysis

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

**Core tip:** We identified 110 differentially expressed miRNAs through mining the datasets of Gene Expression Omnibus database and acquired the miRNAs expression profiles and clinical information of 361 gastric cancer (GC) patients from The Cancer Genome Atlas database. Multiple miRNAs together acting as biomarkers may have a stronger reliability in survival prediction. Our study found that a novel three-miRNA indictor could be used for predicting the prognosis of GC patients.

Zhang C, Zhang CD, Ma MH, Dai DQ. three-miRNA indicator predicts prognosis of gastric cancer patients with bioinformatics analysis.*World J Gastroenterol* 2018; In press

Introduction

Gastric cancer (GC) is the fourth most common cancer in incidence and the second in mortality among all cancers worldwide[1]. Each year a total of 989,600 individuals are newly diagnosed with GC and 738,000 deaths occur, therefore, this disease is a serious public health issue worldwide[2]. Research studies that explore the cellular and molecular mechanisms of GC development and the validation of novel biomarkers are urgently needed to achieve early diagnosis and treatment.

MicroRNAs (miRNAs), which are endogenous small noncoding RNAs (20–22 nt), have been identified as the key regulators of genes at the post-transcriptional level[3]. Increasing studies have found that miRNAs are associated with the development and progression of GC, and can act as important biomarkers in diagnosis[4,5], therapy[6] and prognosis[7,8]. Thus, the identification of differentially expressed miRNAs (DEMs) may contribute to the early diagnosis and the prediction of a survival prognosis in GC.

Several studies have found that a number of miRNAs are differentially expressed in GC and are associated with survival prognosis. However, these studies lack a large sample size or an appropriate proportion of samples. A reliable survival prediction requires large-scale samples that include detailed clinical characteristics. The Gene Expression Omnibus (GEO) database is a public functional genomics data repository that includes array- and sequence-based data and allows users to query and download experiments or curated gene expression profiles[9]. The Cancer Genome Atlas (TCGA, http://cancergenome.nih.gov) project is one of the most useful cancer genomics programs and has generated, analyzed and made available genomic sequence, expression, methylation and copy number variation data on over 11,000 individuals who represent over 30 different types of cancer[10]. In the present study, we identified DEMs between GC and adjacent normal tissues by analyzing the miRNA data of GSE93415 from GEO. In addition, the associations between DEMs and survival prognosis were analyzed using the expression profiles and clinical features downloaded from TCGA.

MATERIALS AND METHODS

**Microarray data processing and DEMs identification**

The microarray data of GSE93415 was downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/) and the miRNA expression data were processed with the limma package in R. Statistically significant DEMs between GC and adjacent normal samples were identified with the cut-off criterion P

< 0.05 and |logFC| > 1.0.

**Association analysis between DEMs and GC patients’ survival**

TCGA (https://cancergenome.nih.gov/) stomach adenocarcinoma and adjacent normal tissue miRNA sequencing data and clinical information were downloaded for analysis. The inclusion criteria included: (1) samples with completed data for analysis; (2) patients had not received preoperative chemoradiation; (3) overall survival time less than 80 mo. Consequently, 361 GC samples were included in the present study. The Kaplan–Meier and log-rank method were conducted to test the prognostic value of DEMs. When P < 0.05, miRNAs were considered significantly associated with the prognosis of patients. Then, we ranked prognosis-related miRNAs according to the median expression level. Subsequently, we scored each GC patient in accordance with a high or low-level of expression, and a risk grade was defined by the total scores. Finally, GC patients were sorted into high and low-risk groups by the risk-score rank. The prognosis-related miRNA indicator was used to analyze overall survival between high and low-risk group patients using a Kaplan–Meier curve.

**Target genes prediction of prognostic DEMs**

We used four online tools to predict the potential target genes of the prognostic related DEMs, including TargetScan (http://www.targetscan.org/vert\_71/), miRDB (http://www.mirdb.org/), miRWalk (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/index.html), and DIANA (http://www.microrna.gr/microT-CDS). In order to obtain the more reliable target genes, the Venn plot was performed to acquire the consensus genes of the four online tools.

**Function analysis of target genes**

FunRich: Functional Enrichment analysis tool (http://www.funrich.org/) is a stand-alone software used for functional enrichment and interaction network analysis of genes and proteins[11]. Enrichment analysis was conducted on the consensus genes using the FunRich tool in the following categories: Biological Process, Cellular Component, Molecular Function and Biological Pathways. P < 0.05 was considered as statistically significant.

**Statistical analysis**

The data of miRNAs expression in GC and adjacent normal samples were performed by unpaired t test. The association between DEMs expression and clinical characteristics were analyzed by The chi-square and t test. Kaplan-Meier survival analysis and the univariate/multivariate Cox regression analysis were used to assess the expression level of DEMs and prognostic features. All the statistical analysis was performed by IBM SPSS version 19.0 and P < 0.05 was defined as statistical significant.

RESULTS

**Identification of DEMs in GC**

The microarray data of GSE93415, including 20 pairs of GC and adjacent normal tissue samples, were obtained from the NCBI-GEO database. After applying cut-off criteria of *P* < 0.05 and |logFC| > 1.0, a total of 110 DEMs were identified between GC and adjacent normal tissues (Table 1). The results of 19 low-expression miRNAs and 91 high-expression miRNAs are displayed in the volcano plot (Figure 1). A heat map of hierarchic cluster analysis showed that DEMs could be discriminated between GC and normal tissues (Figure 2).

***Identification of DEMs related with overall survival in GC***

To identify the DEMs which could be used to predict the overall survival of GC patients, we collected 361 samples from TCGA to assess the relationship between DEMs and the overall survival of GC patients. The patients’ clinical characteristics including age at diagnosis, gender, race, TNM stage, and histologic grade are shown in Table 2. By using a log-rank test and Kaplan–Meier curve, we found that three DEMs (miR-145-3p, miR-125b-5p, and miR-99a-5p) were negatively associated with overall survival (Figure 3). The association analysis between the three DEMs and clinical characteristics indicated that miR-145-3p, miR-125b-5p, and miR-99a-5p were all significantly associated with histologic grade (*P* < 0.05). The detailed results are shown in Table 3.

***Prognostic role of three DEMs indicator in GC patients***

We ranked the three DEMs by the median of expression and then scored each GC patient in accordance with high or low-level expression. A risk grade was defined by the total scores. As a result, all the 361 GC patients were sorted into a high or low-risk group. A survival analysis with the Kaplan–Meier method and log-rank test was conducted. The results indicated that the overall survival between the high-risk and low-risk groups was significantly different (*P* = 0.045). Interestingly, compared to patients in the high-risk group, the low-risk patients tended to have a better prognosis (Figure 4). Furthermore, we performed univariate and multivariate Cox regression analysis to verify the prognostic role of the three-DEM indicator according to clinical features. The univariate analysis showed that pathologic stage (HR = 1.825, *P* < 0.001), T stage (HR = 1.864, *P* = 0.006), N stage (HR = 2.005, *P* = 0.001) and the three-DEM indicator (HR = 1.422, *P* = 0.039) were significantly associated with the prognostic outcome of GC patients. The multivariate analysis revealed that T stage (HR = 1.623, *P* = 0.044) and the three DEMs (HR = 1.451, *P* = 0.032) were all independent factors in predicting the prognostic value of GC patients (Table 4).

***Targets prediction of three DEMs and gene function analysis***

The online target prediction tools TargetScan, miRDB, miRWalk, and DIANA were used to predict the targets genes of miR-145-3p, miR-125b-5p, and miR-99a-5p. We then obtained the consensus genes of each DEM from the four online predictions (Figure 5). As a result, we identified a 107 consensus target genes. Furthermore, we conducted gene enrichment analysis to identify the biological function of common target genes (Figure 6). The Biological Process analysis indicated that the genes were mostly enriched in cell recognition, regulation of nucleic acid and gene silencing. Cellular Component analysis indicated that genes were enriched in the nucleus, RNA-induced silencing complex, chromatin silencing complex and phosphoinositide 3-kinase complex. Molecular Function showed genes were enriched in transmembrane receptor, transcription regulator and transcription factor activity. Biological Pathways were mainly enriched in the VEGFR signaling network, PI3K/Akt signaling and mTOR signaling pathways.

DISCUSSION

Due to the reduction in chronic Helicobacter pylori infection and improvement of sanitation, the incidence and mortality rates of GC have been declined in recent years[12]. However, there are still almost 460,000 new GC cases and 350,000 GC deaths each year in China[13]. The prognosis of GC patients is poor and the five-year survival rate is 5%-20% despite advances in GC therapy[14]. Thus, to improve the clinical treatment and management of GC patients, it is urgent to identify reliable prognostic biomarkers. In this study, we identified a total of 110 DEMs by analyzing the GSE93415 data and discovered that three miRNAs (miR-145-3p, miR-125b-5p and miR-99a-5p) were negatively associated with overall survival. Additionally, we constructed a three-miRNA indicator to predict the prognosis of GC patients.

For decades, a large number of studies have reported that miRNAs can play oncogenes or tumor suppressor roles in regulating cell biological behaviors of cells[15-18]. At present, several miRNAs are known to be useful in the early diagnosis of cancers, including miR-21[19], miR-486[20], miR-24[21]and miR-125a-5p[22]. In addition, miR-191[23], miR-1908[24], miR-200c[25] and miR-217[26] were found to be potential prognostic indictors in cancer. However, these studies only used a single indictor or a limited number of patients for survival analysis. In this study, we identified DEMs by analyzing the array data from the GEO database and found that three highly expressed miRNAs (miR-145-3p, miR-125b-5p and miR-99a-5p) may be potential prognostic indictors in GC. Kaplan-Meier and Log-rank test survival analysis indicated that the three-miRNA indicator can be used to predict the prognosis of GC patients.

We then searched present publications online to compare and test our findings. Chang *et al*[27] showed that miR-125b-5p was overexpression in GC patients and promoted invasion and metastasis of GC by targeting *STARD13* and *NEU1*. miR-125b-5p also indicated that it could be a potential biomarker for predicting prognoses and clinical outcomes in patients with HER2-positive GC that receive trastuzumab treatment[28]. Wu et al. found that miR-125b-5p promotes cell migration and invasion by targeting PPP1CA-Rb signal pathways and acts as an independent prognostic factor in GC[29]. Furthermore, Zhang *et al*[30] demonstrated that miR-99a-5p might function as a novel molecule to regulate cisplatin resistance by directly targeting the calpain small subunit 1 (CAPNS1)-associated pathway in GC. Interestingly, there are no studies describing relations between miR-145-3p and GC. However, in non-small cell lung cancer, miR-145-3p was found to inhibit cancer cell migration and invasion by targeting *PDK1* via the mTOR signaling pathway[31]. Moreover, miR-145-3p was also identified to be down-regulated in metastatic castration-resistant prostate cancer and targeted four molecules which can be significantly predict survival in prostate cancer[32]. These results may suggest that miR-145-3p has a complicated effect in different cancers and, in future research, we will investigate the role of miR-145-3p in GC.

Dysregulated genes may participate in tumorigenesis and progression by aberrant signaling pathways. In this study, we predicted the target genes of the three miRNAs and performed gene functional enrichment analysis. The results showed that these target genes were associated with the process of gene silencing and cell recognition, as well as focal adhesion, EGFR and PI3K/Akt and mTOR signaling pathways. Xu *et al*[33] suggested that the EGFR-Akt signaling pathway regulates drug resistance in GC patients. The PI3K/Akt pathway was demonstrated to be associated with poor prognosis, tumor progression and resistance to systematic therapy in many cancers including GC[34,35]. In addition, the PI3K/Akt/mTOR pathway is a key signaling pathway that is reported to be involved in GC[36]. Thus, the results of our functional enrichment analysis are in accordance with present studies.

Above all, we identified a three-miRNA indicator for predicting the prognosis of patients with GC and analyzed potential signaling pathways in the development and progression of GC. However, to determine the genesis and development mechanism of GC, more large-scale and systematic investigations are required.

**Article Highlights**

***Research background***

Increasing studies have reported that microRNAs (miRNAs) play an important role in the development and progression of cancers, including gastric cancer (GC). Furthermore, miRNAs can also act as accurate biomarkers in diagnosis and prognosis prediction. In this study, we found that a three-miRNA indictor could be used for predicting the prognosis of GC patients and multiple miRNAs together acting as biomarkers may have a stronger reliability in survival prediction.

***Research motivation***

The worldwide incidence and mortality rates of GC are fairly high. Most of GC patients have been in the advanced stage when diagnosed and endure a poor prognosis. Identifying accuracy biomarkers in predicting prognosis of patients is an urgent issue to be solved, so that patients could have an individualized treatment and an improvement in prognosis.

***Research objectives***

We aim to identify multiple miRNAs for predicting the prognosis of patients with gastric cancer. After present study, we found that a three-miRNA (miR-145-3p, miR-125b-5p and miR-99a-5p) indicator could be used for predicting the prognosis of patients with gastric cancer. This objective could be applied to clinical practices and have a guidance role in improving the prognosis of patients with gastric cancer.

***Research methods***

We obtained the differentially expressed miRNAs by analyzing a microarray datasets from the Gene Expression Omnibus database with the limma package in R. The Kaplan-Meier and log-rank method were used for describing the survival curve. The target genes of the three miRNAs (miR-145-3p, miR-125b-5p and miR-99a-5p) were predicted by the online tools of TargetScan, miRDB, miRWalk and DIANA. Venn plot was performed to obtain the common target genes from these four online tools. Enrichment analysis was conducted on the consensus genes using the *FunRich* tool.

***Research results***

In the present study, we found that a three-miRNA (miR-145-3p, miR-125b-5p and miR-99a-5p) indictor could be used for predicting the prognosis of patients with gastric cancer. Multiple miRNAs together acting as prognosis-related biomarkers may have a stronger reliability and this finding could be useful in clinical treatment according to gastric cancer patients with different prognosis. However, the role of miR-145-3p in the tumorigenesis and progression of gastric cancer is remain unclear. Thus, this problem remains to be solved in our future study.

***Research conclusions***

Our study identified that the three miRNAs (miR-145-3p, miR-125b-5p and miR-99a-5p) were up-regulated in gastric cancer patients by analyzing a microarray datasets. Besides, the novel three-miRNA indictor could be used for predicting the prognosis of patients with gastric cancer. Multiple miRNAs together acting as prognosis-related biomarkers may have a stronger reliability, so that our finding could be useful in clinical treatment according to gastric cancer patients with different prognosis.

***Research perspectives***

This study provides us with a new insight that multiple miRNAs together used for predicting the prognosis of patients with gastric cancer. In order to further confirm the prognostic value of the three-miRNA indictor, our future research may focus on exploring the relation between miR-145-3p and gastric cancer.

References

1 **Piazuelo MB**, Correa P. Gastric cáncer: Overview. *Colomb Med* (Cali) 2013; **44**: 192-201 [PMID: 24892619]

2 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]

3 **Filipowicz W**, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet* 2008; **9**: 102-114 [PMID: 18197166 DOI: 10.1038/nrg2290]

4 **Wang D**, Fan Z, Liu F, Zuo J. Hsa-miR-21 and Hsa-miR-29 in Tissue as Potential Diagnostic and Prognostic Biomarkers for Gastric Cancer. *Cell Physiol Biochem* 2015; **37**: 1454-1462 [PMID: 26509997 DOI: 10.1159/000438514]

5 **Liu X**, Kwong A, Sihoe A, Chu KM. Plasma miR-940 may serve as a novel biomarker for gastric cancer. *Tumour Biol* 2016; **37**: 3589-3597 [PMID: 26456959 DOI: 10.1007/s13277-015-4019-5]

6 **Merhautova J**, Demlova R, Slaby O. MicroRNA-Based Therapy in Animal Models of Selected Gastrointestinal Cancers. *Front Pharmacol* 2016; **7**: 329 [PMID: 27729862 DOI: 10.3389/fphar.2016.00329]

7 **Hou CG**, Luo XY, Li G. Diagnostic and Prognostic Value of Serum MicroRNA-206 in Patients with Gastric Cancer. *Cell Physiol Biochem* 2016; **39**: 1512-1520 [PMID: 27614739 DOI: 10.1159/000447854]

8 **Zhang L**, Huang Z, Zhang H, Zhu M, Zhu W, Zhou X, Liu P. Prognostic value of candidate microRNAs in gastric cancer: A validation study. *Cancer Biomark* 2017; **18**: 221-230 [PMID: 27983528 DOI: 10.3233/CBM-160091]

9 **Barrett T**, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, Yefanov A, Lee H, Zhang N, Robertson CL, Serova N, Davis S, Soboleva A. NCBI GEO: archive for functional genomics data sets--update. *Nucleic Acids Res* 2013; **41**: D991-D995 [PMID: 23193258 DOI: 10.1093/nar/gks1193]

10 **Wang Z**, Jensen MA, Zenklusen JC. A Practical Guide to The Cancer Genome Atlas (TCGA). *Methods Mol Biol* 2016; **1418**: 111-141 [PMID: 27008012 DOI: 10.1007/978-1-4939-3578-9\_6]

11 **Pathan M**, Keerthikumar S, Ang CS, Gangoda L, Quek CY, Williamson NA, Mouradov D, Sieber OM, Simpson RJ, Salim A, Bacic A, Hill AF, Stroud DA, Ryan MT, Agbinya JI, Mariadason JM, Burgess AW, Mathivanan S. FunRich: An open access standalone functional enrichment and interaction network analysis tool. *Proteomics* 2015; **15**: 2597-2601 [PMID: 25921073 DOI: 10.1002/pmic.201400515]

12 **Parkin DM**. The global health burden of infection-associated cancers in the year 2002. *Int J Cancer* 2006; **118**: 3030-3044 [PMID: 16404738 DOI: 10.1002/ijc.21731]

13 **Chen W**, Zheng R, Zhang S, Zhao P, Li G, Wu L, He J. Report of incidence and mortality in China cancer registries, 2009. *Chin J Cancer Res* 2013; **25**: 10-21 [PMID: 23372337 DOI: 10.3978/j.issn.1000-9604.2012.12.04]

14 **Wang Z**, Cai Q, Jiang Z, Liu B, Zhu Z, Li C. Prognostic role of microRNA-21 in gastric cancer: a meta-analysis. *Med Sci Monit* 2014; **20**: 1668-1674 [PMID: 25230738 DOI: 10.12659/MSM.892096]

15 **Zhang B**, Pan X, Cobb GP, Anderson TA. microRNAs as oncogenes and tumor suppressors. *Dev Biol* 2007; **302**: 1-12 [PMID: 16989803 DOI: 10.1016/j.ydbio.2006.08.028]

16 **Nohata N**, Hanazawa T, Kinoshita T, Okamoto Y, Seki N. MicroRNAs function as tumor suppressors or oncogenes: aberrant expression of microRNAs in head and neck squamous cell carcinoma. *Auris Nasus Larynx* 2013; **40**: 143-149 [PMID: 22831895 DOI: 10.1016/j.anl.2012.07.001]

17 **Chen Y**, Fu LL, Wen X, Liu B, Huang J, Wang JH, Wei YQ. Oncogenic and tumor suppressive roles of microRNAs in apoptosis and autophagy. *Apoptosis* 2014; **19**: 1177-1189 [PMID: 24850099 DOI: 10.1007/s10495-014-0999-7]

18 **Babashah S**, Soleimani M. The oncogenic and tumour suppressive roles of microRNAs in cancer and apoptosis. *Eur J Cancer* 2011; **47**: 1127-1137 [PMID: 21402473 DOI: 10.1016/j.ejca.2011.02.008]

19 **Zeng Z**, Wang J, Zhao L, Hu P, Zhang H, Tang X, He D, Tang S, Zeng Z. Potential role of microRNA-21 in the diagnosis of gastric cancer: a meta-analysis. *PLoS One* 2013; **8**: e73278 [PMID: 24023850 DOI: 10.1371/journal.pone.0073278]

20 **Li W**, Wang Y, Zhang Q, Tang L, Liu X, Dai Y, Xiao L, Huang S, Chen L, Guo Z, Lu J, Yuan K. MicroRNA-486 as a Biomarker for Early Diagnosis and Recurrence of Non-Small Cell Lung Cancer. *PLoS One* 2015; **10**: e0134220 [PMID: 26237047 DOI: 10.1371/journal.pone.0134220]

21 **Fang Z**, Tang J, Bai Y, Lin H, You H, Jin H, Lin L, You P, Li J, Dai Z, Liang X, Su Y, Hu Q, Wang F, Zhang ZY. Plasma levels of microRNA-24, microRNA-320a, and microRNA-423-5p are potential biomarkers for colorectal carcinoma. *J Exp Clin Cancer Res* 2015; **34**: 86 [PMID: 26297223 DOI: 10.1186/s13046-015-0198-6]

22 **Wang P**, Yang D, Zhang H, Wei X, Ma T, Cheng Z, Hong Q, Hu J, Zhuo H, Song Y, Jia C, Jing F, Jin Q, Bai C, Mao H, Zhao J. Early Detection of Lung Cancer in Serum by a Panel of MicroRNA Biomarkers. *Clin Lung Cancer* 2015; **16**: 313-9.e1 [PMID: 25639977 DOI: 10.1016/j.cllc.2014.12.006]

23 **Gao X**, Xie Z, Wang Z, Cheng K, Liang K, Song Z. Overexpression of miR-191 Predicts Poor Prognosis and Promotes Proliferation and Invasion in Esophageal Squamous Cell Carcinoma. *Yonsei Med J* 2017; **58**: 1101-1110 [PMID: 29047233 DOI: 10.3349/ymj.2017.58.6.1101]

24 **Teng C**, Zheng H. Low expression of microRNA-1908 predicts a poor prognosis for patients with ovarian cancer. *Oncol Lett* 2017; **14**: 4277-4281 [PMID: 28943939 DOI: 10.3892/ol.2017.6714]

25 **Si L**, Tian H, Yue W, Li L, Li S, Gao C, Qi L. Potential use of microRNA-200c as a prognostic marker in non-small cell lung cancer. *Oncol Lett* 2017; **14**: 4325-4330 [PMID: 28943946 DOI: 10.3892/ol.2017.6667]

26 **Yang J**, Zhang HF, Qin CF. MicroRNA-217 functions as a prognosis predictor and inhibits pancreatic cancer cell proliferation and invasion via targeting E2F3. *Eur Rev Med Pharmacol Sci* 2017; **21**: 4050-4057 [PMID: 29028097]

27 **Chang S**, He S, Qiu G, Lu J, Wang J, Liu J, Fan L, Zhao W, Che X. MicroRNA-125b promotes invasion and metastasis of gastric cancer by targeting STARD13 and NEU1. *Tumour Biol* 2016; **37**: 12141-12151 [PMID: 27220320 DOI: 10.1007/s13277-016-5094-y]

28 **Sui M**, Jiao A, Zhai H, Wang Y, Wang Y, Sun D, Li P. Upregulation of miR-125b is associated with poor prognosis and trastuzumab resistance in HER2-positive gastric cancer. *Exp Ther Med* 2017; **14**: 657-663 [PMID: 28672982 DOI: 10.3892/etm.2017.4548]

29 **Wu JG**, Wang JJ, Jiang X, Lan JP, He XJ, Wang HJ, Ma YY, Xia YJ, Ru GQ, Ma J, Zhao ZS, Zhou R. MiR-125b promotes cell migration and invasion by targeting PPP1CA-Rb signal pathways in gastric cancer, resulting in a poor prognosis. *Gastric Cancer* 2015; **18**: 729-739 [PMID: 25240408 DOI: 10.1007/s10120-014-0421-8]

30 **Zhang Y**, Xu W, Ni P, Li A, Zhou J, Xu S. MiR-99a and MiR-491 Regulate Cisplatin Resistance in Human Gastric Cancer Cells by Targeting CAPNS1. *Int J Biol Sci* 2016; **12**: 1437-1447 [PMID: 27994509 DOI: 10.7150/ijbs.16529]

31 **Chen GM**, Zheng AJ, Cai J, Han P, Ji HB, Wang LL. microRNA-145-3p inhibits non-small cell lung cancer cell migration and invasion by targeting PDK1 via the mTOR signaling pathway. *J Cell Biochem* 2018; **119**: 885-895 [PMID: 28661070 DOI: 10.1002/jcb.26252]

32 **Goto Y**, Kurozumi A, Arai T, Nohata N, Kojima S, Okato A, Kato M, Yamazaki K, Ishida Y, Naya Y, Ichikawa T, Seki N. Impact of novel miR-145-3p regulatory networks on survival in patients with castration-resistant prostate cancer. *Br J Cancer* 2017; **117**: 409-420 [PMID: 28641312 DOI: 10.1038/bjc.2017.191]

33 **Xu H**, Miao ZF, Wang ZN, Zhao TT, Xu YY, Song YX, Huang JY, Zhang JY, Liu XY, Wu JH, Xu HM. HCRP1 downregulation confers poor prognosis and induces chemoresistance through regulation of EGFR-AKT pathway in human gastric cancer. *Virchows Arch* 2017; **471**: 743-751 [PMID: 28963677 DOI: 10.1007/s00428-017-2237-5]

34 **Lin HL**, Yang MH, Wu CW, Chen PM, Yang YP, Chu YR, Kao CL, Ku HH, Lo JF, Liou JP, Chi CW, Chiou SH. 2-Methoxyestradiol attenuates phosphatidylinositol 3-kinase/Akt pathway-mediated metastasis of gastric cancer. *Int J Cancer* 2007; **121**: 2547-2555 [PMID: 17680560 DOI: 10.1002/ijc.22963]

35 **Altomare DA**, Testa JR. Perturbations of the AKT signaling pathway in human cancer. *Oncogene* 2005; **24**: 7455-7464 [PMID: 16288292 DOI: 10.1038/sj.onc.1209085]

36 **Liu M**, Li CM, Chen ZF, Ji R, Guo QH, Li Q, Zhang HL, Zhou YN. Celecoxib regulates apoptosis and autophagy via the PI3K/Akt signaling pathway in SGC-7901 gastric cancer cells. *Int J Mol Med* 2014; **33**: 1451-1458 [PMID: 24676394 DOI: 10.3892/ijmm.2014.1713]

**P-Reviewer:** Kimura A, Matowicka-Karna J **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): B

Grade C (Good): 0

Grade D (Fair): D

Grade E (Poor): 0



**Figure 1 The volcano plot of the differentially expressed miRNAs.** A total of 110 DEMs were identified between 20 pairs of GC patients and adjacent normal tissues (cut-off criteria are *P* < 0.05 and |logFC| >1.0). The green and red spots represent low and high expression miRNAs respectively. DEMs: Differentially expressed miRNAs; GC: Gastric cancer.



**Figure 2 Hierarchical clustering analysis of the differentially expressed miRNAs between 20 pairs of gastric cancer and adjacent normal sample.** Each row represents the expression of miRNA and each column represents a sample: orange color for gastric cancer, blue for normal.



**Figure 3 Three differentially expressed miRNAs were negatively associated with overall survival in gastric cancer.** A: Patients with high level of miR-145-3p (*n* = 180) have a poorer prognosis than that with low level of miR-145-3p (*n* = 181) (*P* = 0.0049). B: Patients with high level of miR-125b-5p (*n* = 180) have a poorer prognosis than that with low level of miR-125b-5p (*n* = 181) (*P* = 0.0063). C: Patients with high level of miR-99a-5p (*n* = 180) have a poorer prognosis than that with low level of miR-99a-5p (*n* = 181) (*P* = 0.047).



**Figure 4 The Kaplan–Meier curve for the three-miRNA indicator in gastric cancer.** The three differentially expressed miRNAs were ranked by the median of expression and then scored for each gastric cancer patient in accordance with high or low-level expression. The low risk group (*n* = 175) and high risk group (*n* = 186) were defined by the total scores. Compare to low risk group, patients in high risk group have a poorer prognosis (*P* = 0.045).



Figure 5 The consensus target genes of each differentially expressed miRNA. The target genes of the three miRNAs were predicted by four online websites (TargetScan, miRDB, miRWalk and DIANA).



Figure 6 Genetic functional enrichment analysis. A total of 108 consensus genes were used for functional enrichment analysis with the tool of *FunRich*. A: Biological process analysis showed that the genes are involved in cell recognition and gene silencing; B: Cellular component analysis indicated that the genes are portion of nucleus, chromatin silencing complex and TORC1/2 complex; C: Molecular function results showed that are involved in nucleic acid bingding, transcription factor activity, transmembrane receptor activity; D: Biological pathway analysis indicated that the genes participate in focal adhesion, PI3K and mTOR cancer-related signaling pathway. TORC1/2: Target of rapamycin 1/2; PI3K: phosphatidylinositide 3-kinases; mTOR: Mammalian target of rapamycin.

**Table 1 The differentially expressed miRNAs identified between gastric cancer and adjacent normal tissues**

|  |  |  |  |
| --- | --- | --- | --- |
| **DEMs of high expression1** | ***P* value** | **DEMs of low expression** | ***P* value** |
| hsa-miR-199a-3p/hsa-miR-199b-3p | 7.10E-05 | hsa-miR-652-5p | 0.000135 |
| hsa-miR-125b-5p | 2.99E-05 | hsa-miR-1269b | 1.13E-09 |
| hsa-miR-199a-5p | 7.65E-07 | hsa-miR-665 | 2.86E-06 |
| hsa-miR-223-3p | 7.49E-06 | hsa-miR-375 | 0.003304 |
| hsa-miR-196a-5p | 3.22E-07 | hsa-miR-4501 | 1.64E-06 |
| hsa-miR-27a-3p | 0.000112 | hsa-miR-4279 | 1.94E-10 |
| hsa-miR-23b-3p | 4.71E-05 | hsa-miR-943 | 2.46E-05 |
| hsa-miR-21-5p | 1.36E-05 | hsa-miR-148a-3p | 1.53E-05 |
| hsa-miR-100-5p | 0.000197 | hsa-miR-1275 | 0.000349 |
| hsa-miR-20a-5p | 0.000102 | hsa-miR-4290 | 8.91E-11 |
| hsa-miR-23a-3p | 5.15E-10 | hsa-miR-4268 | 9.16E-11 |
| hsa-miR-1 | 0.002694 | hsa-miR-891a | 7.29E-10 |
| hsa-miR-214-3p | 1.23E-08 | hsa-miR-4795-3p | 3.07E-05 |
| hsa-miR-10a-5p | 2.33E-05 | hsa-miR-1298 | 3.00E-06 |
| hsa-miR-135b-5p | 1.04E-05 | hsa-miR-660-3p | 1.75E-08 |
| hsa-miR-99a-5p | 0.001109 | hsa-miR-4661-5p | 9.22E-08 |
| hsa-miR-20b-5p | 0.000365 | hsa-miR-4539 | 2.37E-09 |
| hsa-miR-199b-5p | 0.000291 | hsa-let-7d-3p | 1.10E-08 |
| hsa-miR-10b-5p | 1.83E-05 | hsa-miR-4636 | 1.94E-06 |
| hsa-miR-27b-3p | 1.95E-05 |
| hsa-miR-126-3p | 0.004079 |
| hsa-miR-130a-3p | 0.000442 |
| hsa-miR-142-3p | 0.002661 |
| hsa-miR-4291 | 1.12E-09 |
| hsa-miR-24-3p | 6.14E-06 |
| hsa-let-7a-5p | 0.0059 |
| hsa-miR-145-5p | 0.00066 |
| hsa-miR-17-5p | 3.84E-05 |
| hsa-miR-143-5p | 6.07E-05 |
| hsa-let-7f-5p | 0.001364 |
| hsa-miR-4328 | 0.001281 |
| hsa-miR-4324 | 3.91E-05 |
| hsa-miR-145-3p | 0.000389 |
| hsa-miR-143-3p | 0.006402 |
| hsa-miR-95 | 0.000106 |

1DEMs of high expression were listed according to the rank of fold changes. DEMs: differentially expressed miRNAs.

**Table 2 Clinical features of gastric cancer patients**

|  |  |
| --- | --- |
| **Variables** | **Case, *n* (%)** |
| Age at diagnosis |  |
| < 60 | 113 (31.3) |
| ≥ 60 | 248 (68.7) |
| Gender |  |
| Male | 241 (66.8) |
| Female | 120 (33.2) |
| T stage |  |
| T1 + T2 | 88 (24.4) |
| T3 + T4 | 273 (75.6) |
| Histologic grade |  |
| g1 + g2 | 134 (37.1) |
| g3 + g4 | 227 (62.9) |
| Race |  |
| White | 234 (64.8) |
| Asian | 83 (23.0) |
| Black or African American | 12 (3.3) |
| NA | 32 (8.9) |
| Pathologic stage |  |
| Stage I | 44 (12.2) |
| Stage II | 117 (32.4) |
| Stage III | 162 (44.9) |
| Stage IV | 30 (8.3) |
| NA | 8 (2.2) |
| Node status |  |
| N0 | 111 (30.7) |
| N1-3 | 249 (69.0) |
| NA | 1 (0.3) |
| Metastasis |  |
| M0 | 325 (90.0) |
| M1 | 22 (6.1) |
| Mx | 14 (3.9) |

NA: Not available.

**Table 3 Association between the three differentially expressed miRNAs and clinical features**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Variables** | **miR-145-3p expression** | | ***P* value** | **miR-125b-5p expression** | | | ***P* value** | **miR-99a-5p expression** | | | ***P* value** |
| Age at diagnosis | Low | High |  | Low | High | |  | Low | | High |  |
| < 60 | 51 | 62 | 0.225 | 42 | 71 | | 0.001a | 49 | | 64 | 0.096 |
| ≥ 60 | 129 | 119 | 138 | 110 | | 131 | | 117 |
| T stage |  |  |  |  |  |  | |  |  | |  |
| T1 + T2 | 40 | 48 | 0.342 | 51 | 37 | | 0.081 | 46 | | 42 | 0.603 |
| T3 + T4 | 140 | 133 | 129 | 144 | | 134 | | 139 |
| N stage |  |  |  |  |  |  | |  |  | |  |
| N0 | 58 | 53 | 0.567 | 57 | 54 | | 0.731 | 57 | | 54 | 0.731 |
| N1-3 | 119 | 124 | 120 | 123 | | 120 | | 123 |
| M stage |  |  |  |  |  |  | |  |  | |  |
| M0 | 163 | 162 | 0.670 | 165 | 160 | | 0.191 | 164 | | 161 | 0.386 |
| M1 | 10 | 12 | 8 | 14 | | 9 | | 13 |
| Histologic grade |  |  |  |  |  |  | |  |  | |  |
| g1 + g2 | 77 | 57 | 0.026a | 87 | 47 | | < 0.001a | 80 | | 54 | 0.004a |
| g3 + g4 | 103 | 124 | 93 | 134 | | 100 | | 127 |
| Pathologic stage |  |  |  |  |  |  | |  |  | |  |
| I + II | 81 | 80 | 0.876 | 86 | 75 | | 0.221 | 80 | | 81 | 0.954 |
| III + IV | 95 | 97 | 90 | 102 | | 96 | | 96 |

a*P* < 0.05, statistically significant.

**Table 4 Univariate and multivariate Cox regression between the three differentially expressed miRNAs and clinical features**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variables** | **Univariate analysis** | | **Multivariate analysis** | |
| **HR (95%CI)** | ***P* value** | **HR (95%CI)** | ***P* value** |
| Age at diagnosis (≥ 60 *vs* < 60) | 1.373 (0.948-1.988) | 0.094 | 1.642 (1.122-2.401) | 0.011a |
| Pathologic stage (III + IV *vs* I + II) | 1.825 (1.332-2.499) | < 0.001a | 1.252 (0.812-1.929) | 0.309 |
| T stage (T3 + T4 *vs* T1 + T2) | 1.864 (1.197-2.902) | 0.006a | 1.623 (1.012-2.603) | 0.044a |
| N stage (N1-2 *vs* N0) | 2.005 (1.328-3.026) | 0.001a | 1.602 (0.935-2.744) | 0.086 |
| M stage (M1 *vs* M0) | 1.368 (0.964-1.941) | 0.080 | 1.313 (0.919-1.875) | 0.134 |
| Three DEMs indicator (high *vs* low risk) | 1.442 (1.018-1.988) | 0.039a | 1.451 (1.033-2.040) | 0.032a |

a*P* < 0.05, statistically significant. DEMs: differentially expressed miRNAs.