ORIGINAL ARTICLE

Exosomal miR-1246 in serum as a potential biomarker for early diagnosis of gastric cancer

Yuntao Shi[1,2] · Zhonghong Wang[3] · Xiaojuan Zhu[1] · Ling Chen[1] · Yilan Ma[1] · Jiayan Wang[1] · Xiaozhong Yang[2] · Zheng Liu[1]

Abstract

Background Gastric cancer (GC) patients are usually diagnosed in advanced stages which results in high mortality. This study aimed to identify novel circulating miRNAs as biomarkers for the early detection of GC.

Methods Candidate miRNA was identified after integrated analysis of two Gene Expression Omnibus (GEO) datasets and clinical serum samples. Exosomes extracted were verified using transmission electron microscopy (TEM) and western blot. The expressions of miRNAs were tested through qRT-PCR. Receiver operating characteristic curve (ROC) analysis was used to explore the diagnostic utility of miRNAs. RNA pull-down assay was used to find RNA binding proteins (RBPs) which transport candidate miRNA into exosomes. Bioinformatics analysis of candidate miRNA was conducted using DAVID and Cytoscape.

Results After integrated analysis of two GEO datasets, six circulating miRNAs were found to be consistently upregulated in GC patients. Then, qRT-PCR demonstrated that serum miR-1246 was the one with the largest fold change. Studies in vitro revealed that elevated serum miR-1246 was tumor-derived by being packaged into exosomes with the help of ELAVL1. Thereafter, we discovered that exosomal miR-1246 expressions in serum could differentiate GC patients with TNM stage I from healthy controls (HCs) and patients with benign diseases (BDs) with area under the curve (AUC) of 0.843 and 0.811, respectively. Bioinformatics analysis revealed miR-1246, as a tumor suppressor in GC, could regulate several signaling pathways.

Conclusion Circulating exosomal miR-1246 was a potential biomarker for the early diagnosis of GC.

Keywords Exosomes · miR-1246 · Gastric cancer · Diagnosis · Biomarker

Introduction

Gastric cancer (GC), as one of the most common cancers worldwide, ranks the fourth in incidence and second in cancer-related mortality [1]. Currently, gastroscopy is still the criterion standard test for diagnosing GC [2]. However, this screening method is too expensive and invasive to be popularized in China. Moreover, non-invasive or minimally invasive markers such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) have been widely used in clinical, but these similar markers are neither sensitive nor specific enough to facilitate early detection of GC [3]. Due to the lack of early diagnosis and non-specific symptoms, a large number of GC patients were diagnosed at advanced stages [4, 5]. In spite of the development of surgery, chemotherapy, and other therapies in the management of GC patients, their prognosis remains

Yuntao Shi and Zhonghong Wang contribute to the study equally.

✉ Xiaozhong Yang
  xz_yang1023@aliyun.com

✉ Zheng Liu
  liuzhenghjk@163.com

1 Medical Center for Digestive Diseases, The Second Affiliated Hospital of Nanjing Medical University, No.121, Jiangjiayuan Road, Nanjing 210011, China

2 Department of Gastroenterology, The Affiliated Huaian No. 1 People's Hospital of Nanjing Medical University, No.1, Huanghe West Road, Huaian 223300, China

3 Department of Gastroenterology, Huaian People's Hospital of Hongze District, Huaian 223100, China

poor [6]. Therefore, non-invasive and cost-effective biomarkers are urgently needed for early detection of GC.

MicroRNAs (miRNAs) are a class of endogenous noncoding RNAs (about 22 nt) that post-transcriptionally modulate genes expression by degrading target mRNAs or blocking their translation [7, 8]. Exosomes, a class of 30–150 nm vesicles secreted by a wide range of mammalian cell types, consist of a lipid bilayer membrane surrounding a small cytosol and are devoid of cellular organelles [9]. Exosomes contain a range of constituents derived from parent cells, such as proteins, adiposes, mRNAs as well as miRNAs [10]. It has been widely reported that cancer cells could release miRNAs through exosomes to promote proliferation and migration [11–14]. Exosomal miRNAs secreted into biofluids, such as serum, plasma, urine, tear, and gastric juice, could avoid the degradation by RNases [15]. Furthermore, circulating miRNA expression profile was more accurate than circulating mRNA expression profiles [16]. Up to date, emerging studies have declared the biomarker value of circulating miRNAs used for diagnosis and predicting prognosis of patients with various tumors [17–19].

In this study, after integrated analysis of two GEO datasets, six circulating miRNAs were founded to be consistently upregulated in GC patients among which miR-1246 was the one with the largest fold change verified by qRT-PCR. Subsequently, we demonstrated that elevated serum miR-1246 was tumor-derived by being packaged into exosomes with the help of ELAVL1 (HuR) for the first time. Then we discovered the diagnostic value of serum exosomal miR-1246 to discriminate GC patients with TNM stage I from healthy controls (HCs) and patients with benign diseases (BDs) was remarkably high. Besides, bioinformatics analysis exhibited that potential targets of miR-1246 mainly located in cellular membrane and were significantly associated with several pathways in GC, such as Hippo signaling pathway.

Materials and methods

Clinical patients

All patients with GC ($n = 117$), gender/age matched healthy controls ($n = 82$) and patients with benign gastric diseases (gastric ulcer, atrophic gastritis, gastric polyps, $n = 30$) were enrolled from The Second Affiliated Hospital of Nanjing Medical University. GC Patients were confirmed through histopathological analysis of surgical resected tumor. All the procedures were approved by the Research and Ethical Committee of The Second Affiliated Hospital of Nanjing Medical University, and the written informed consent was obtained from each participant.

Samples processing

Serum samples collected from venous blood were stored at – 80 °C after being centrifuged at 4000 rpm for 10 min. GC tissues and adjacent normal tissues (ANTs) were frozen in liquid nitrogen immediately after resection. Total RNA was isolated using Trizol LS reagent (Invitrogen, USA) according to the manufacturer's protocol. Cel-miR-39-3p (Takara, Japan), acting as external reference, was added into each serum sample at a final concentration of 10^{-4} pmol/ul. U6 (GenePharma, Shanghai) was chosen as the internal control for miRNA expression.

Cell culture

GC cells (AGS) were cultured in RPMI1640 (KeyGEN BioTECH, China) containing 10% fetal bovine serum (FBS) and antibiotics (100 U/mL streptomycin and 100 U/mL penicillin) in 37 °C with 5% CO_2. When growing at 80% confluency, AGS cells were treated with 10 uM GW4869, an exosomes inhibitor (Sigma, USA), for 2 h.

Exosomes purification

Exosomes were isolated from cultured media using RiboTM Exosome Isolation Reagent Kit according to the manufacturer's instruction (RioBoBio, China). Briefly, after collecting media (serum), cellular debris were removed by centrifugation at 2000g for 30 min. Then media (serum) was transferred to a fresh tube and exosomes were precipitated using precipitation solution at 4 °C overnight (3 h). The mixture was centrifuged at 1500g for 30 min at 4 °C to pellet exosomes which were resuspended in PBS subsequently.

Transmission electron microscopy analysis

Isolated exosomes were re-suspended in 100 µl phosphate buffer (pH 7.4) and fixed in 50 µl glutaraldehyde. Subsequently, fixed exosomes were dropped onto a formvar-carbon coated grid and left to dry at room temperature for 5 min and stained with phosphotungstic acidoxalate for 1 min. Then, excess liquid was removed. The grid was dried at room temperature for 10 min and visualized on Tecnai G2 F20 transmission electron microscope (TEM) (FEI, United States) at 185 kV.

qRT-PCR for miRNA quantitation

Reverse transcription and qRT-PCR for miR-1246, external reference miR-39 and endogenous control U6 snRNA were performed using Hairpin-itTM microRNA RT-PCR

Quantitation Kit (GenePharma, China) according the manufactures' instructions. The reactions were initiated with denaturation at 95 °C for 3 min, followed by 40 cycles of 95 °C for 15 s and 62 °C for 34 s. The relative expression of miRNA was calculated by $2^{-\Delta\Delta Ct}$ method.

Transfection

miR-1246 mimics labeled with biotin at 3′ end and negative control (NC) were commercially synthesized (RioBo-Bio, China). SiELAVL1, a small interfering RNA against ELAVL1, were synthesized by GenePharma (GenePharma, China). Transfection was carried out with a final concentration of 50 nM biotin-labeled-miR-1246 mimics, 100 nM siELAVL1, and corresponding NCs using the Lipofectamine 2000 reagent (Invitrogen, USA) following the manufacturer's protocol.

RNA pull-down

After transfecting AGS cells with NC and 3′ biotin-labeled mimics at a final concentration of 50 nM, cells were scraped to prepare fresh lysates supplemented with protease inhibitor (PI) and RNase inhibitor in the lysis buffer. Then wash the beads three times with 1 ml lysis buffer and incubate the Streptavidin-Dyna beads (RioBoBio, China) with lysis buffer on a rotator at 4 °C for 2 h. Put the tube containing the beads on the magnetic stand, take out the supernatant and wash the beads for twice. Mix 500 µl lysates with the beads and incubate at 4 °C on the rotator overnight. 50 µl lysates left were saved at − 80 °C as input. Centrifuge the tube and put it on magnetic stand and remove the supernatant briefly. Wash the beads four times with 1 ml lysis buffer and eluted in PBS. The eluted protein complexes were denatured and prepared for western blot.

Western blot

Briefly, harvested and denatured proteins were separated by 10% SDS-PAGE, transferred to PVDF membranes. The membranes were blocked with TBST that contains 5% skimmed milk, then blocked with primary antibodies (mouse anti-CD63, 1:1000, ab59479; mouse anti-TSG101, 1:1000, ab83; Rabbit anti-AGO2, 1:1000, 10,686–1-AP; Rabbit anti-HuR, 1:2000, 11,910–1-AP; Rabbit anti-beta-actin, 1:5000, 20,536–1-AP) overnight at 4 °C. After being washed four times, the membranes were incubated with anti-IgG conjugated to horseradish peroxidase at room temperature for 1 h. Bands were visualized using the enhanced chemiluminescence system (ECL) reagent (KeyGEN BioTECH, China).

GEO datasets analysis

After searching GEO datasets using keywords "gastric cancer" and "miRNA", GSE106817 and GSE113486 were enrolled. GSE106817 included 300 serum samples collected from 115 healthy controls and 115 GC patients. GSE113486 included 141 serum samples collected from 101 healthy controls and 40 GC patients. The platform of both GSE106817 and GSE113486 were 3D-Gene Human miRNA V21_1.0.0 (GPL21263).

Bioinformatics analysis

The significantly dysregulated miRNAs were calculated using GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/), an online analysis tool in GEO database. The targets of miR-1246 in GC were identified by integrated analysis of public bioinformatics algorithms TargetScan (https://www.targetscan.org/vert_72/) and a GEO dataset. GO, as a common bioinformatics tool, could provide comprehensive information on genes. GO contains three items: molecular functions (MF), biological processes (BP) and cellular components (CC) [20]. The KEGG is a reference resource for biological interpretation of high-throughput genes and protein pathways [21]. The GO and KEGG analysis were available in the online tool DAVID (https://david.ncifcrf.gov/), which is a bioinformatics database composing functional annotation of large quantities of genes [22]. Protein–protein interaction (PPI) network was constructed using online tool String [23] and Cytoscape.

Statistical analysis

Statistical analysis was performed using SPSS 19.0 (IBM, USA) and GraphPad Prism 8.0 (GraphPad Software, USA) using the Student's paired or unpaired t test, and Chi-square test. The ROC was performed to evaluate the utility of miR-1246 as diagnostic biomarker of GC. Cut-off value of the relative expression of miRNAs were determined by Youden index from ROC curves. P value < 0.05 was considered to be statistically significant.

Results

Identifying circulating miR-1246 as candidate

After integrated analysis of GSE106817 and GSE113486, 1366 and 1080 miRNAs were found to be upregulated with logFC ≥ 1 and $p < 0.05$. As shown in Fig. 1a, among top 10 upregulated miRNAs, six circulating miRNAs (miR-29b-1-5p, miR-191-5p, miR-1246, miR-6131, miR-451a, and miR-103a-3p) were consistently upregulated in GC patients.
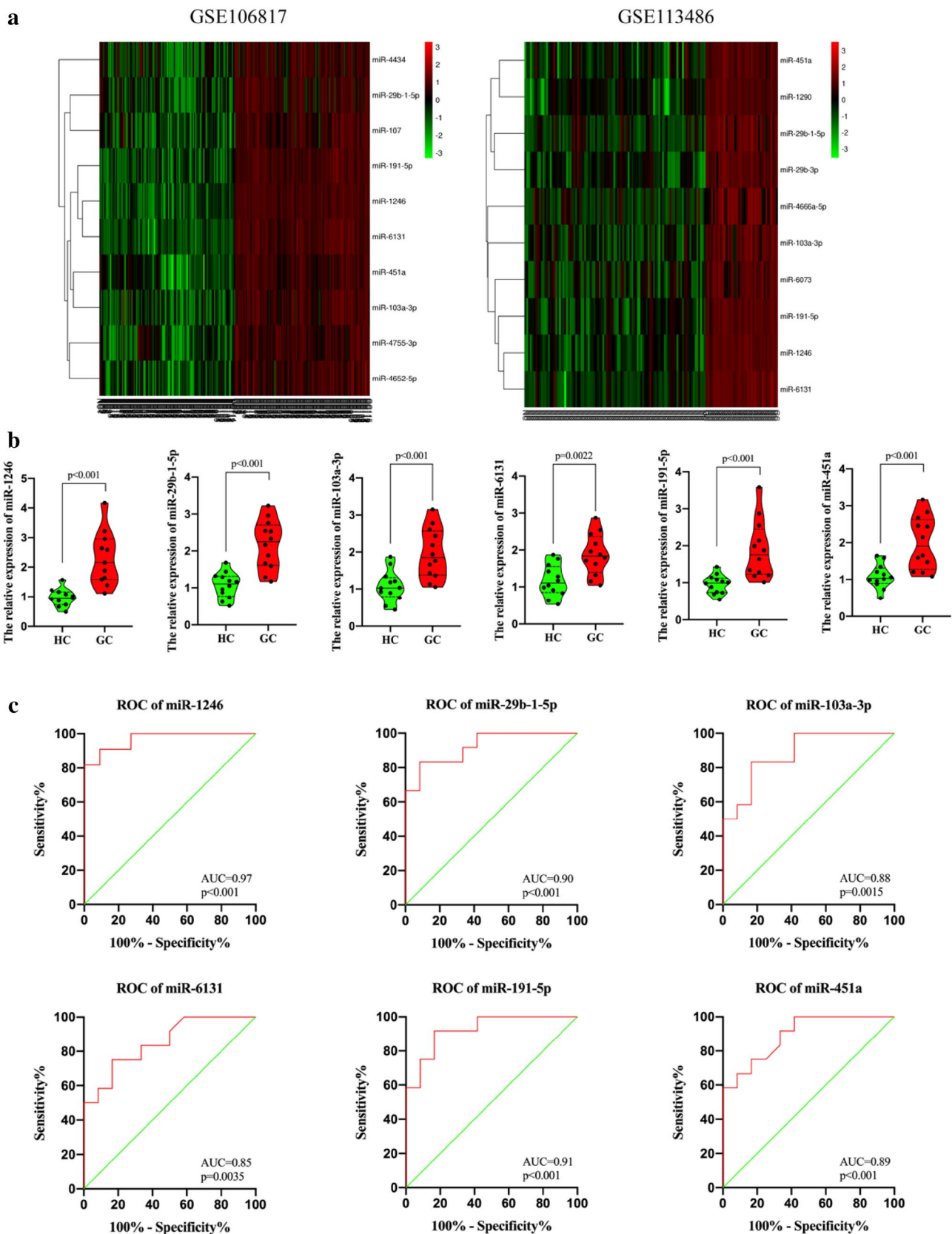
Fig. 1 Identifying circulating miR-1246 as candidate. **a** Six circulating miRNAs (miR-29b-1-5p, miR-191-5p, miR-1246, miR-6131, miR-451a, and miR-103a-3p) were consistently upregulated in GC patients. **b** The expressions of six circulating miRNAs analyzed by qRT-PCR. **c** ROC of six circulating miRNAs to discriminate GC patients from HCs
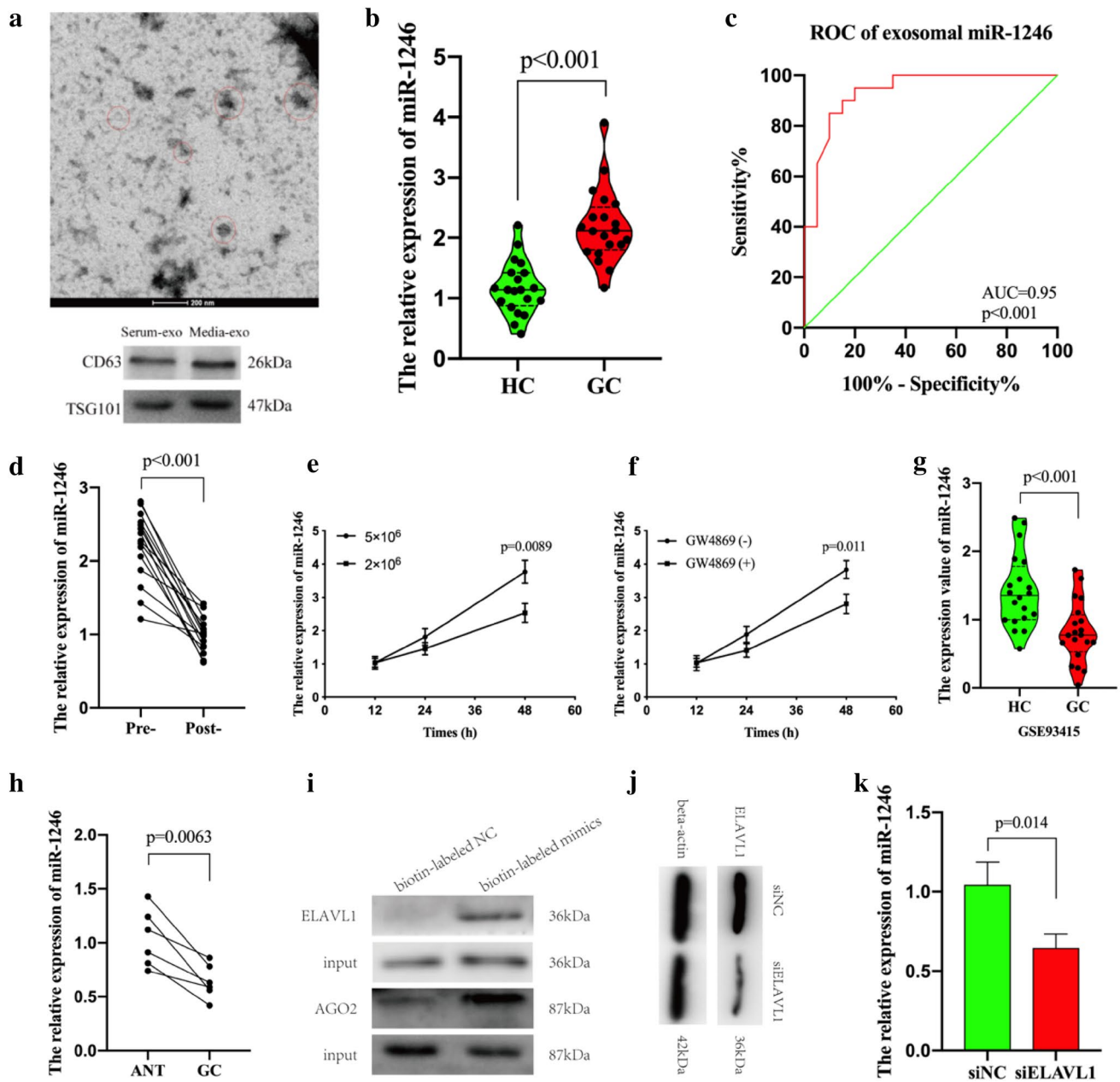
Fig. 2 Elevated serum miR-1246 was tumor-derived by being packaged into exosomes. **a** Exosomes verified by TEM and western blot. **b** Circulating exosomal miR-1246 expressions were significantly upregulated in GC patients. **c** ROC of circulating exosomal miR-1246 to discriminate GC patients from HCs. **d** Serum exosomal miR-1246 expressions were significantly downregulated after tumor resection. **e** Exosomal miR-1246 expressions in the culture media increased with time and with increasing numbers of cells. **f** GW4860 significantly inhibited exosomal miR-1246 expressions in the culture media. **g** GSE93415 and **h** qRT-PCR verified that miR-1246 expressions were significantly downregulated in GC tissues. **i** Biotin-labeled miRNA pull-down assay demonstrated that miR-1246 could bind ELAVL1. **j** SiELAVL1 significantly knocked down the expressions of ELAVL1. **k** Exosomal miR-1246 was significantly downregulated in media of cells transfected with siELAVL1

qRT-PCR demonstrated miR-1246 was the one with the largest fold change (Fig. 1b). ROC analysis showed that serum miR-1246 had a remarkably high value to differentiate GC patients from HCs when compared to other miRNAs (Fig. 1c). Therefore, serum miR-1246 was selected as candidate to explore whether it could serve as a biomarker for the early diagnosis of GC patients.

Elevated serum miR-1246 was tumor-derived by being packaged into exosomes

Li et al. reported that breast cancer cells could secrete exosomal miR-1246 to promote proliferation, migration, and drug resistance [24]. We wondered whether elevated serum miR-1246 in GC patients was released by tumor in the form of exosomes. Exosomes isolated from serum and cultured media were verified by TEM and western blot (Fig. 2a). qRT-PCR showed that circulating exosomal miR-1246 expressions were significantly upregulated in GC patients (Fig. 2b) and could discriminate GC patients from HCs with an AUC value of 0.95 (Fig. 2c). In addition, serum exosomal miR-1246 expressions were significantly downregulated after tumor resection (Fig. 2d). Next, different amounts of AGS cells were cultured and exosomes in media were collected in different time points. As shown in Fig. 2e, we observed that exosomal miR-1246 expressions in the culture media increased with time and with increasing numbers of cells. Moreover, after AGS cells being treated with GW4860, exosomal miR-1246 expressions in the culture media were significantly downregulated (Fig. 2f). Interestingly, analysis results of GSE93415 showed that miR-1246 expressions were significantly downregulated in GC tissues (Fig. 2g). qRT-PCR verified that compared to ANTs, miR-1246 expressions were significantly downregulated in GC tissues (Fig. 2h). It has been reported that several miRNAs were downregulated in cells, but they can be sorted into exosomes through RNA binding proteins (RBPs), such as SYNCRIP and hnRNP A2/B1, and be released into circulating system [25, 26]. We wondered whether exosomal miR-1246 was mediated by specific RBP. Firstly, potential RBPs binding miR-1246 were searched in RBPDB (https://rbpdb.ccbr.utoronto.ca/index.php). SFRS1 and ELAVL1 were identified. Then biotin-labeled miRNA pull-down assay demonstrated that miR-1246 could bind ELAVL1 through AUUUU motif (Fig. 2i). Then we knocked down the expressions of ELAVL1 by transfecting cells with siELAVL1 (Fig. 2j) and found that exosomal miR-1246 was significantly downregulated in media of cells transfected with siELAVL1 (Fig. 2k).

High levels and diagnostic utility of exosomal miR-1246 in serum validated in an independent cohort

A total of 165 serum samples, including those from GC patients ($n = 85$), BD patients ($n = 30$) and HCs ($n = 50$), were tested to validate the diagnostic potential of exosomal miR-1246. The clinicopathological characteristics of participants are summarized in Table 1. As shown in Fig. 3a, the relative expressions of exosomal miR-1246 in serum were significantly overexpressed in GC patients compared to that in HCs. ROC analysis demonstrated that circulating exosomal miR-1246 expressions could differentiate GC patients

Table 1 The clinicopathological characteristics of participants

Variables	HC	BD	GC
Number	50	30	85
Age (mean ± SD)	58.1 ± 12.4	57.6 ± 13.7	59.7 ± 11.3
Gender			
Male	34	20	56
Female	16	10	29
TNM stage			
I			28
II			32
III			14
IV			11
Differentiation			
Well/moderate			62
Poor			23
Distant metastasis			
No			57
Yes			28

HC healthy control, *BD* benign disease, *GC* gastric cancer

from HCs with an AUC value of 0.911 (95% CI 0.86–0.96, $p < 0.001$) (Fig. 3d). The sensitivity and specificity were 82.35% and 86%, respectively. The cut-off value was 1.67. Moreover, the gradual increase of circulating exosomal miR-1246 levels was clearly discernible with significantly higher levels in stage III-IV patients compared with stage I–II patients (Fig. 3b). We further explored whether exosomal miR-1246 in serum could differentiate GC patients in TNM stage I from HCs working for early diagnosis of GC. ROC analysis demonstrated that circulating exosomal miR-1246 expressions could differentiate GC patients in TNM stage I from HCs with an AUC value of 0.843 (95% CI 0.75–0.93, $p < 0.001$) (Fig. 3e). The sensitivity and specificity were 85.71% and 74%, respectively. The cut-off value was 1.43. When compared to patients with BDs, circulating exosomal miR-1246 levels were also significantly higher in GC patients with TNM stage I (Fig. 3c). ROC analysis demonstrated that circulating exosomal miR-1246 expressions could differentiate GC patients in TNM stage I from BDs patients with an AUC value of 0.811 (95% CI 0.71–0.93, $p < 0.001$) (Fig. 3f). The sensitivity and specificity were 78.57% and 80%, respectively. The cut-off value was 1.53. Soon afterwards, 85 GC patients were divided into high miR-1246 group ($n = 42$) and low miR-1246 group ($n = 43$) according to the median value of exosomal miR-1246 expressions. Chi-square test showed that high exosomal miR-1246 expressions were significantly correlated with advanced TNM stage ($p = 0.033$) and distant metastasis ($p = 0.035$) (Table 2).

Bioinformatics analysis of miR-1246

Considering that miR-1246 was significantly downregulated in GC tissues and that the regulatory mechanisms of miR-1246 had not been reported in GC, we conducted a bioinformatics analysis of miR-1246 in GC. Firstly, 659 upregulated genes and 1818 downregulated genes with |logFC| > 1 were found in another GEO dataset (GSE112369) which means these genes may modulate the development and progression of GC (Fig. 4a). Potential targets of miR-1246 were searched by public bioinformatics algorithms (TargetScan). Among these predicted targets of miR-1246, 285 targets were significantly dysregulated in GC tissues (Fig. 4b). Thereafter, we carried out functional and pathway enrichment analysis of these significantly dysregulated targets. GO term enrichment analysis showed that in the BP category, targets of miR-1246 were significantly enriched in positive regulation of cell proliferation, positive regulation of transcription, positive

regulation of gene expression, palate development, and negative regulation of apoptotic process (Fig. 4c). CC analysis showed that most targets were located in plasma membrane (Fig. 4d). According to the results of MF analysis, targets of miR-1246 were associated with protein binding, including transforming growth factor beta binding, sequence-specific DNA binding, and growth factor binding (Fig. 4e). Additionally, KEGG pathway analysis discovered that miR-1246 participated in several pathways in cancer, including Hippo signaling pathway, FoxO signaling pathway, PI3K-Akt signaling pathway, and GnRH signaling pathway (Fig. 4f). If there were more than ten terms enriched in these categories, the top ten terms based on p value were chosen. Furthermore, we carried out a PPI network analysis (Fig. 4g) and identified a molecular complex (Fig. 4h) using Cytoscape.
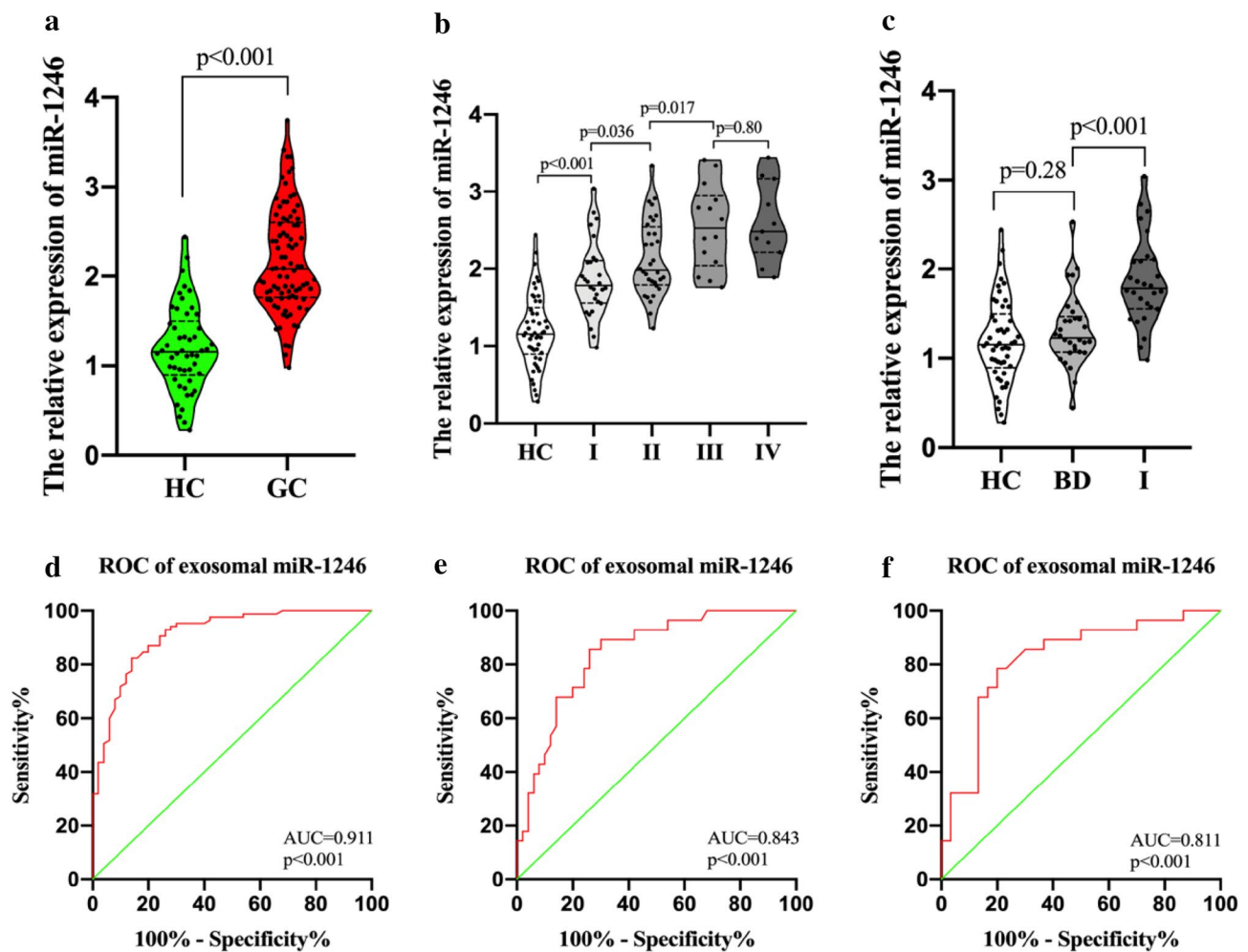

Fig. 3 High levels and diagnostic utility of exosomal miR-1246 in serum validated in an independent cohort. **a, b** The expression levels of exosomal miR-1246 in serum in GC patients and HCs. **c** The expression levels of exosomal miR-1246 in serum in GC patients with TNM stage I, patients with BDs, and HCs. **d** ROC of circulating exosomal miR-1246 expressions to differentiate GC patients from HCs. **e–f** ROC of circulating exosomal miR-1246 expressions to differentiate GC patients with TNM stage I from HCs and patients with BDs

Discussion

In this study, we discovered that circulating exosomal miR-1246 were significantly upregulated in GC patients and elevated exosomal miR-1246 in serum were tumor-derived. ROC analysis exhibited that circulating exosomal miR-1246 expressions could differentiate GC patients in TNM stage I from patients with BDs and HCs with remarkable value. Besides, based on bioinformatics analysis, we discovered targets of miR-1246 mainly located in cellular membrane and regulated protein binding to promote proliferation and transcription of GC cells through several signaling pathways.

Dysregulated miRNAs in peripheral blood acting as diagnostic biomarkers have been explored in various cancers. Liu et al. reported that circulating miR-182 could serve as diagnostic biomarker of colorectal cancer [27]; Zhang et al. discovered serum miR-100 was a potential biomarker for detection and outcome prediction of glioblastoma patients [28]; Orangi et al. evaluated that miRNA-9 and miRNA-34a may be potential biomarkers for diagnosis of breast cancer in Iranian women [29]. Circulating miR-1246 has been regarded as a novel biomarker for oesophageal squamous cell carcinoma [30], high-grade serous ovarian carcinoma [31], aggressive prostate cancer [32], and hepatocellular carcinoma [33]. Based on a systematic review and meta-analysis, Wei et al. declared exosomal miR-1246 in body fluids is a potential biomarker for gastrointestinal cancer [34]. However, gastrointestinal cancer enrolled in their meta-analysis only consists of pancreatic cancer [35], colon cancer [36], and pancreatobiliary tract cancer [37]. To the best of our knowledge, the diagnostic value of circulating exosomal miR-1246 in GC has not been reported till now. Our study demonstrated that elevated exosomal miR-1246 in serum were tumor-derived and remarkably higher in GC patients than HCs as well as patients with BDs which provided a novel potential biomarker for the early diagnosis of GC patients. Unexpectedly, compared to normal tissues, the expressions of miR-1246 in GC tissues were significantly downregulated. In addition to our finding that GC cells could forwardly sort miR-1246 into exosomes and release it into peripheral blood, a various of cancers have been verified to release exosomal miR-1246, including oral squamous cell carcinoma, breast cancer [24, 38–40]. For example, exosomal miR-1246 secreted from breast cancer cells could be transferred into non-malignant breast cells through direct uptake to promote cell proliferation, invasion and drug resistance by targeting CCNG2 [24]. Besides, several RNA binding proteins have been proved to control the sorting of specific miRNAs into exosomes, regardless of the downregulated expressions of miRNAs, such as hnRNPA2B1 [25, 26]. Xu et al. discovered exosome miR-1246 is derived from RNU2-1 degradation [41]. We speculated that in spite

Table 2 Associations between exosomal miR-1246 expressions in serum and clinicopathological parameters of GC patients

Variables	miR-1246 expression		P value
	High	Low	
Gender			
Male	22	19	
Female	20	24	0.45
Age			
> 61	16	14	
≤ 61	26	29	0.59
TNM stage			
I + II	23	33	
III + IV	19	10	0.033
Histologic differentiation			
Well	18	21	
Poor	24	22	0.58
Distant metastasis			
No	28	37	
Yes	14	6	0.035

of downregulated expression in GC cells, miR-1246 was regarded as a kind of metabolite. Because the metabolism of tumorous cells was faster than normal ones [42], to keep homeostasis, tumors had to secret miR-1246 into extracellular environment. Therefore, it is meaningful to investigate the relationship between exosomal miR-1246 and tumor microenvironment in the future.

Several studies have reported the biological functions of miR-1246 in cancers. Lin et al. discovered that miR-1246 expressions were significantly upregulated in oral carcinomas and enhanced cancer stemness and chemoresistance by targeting CCNG2 [43]. In addition, Zhang et al. revealed miR-1246 was a tumor-initiating miRNA and promoted non-small cell lung cancer progression [44]. However, the regulatory mechanisms of miR-1246 in GC have not been investigated before. Our study demonstrated that miR-1246 expressions were significantly downregulated in GC tissues. Functional and pathway enrichment analysis revealed miR-1246 could regulate protein binding to promote proliferation and gene transcription through several vital signaling pathways, such as Hippo signaling pathway. PPI network of targets revealed a molecular complex which consisted of KRAS, SOX2, ABCG2, NT5E, KITLG, MME, PROM1, and KIT. Tian et al. reported SOX2 is a commonly activated tumor promoter that activate AKT signaling in GC [45]. Polom et al. declared KRAS mutation was significantly associated with the prognosis of GC patients [46]. Besides, Jiang et al. showed that high expression of NT5E was significantly associated with lymph node metastases and a promising
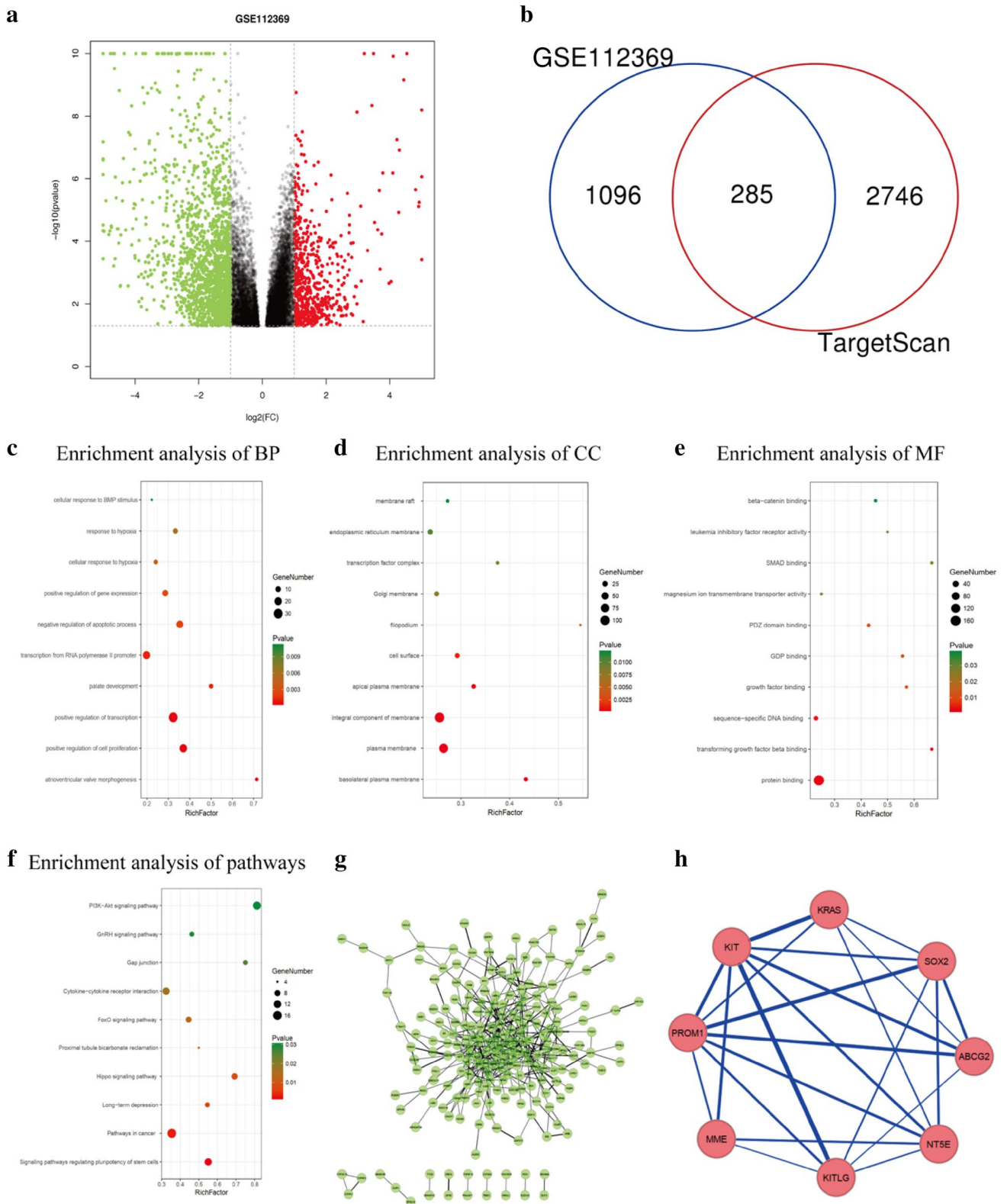
Fig. 4 Bioinformatics analysis of miR-1246. **a** Differentially expressed genes in GSE112369. **b** Potential targets that were significantly dysregulated in GC tissues. **c–e** Functional enrichment analysis, **f** KEGG pathway analysis, and **g** PPI network analysis of dysregulated targets of miR-1246. **h** Molecular complex identified in PPI network

prognostic factor in different types of cancer [47]. Our study suggested that targeting this molecular complex may be a novel therapeutic strategy of GC.

Taken together, we demonstrated that exosomal miR-1246 in serum was a novel potential biomarker for the early diagnosis of GC patients.

Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest in this work.

Ethical approval This study was approved by the Research and Ethical Committee of The Second Affiliated Hospital of Nanjing Medical University.

Informed consent Written informed consent was obtained from all participants

References

1. Van Cutsem E, Sagaert X, Topal B et al (2016) Gastric cancer. Lancet 388:2654–2664
2. Veitch AM, Uedo N, Yao K et al (2015) Optimizing early upper gastrointestinal cancer detection at endoscopy. Nat Rev Gastroenterol Hepatol 12:660–667
3. Carpelan-Holmstrom M, Louhimo J, Stenman UH et al (2002) CEA, CA 19–9 and CA 72–4 improve the diagnostic accuracy in gastrointestinal cancers. Anticancer Res 22:2311–2316
4. Hundahl SA, Phillips JL, Menck HR (2000) The National Cancer Data Base Report on poor survival of U.S. gastric carcinoma patients treated with gastrectomy: Fifth Edition American Joint Committee on Cancer staging, proximal disease, and the "different disease" hypothesis. Cancer 88:921–932
5. Hartgrink HH, Jansen EP, van Grieken NC et al (2009) Gastric cancer. Lancet 374:477–490
6. Newton AD, Datta J, Loaiza-Bonilla A et al (2015) Neoadjuvant therapy for gastric cancer: current evidence and future directions. J Gastrointest Oncol 6:534–543
7. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116:281–297
8. Caldas C, Brenton JD (2005) Sizing up miRNAs as cancer genes. Nat Med 11:712–714
9. Trams EG, Lauter CJ, Salem N Jr et al (1981) Exfoliation of membrane ecto-enzymes in the form of micro-vesicles. Biochim Biophys Acta 645:63–70
10. Conigliaro A, Fontana S, Raimondo S et al (2017) Exosomes: nanocarriers of biological messages. Adv Exp Med Biol 998:23–43
11. Zeng Z, Li Y, Pan Y et al (2018) Cancer-derived exosomal miR-25-3p promotes pre-metastatic niche formation by inducing vascular permeability and angiogenesis. Nat Commun 9:5395
12. Naseri Z, Oskuee RK, Jaafari MR et al (2018) Exosome-mediated delivery of functionally active miRNA-142-3p inhibitor reduces tumorigenicity of breast cancer in vitro and in vivo. Int J Nanomed 13:7727–7747
13. Li Z, Tao Y, Wang X et al (2018) Tumor-secreted exosomal miR-222 promotes tumor progression via regulating P27 expression and re-localization in pancreatic cancer. Cell Physiol Biochem 51:610–629
14. Yoshimura A, Sawada K, Nakamura K et al (2018) Exosomal miR-99a-5p is elevated in sera of ovarian cancer patients and promotes cancer cell invasion by increasing fibronectin and vitronectin expression in neighboring peritoneal mesothelial cells. BMC Cancer 18:1065
15. Gilad S, Meiri E, Yogev Y et al (2008) Serum microRNAs are promising novel biomarkers. PLoS ONE 3:e3148
16. Lu J, Getz G, Miska EA et al (2005) MicroRNA expression profiles classify human cancers. Nature 435:834–838
17. Zhong Y, Chen Z, Guo S et al (2017) TUG1, SPRY4-IT1, and HULC as valuable prognostic biomarkers of survival in cancer: a PRISMA-compliant meta-analysis. Medicine (Baltimore) 96:e8583
18. Shin VY, Ng EK, Chan VW et al (2015) A three-miRNA signature as promising non-invasive diagnostic marker for gastric cancer. Mol Cancer 14:202
19. Yuan HL, Wang T, Zhang KH (2018) MicroRNAs as potential biomarkers for diagnosis, therapy and prognosis of gastric cancer. Onco Targets Ther 11:3891–3900
20. Blake JA, Christie KR, Dolan ME et al (2015) Gene ontology consortium: going forward. Nucleic Acids Res 43:D1049–D1056
21. Kanehisa M, Sato Y, Kawashima M et al (2016) KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res 44:D457–462
22. da Huang W, Sherman BT, Lempicki RA (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4:44–57
23. Szklarczyk D, Franceschini A, Wyder S et al (2015) STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res 43:D447–452
24. Li XJ, Ren ZJ, Tang JH et al (2017) Exosomal MicroRNA MiR-1246 promotes cell proliferation, invasion and drug resistance by targeting CCNG2 in breast cancer. Cell Physiol Biochem 44:1741–1748
25. Santangelo L, Giurato G, Cicchini C et al (2016) The RNA-binding protein SYNCRIP is a component of the hepatocyte exosomal machinery controlling MicroRNA sorting. Cell Rep 17:799–808
26. Villarroya-Beltri C, Gutierrez-Vazquez C, Sanchez-Cabo F et al (2013) Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs. Nat Commun 4:2980
27. Liu X, Xu T, Hu X et al (2018) Elevated circulating miR-182 acts as a diagnostic biomarker for early colorectal cancer. Cancer Manag Res 10:857–865
28. Zhang H, Wang J, Wang Z et al (2018) Serum miR-100 is a potential biomarker for detection and outcome prediction of glioblastoma patients. Cancer Biomark 24:43–49
29. Orangi E, Motovali-Bashi M (2018) Evaluation of miRNA-9 and miRNA-34a as potential biomarkers for diagnosis of breast cancer in Iranian women. Gene 687:272–279
30. Takeshita N, Hoshino I, Mori M et al (2013) Serum microRNA expression profile: miR-1246 as a novel diagnostic and prognostic biomarker for oesophageal squamous cell carcinoma. Br J Cancer 108:644–652
31. Todeschini P, Salviato E, Paracchini L et al (2017) Circulating miRNA landscape identifies miR-1246 as promising diagnostic biomarker in high-grade serous ovarian carcinoma: a validation across two independent cohorts. Cancer Lett 388:320–327

32. Bhagirath D, Yang TL, Bucay N et al (2018) microRNA-1246 is an exosomal biomarker for aggressive prostate cancer. Cancer Res 78:1833–1844

33. Moshiri F, Salvi A, Gramantieri L et al (2018) Circulating miR-106b-3p, miR-101-3p and miR-1246 as diagnostic biomarkers of hepatocellular carcinoma. Oncotarget 9:15350–15364

34. Wei C, Li Y, Huang K et al (2018) Exosomal miR-1246 in body fluids is a potential biomarker for gastrointestinal cancer. Biomark Med 12:1185–1196

35. Madhavan B, Yue S, Galli U et al (2015) Combined evaluation of a panel of protein and miRNA serum-exosome biomarkers for pancreatic cancer diagnosis increases sensitivity and specificity. Int J Cancer 136:2616–2627

36. Ogata-Kawata H, Izumiya M, Kurioka D et al (2014) Circulating exosomal microRNAs as biomarkers of colon cancer. PLoS ONE 9:e92921

37. Machida T, Tomofuji T, Maruyama T et al (2016) miR1246 and miR4644 in salivary exosome as potential biomarkers for pancreatobiliary tract cancer. Oncol Rep 36:2375–2381

38. Mo LJ, Song M, Huang QH et al (2018) Exosome-packaged miR-1246 contributes to bystander DNA damage by targeting LIG4. Br J Cancer 119:492–502

39. Sakha S, Muramatsu T, Ueda K et al (2016) Exosomal microRNA miR-1246 induces cell motility and invasion through the regulation of DENND2D in oral squamous cell carcinoma. Sci Rep 6:38750

40. Yuan D, Xu J, Wang J et al (2016) Extracellular miR-1246 promotes lung cancer cell proliferation and enhances radioresistance by directly targeting DR5. Oncotarget 7:32707–32722

41. Xu YF, Hannafon BN, Khatri U et al (2019) The origin of exosomal miR-1246 in human cancer cells. RNA Biol 16:770–784

42. Kahlert UD, Mooney SM, Natsumeda M et al (2017) Targeting cancer stem-like cells in glioblastoma and colorectal cancer through metabolic pathways. Int J Cancer 140:10–22

43. Lin SS, Peng CY, Liao YW et al (2018) miR-1246 targets CCNG2 to enhance cancer stemness and chemoresistance in oral carcinomas. Cancers (Basel) 10:272

44. Zhang WC, Chin TM, Yang H et al (2016) Tumour-initiating cell-specific miR-1246 and miR-1290 expression converge to promote non-small cell lung cancer progression. Nat Commun 7:11702

45. Tian Y, Jia X, Wang S et al (2014) SOX2 oncogenes amplified and operate to activate AKT signaling in gastric cancer and predict immunotherapy responsiveness. J Cancer Res Clin Oncol 140:1117–1124

46. Polom K, Das K, Marrelli D et al (2019) KRAS mutation in gastric cancer and prognostication associated with microsatellite instability status. Pathol Oncol Res 25:333–340

47. Jiang T, Xu X, Qiao M et al (2018) Comprehensive evaluation of NT5E/CD73 expression and its prognostic significance in distinct types of cancers. BMC Cancer 18:267