Circulating Exosomal miR-17-92 Cluster Serves as a Novel Non-Invasive Diagnostic Marker for Gastric Cancer Patients

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Abstract

Gastric cancer (GC) is one of the most common malignant tumors with a leading cause of cancer-related mortality worldwide. Exosomal miRNAs are considered as promising non-invasive biomarkers for the diagnosis of malignant tumors. In this study, we aimed to investigate the expression of exosomal miR-17-92 cluster and develop a potential biomarker for the diagnosis of GC. Exosomal RNAs were extracted and the expression profile of miR-17-92 cluster was detected using quantitative polymerase chain reaction (qRT-PCR). The ROC (receiver-operating characteristic) curve and AUC (area under the ROC curve) analysis were used to explore the diagnostic utility of miRNAs. Statistical was used to analyze the expression of serum exosomal miR-17-92 cluster with the clinical pathological parameters of GC patients. The results showed that the expressions of four members of the exosomal miR-17-92 cluster in the serum samples of GC patients were significantly upregulated compared with those of healthy controls. The AUC for serum exosomal miR-17, miR-18, miR-19a and miR-92 was 0.750 (95%CI=0.626-0.874, sensitivity=84.7%, specificity=70.0%), 0.736 (95%CI=0.590-0.881, sensitivity=88.9%, specificity=65.0%), 0.700 (95%CI=0.562-0.838, sensitivity=62.5%, specificity=80.0%), 0.689 (95%CI=0.567-0.811, sensitivity=45.8%, specificity=90.0%), respectively. The AUC for the newly combined panel consisting of miR-17, miR-18, miR-19a and miR-92 was 0.808 (95%CI=0.680-0.937), with sensitivity of 90.3% and specificity of 70.0%, which showed much higher clinical diagnostic value for GC than any of the four alone or any pair. Besides, the AUC for the newly developed panel consisting of the two traditional tumor biomarkers including CEA (carcinoembryonic antigen) and CA19-9 (carbohydrate antigen 19-9) and the four miR-17-92 cluster members was 0.881 (95%CI, 0.765-0.998) with sensitivity of 91.7% and specificity of 90.0%, which showed the greatest powerful clinical diagnostic value for GC. Moreover, the elevated exosomal miR-17-92 expressions were closely correlated with tumor size, tumor depth, lymph node metastasis, distant metastasis and TNM stage of GC patients. In conclusion, our findings revealed that circulating exosomal miR-17-92 cluster may be used as a novel potential non-invasive biomarker to improve the diagnostic efficiency in GC.

Introduction

Gastric cancer (GC) ranks fifth among the most common types of cancer and is the third leading cause of cancer-related mortality worldwide [1]. Although great progress has been made in the GC therapy, the 5-year survival rate of GC patients is still not satisfactory [2]. Early diagnosis could significantly improve the prognosis of GC, however, most patients has progressed to advanced stage when firstly diagnosed [3, 4]. Carcinoembryonic antigen (CEA) and carbohydrate antigen 19 – 9 (CA19-9), the conventional serum tumor biomarkers, have been proved to be useful indicators for the early diagnosis, prognosis and recurrence in GC [5, 6]. However, most of them are not ideal serum biomarkers for the early stages of GC diagnosis due to the limited specificity and sensitivity [7]. Therefore, it is an urgent need to identify an early screening and definitive diagnostic marker for GC. The imageological screening is generally consuming and its sensitivity is really low in identifying early stage of GC. Additionally, biopsy for histopathologic examination is not widely used for the GC screening due to its invasiveness. By contrast, peripheral blood test shows superior advantages in screening cancer due to the characters of non-invasive, easily obtained, high-efficiency and so on. However, the putative biomarkers with high sensitivity and/or specificity for GC detection and monitoring are still rare. Thus, there is an urgent need to identify more original and effective biomarkers for the early diagnosis of GC.

MicroRNAs (miRNAs) are small single-stranded, non-coding RNAs which can regulate the expression of their target genes [8]. miRNAs have been reported to play pivotal roles in several cellular processes, including cell growth, proliferation, apoptosis, migration and metabolism [9–12]. The miR-17-92 cluster, is one of the most well-known oncogenic miRNAs, including miR-17, miR-18, miR-19a, miR-19b, miR-20 and miR-92 [13]. Human miR-17-92 is frequently upregulated in a wide range of cancers, such as lung cancer, breast cancer, prostate and thyroid cancer, as well as GC [14].

Exosomes are membrane-enclosed nanoscale vesicles of endosomal origin whose diameters ranged from 30 to 150 nm [15]. They are generated from the internal vesicles of multivesicular bodies (MVBs). They can be secreted by numerous kinds of cells and present abundantly in blood, urine, and other body fluids [16]. Recently, studies about exosomes in cancer emerge rapidly due to its features of noninvasiveness and easy collection and detection [17–19]. Exosomes are reported to be involved in many pathological processes, such as tumor invasion, angiogenesis, metastasis, and chemoresistance [20–23]. Exosomal miRNAs, such as miR-155, the miR-17-92 cluster, and miR-1246 provide valuable potential in the diagnosis and/or prognosis in many types of cancers [24].

In this study, we systematically investigated the expression profiles of the exosomal miR-17-92 cluster from the serum of GC patients. In addition, we tried to build a potential diagnostic panel combined with traditional serum biomarkers, CEA and CA19-9, in order to achieve an optimal diagnosis of GC with high sensitivity and/or specificity. Our present study focused on the clinical diagnostic value of the expression of circulating exosomal miR-17-92 cluster as a novel non-invasive diagnostic marker for gastric cancer patients.

Materials And Methods

Clinical samples

Peripheral blood samples were obtained from 72 GC patients at the Department of General Surgery, the First Affiliated Hospital of Soochow University, from August 2019 to December 2020. The GC patients did not receive chemotherapy or radiotherapy before serum collection. Samples of 20 healthy controls were acquired from people for health examination. Informed consent was given in all the patients enrolled. All GC specimens were confirmed by pathological examination. Histological grade was defined according to the World Health Organization classification. This study was approved by the institutional ethics committee of the First Affiliated Hospital of Soochow University.

Exosome isolation

According to manufacturer's instructions, serum exosomes were obtained using ExoQuick exosome precipitation solution (System Biosciences, CA, USA). In brief, the serum samples were isolated from the whole blood samples by centrifugation of 3,000 rpm for 15 min at 4˚C. The cellular fractions of the serum were removed by centrifugation of 12,000g for 10 min at 4˚C. Then 250 μL serum were mixed with 63 μL of ExoQuick solution and were incubated overnight at 4˚C. After centrifugation at 1,500 g for 30 min, the exosomes precipitation was suspended in 50 μL PBS. The isolated exosomes were stored at -80˚C for use.

RNA extraction and real-time PCR (qRT-PCR)

Exosomal miRNAs in the serum were extracted by using miRNeasy Serum/Plasma Kit (Qiagen, Germany) according to the manufacturer's instructions. The exosomal miRNAs were then reverse transcribed with miScript II RT Kit (Qiagen, Germany). miScript SYBR Green PCR Kit (Qiagen, Germany) were used to detect the expression of exosomal miRNAs by the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA). The relative expression levels of miR-17-92 in serum exosomes of GC patients were normalized to that of miR-16. For the relative expression of serum exosomal miR-17-92 in GC patients, $\Delta Ct=Ct_{miR\text{-}17\text{-}92}\text{-}Ct_{miR\text{-}16}$, $\Delta\Delta Ct=\Delta Ct_{gastric\ cancer\ patients}\text{-}\Delta Ct_{healthy\ controls}$. The $2^{-\Delta\Delta Ct}$ method was used to analyze the relative expression of exosomal miR-17-92 in GC patients and healthy controls. The $2^{-\Delta Ct}$ method was used to analyze the relationship between the expression of exosomal miR-17-92 and clinicopathological characteristics. The primers for miRNA detection were supplied by Invitrogen. The primer sequences used in qRT-PCR analysis were listed in Table 1.

Transmission electron microscopy (TEM)

20 μL of freshly extracted exosomes were added onto formvar carbon-coated 200-mesh copper grids and were adsorbed for 10 min. The adsorbed exosomes were negatively stained with 2% (w/v) phospphotungstic acid (pH 6.8) for 5 min, and then air-dried under the incandescent lamp. The morphology of the exosomes was observed with a transmission electron microscopy (FEI Tecnai 12, Philips).

Nanoparticle Tracking Analysis (NTA)

The extracted exosomes were suspended in PBS and were analyzed using a Malven Zetasizer Nano ZS90 (Malvern, UK) according to the manufacturer's protocol. The size distribution and concentration of exosomes were then analyzed by the Nanoparticle Tracking Analysis 2.0 (NTA 2.0) software.

Western blot analysis

Total protein of serum exosomes was extracted with RIPA buffer supplemented with proteinase inhibitors according to the manufacturer's instructions. Equal amounts of proteins were loaded and separated on a 12% SDS-PAGE gel. Following electrophoresis, the proteins were transferred to a PVDF (polyvinylidene difluoride) membrane, blocked in 5% (w/v) non-fat milk and incubated with the primary antibodies (Abs). Membranes were then washed and incubated with the appropriate secondary antibodies. Proteins were detected and scanned with the Gel Imaging system (PeiQing, China). Abs against CD9 (#ab92726) and CD81 (#ab109201) were obtained from Abcam. Secondary Abs against rabbit IgG, HRP- linked antibody (#7074) was obtained from Cell Signaling Technology.

Statistical analysis

Statistical analysis was performed with SPSS 20.0 software (IBM, Chicago, IL, USA). GraphPad Prism 5 (San Diego, CA, USA) was used to generate scatter diagrams. All experiments were performed at least three times. Differences of exosomal miRNA levels in serum of GC patients and healthy controls were analyzed using MannWhitney U test. The AUC was utilized to evaluate the feasibility of miRNA to differentially diagnose GC patients and healthy controls. A P-value of <0.05 was considered significant and a P-value of <0.01 as highly significant.

Results

Identification and characterization of exosomes in cell-free serum specimens.

Transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA) and western blot analysis were performed to determine whether exosomes were extracted efficiently from serum samples collected from 72 GC patients and 20 healthy controls. The morphology of exosomes

was directly observed by TEM. The exosomes showed spherical or oval vesicle shape and were found to have the lipid bilayer membrane structure with a diameter of 50–100 nm (Fig. 1a). Meanwhile, the results of NTA showed that the diameter of exosomes from the serum of GC patients was similar to that from healthy controls, and the average size of exosomes was 50–100 nm (Fig. 1b). The concentration of exosomes from the serum of GC patients was higher than that from healthy controls ($P < 0.01$) (Fig. 1c). Furthermore, we also confirmed that the isolated exosomes from the serum of GC patients and healthy controls expressed specific exosomal markers such as CD9 and CD81 by using western blot analysis (Fig. 1d). Taken together, these results showed that exosomes were successfully isolated from the serum samples, which provided a solid foundation for the further study of exosomal biomarkers.

Screening and evaluation of exosomal miR-17-92 cluster in the serum samples of gastric cancer patients.

The expression profiles of the serum exosomal miR-17-92 cluster, including miR-17, miR-18, miR-19a, miR-19b, miR-20 and miR-92, were detected by RT-qPCR analysis. As shown in Fig. 2, all of the six members of the exosomal miR-17-92 cluster could be clearly detected in the serum samples of human GC patients and healthy controls, albeit with different levels. Our data showed that the expression level of serum exosomal miR-17 in GC patients was significantly higher than that in healthy controls ($P < 0.001$) (Fig. 2a). Accordingly, the expressions of serum exosomal miR-18, miR-19a and miR-92 were also statistically much higher in GC patients compared with those of healthy controls (Fig. 2b, c, f). However, there were no significant differences in the expression of serum exosomal miR-19b and miR-20 between GC patients and healthy controls ($P > 0.05$) (Fig. 2d, e). Therefore, our results showed that the expression profile of the serum exosomal miR-17-92 cluster in GC patients was different from the healthy controls.

Evaluation of the diagnostic potential of serum exosomal miR-17-92 cluster for gastric cancer.

ROC (receiver-operating characteristic curve) analysis was used to explore the diagnostic utility of the serum exosomal miR-17-92 cluster for GC, with the sensitivity as the y axis and the 1-specificity as the x axis. The AUC (area under the curve) for serum exosomal miR-17 was 0.750 (95%CI, 0.626–0.874), the sensitivity was 84.7% and the specificity was 70.0% (Fig. 3a). Besides, the AUC for miR-18 was 0.736 (95%CI, 0.590–0.881) with sensitivity of 88.9% and specificity of 65.0% (Fig. 3b). Accordingly, the AUC for miR-19a and miR-92 was 0.700 (95%CI, 0.562–0.838) and 0.689 (95%CI, 0.567–0.811), respectively (Fig. 3c, d). These results showed that the AUC of serum exosomal miR-17 was the highest among the four miR-17-92 cluster members, indicating that miR-17 had the most powerful diagnostic efficacy for GC. In addition, we further explored whether combination of the exosomal miR-17-92 could be used to better distinguish GC patients from healthy controls. As shown in Fig. 3e, the combined detection of miR-17 and miR-18, which was the top two up-regulated miR-17-92 cluster members in GC patients, received the AUC of 0.774 (95%CI, 0.638–0.911), with sensitivity of 87.5% and specificity of 70.0%. It seemed that the combination of miR-17 and miR-18 showed much higher predictive value for the diagnosis of GC than each alone. Furthermore, we wondered whether the combination of all the four members could be the most powerful predictive marker for the diagnosis of GC. As expected, the combined panel consisting of miR-17, miR-18, miR-19a and miR-92 received the AUC of 0.808 (95%CI, 0.680–0.937), with sensitivity of 90.3% and specificity of 70.0% (Fig. 3f), which showed much higher clinical diagnostic value for GC than any of the four alone or any pair. Taken together, these results indicated that the serum exosomal miR-17-92 cluster could serve as potential predictive biomarker for the diagnosis of GC, and the exosomal miRNA combination panel could enhance the diagnostic power for GC.

Combining multiple biomarkers enhances the diagnostic power for gastric cancer.

To better evaluate the diagnostic potential of exosomal miR-17-92, we collected the clinical data from the 72 GC patients and 20 healthy controls for further study. The traditional tumor biomarkers such as CEA and CA19-9, which were commonly used in our clinical work, were calculated between the GC patients and healthy controls. As shown in Fig. 4a, the expression level of CEA was significantly upregulated in the serum of GC patients compared with that of healthy controls. Similarly, the expression of CA19-9 was also statistically upregulated in the GC patients than that of healthy controls (Fig. 4b). Then, ROC analysis was performed to explore the diagnostic efficiency of the two traditional tumor biomarkers. The AUC for CEA was 0.697 (95%CI, 0.569–0.825), the sensitivity was 56.9% and the specificity was 85.0% (Fig. 4c). The AUC for CA19-9 was 0.676 (95%CI, 0.547–0.805) with sensitivity of 70.8% and specificity of 65.0% (Fig. 4d). Besides, the combined panel of CEA and CA19-9 was built to further investigate the diagnostic efficiency for GC. As expected, the combination of CEA and CA19-9 showed much higher diagnostic value with the AUC of 0.738 (95%CI, 0.615–0.861) than any of the two alone (Fig. 4e). Furthermore, we also built the panel consisting of the two traditional tumor biomarkers and the four miR-17-92 cluster members, and the ROC of the newly developed diagnostic panel was calculated. Our results showed that the AUC for the newly developed panel was 0.881 (95%CI, 0.765–0.998) with sensitivity of 91.7% and specificity of 90.0% (Fig. 4f), which showed the greatest powerful clinical diagnostic value for GC. Taken together, these results suggested that the serum exosomal miR-17-92 cluster, together with traditional tumor biomarkers, could significantly enhance the diagnostic power for GC.

Correlation of serum exosomal miR-17-92 expression with clinicopathological parameters of gastric cancer patients.

In order to better understand the potential role of the serum exosomal miR-17-92 cluster in the development of GC, the association of the expression levels of each member of the miR-17-92 cluster with various clinicopathological features of GC patients was further analyzed. As shown in Table 2, the serum exosomal miR-17 expression was strongly related to the tumor size, tumor depth, distant metastasis and TNM stage of GC patients. However, there was no significant correlation between the expression of serum exosomal miR-17 and other clinical pathological characteristics such as age, gender, histological grade, lymph node metastasis and venous invasion of GC patients. Correspondingly, the serum exosomal miR-18 expression was significantly associated with tumor size, distant metastasis and TNM stage of GC patients. The serum exosomal miR-19a expression was markedly relevant to tumor depth, lymph node metastasis and TNM stage of GC patients. The serum exosomal miR-92 expression was greatly related to tumor depth, distant metastasis and TNM stage but not to the other clinicopathological features of GC patients. Taken together, these results suggested that exosomal miR-17-92 cluster might play an oncogenic role in the progression of GC.

Discussion

In this study, we discovered that circulating exosomal miR-17-92 cluster was significantly upregulated in GC patients compared with that of healthy controls. Elevated circulating exosomal miR-17-92 expressions were positively correlated with tumor size, tumor depth, lymph node metastasis, distant metastasis and TNM stage of GC patients. ROC analysis exhibited that a panel of the circulating exosomal miR-17-92 cluster including miR-17, miR-18, miR-19a and miR-92 had higher diagnostic efficiency than traditional biomarkers such as CEA and CA19-9 in GC. Meanwhile, synergistic effects were also found in combining of traditional biomarkers with the exosomal miR-17-92 biomarkers. These results provided evidence for exosomal miR-17-92 as a novel non-invasive candidate for GC diagnosis.

At present, gastroscopy, computed tomography, x-ray and serological examination are the main methods for GC diagnosis. Especially, gastroscopy together with the pathological biopsy, is the golden standard method for GC diagnosis. However, it is expensive and invasive. Compared with traditional invasive methods, liquid biopsy, such as circulating exosomes are non-invasive and can provide comprehensive and dynamic information of the whole stage of GC.

Emerging evidence have suggested that exosomal miRNAs can serve as promising biomarkers in various cancers, and are eligible for diagnosis, predicting recurrences, and providing prognostic information [25, 26]. It is reported that serum exosomal miR-19b and miR-106a were significantly upregulated in the GC patients, and they are potential biomarkers with higher diagnostic sensitivity and specificity for detecting GC [27]. In addition, serum exosomal miR-92a-3p was significantly lower in GC patients. Combined detection of serum exosomal miR-92a-3p, CEA and CA19-9 were more sensitive in GC diagnosis [28]. When combined with CEA, circulating exosomal miR-125a-3p could significantly improve the potential of screening early-stage colon cancer both in the sensitivity and specificity [29]. Report revealed that high plasma exosomal miR-1290 and miR-375 are significantly associated with poor overall survival for castration-resistant prostate cancer (CRPC) patients [30].

In our study, we found that the expressions of four members of circulating exosomal miR-17-92 cluster were significantly higher than those of healthy controls. Circulating exosomal miR-17-92 cluster could serve as a novel diagnostic marker for GC patients. miR-17-92 has been confirmed to play important roles in cancer proliferation, invasion, and migration. miR19 is reported to be highly expressed in gastric and prostate cancer [31]. miR18a was found to be highly expressed and promoted tumorigenesis through suppressing STK4 in prostate cancer [32]. Plasma miR-18a was upregulated in patients with GC, and higher miR-18a expression was associated with shorter disease-free survival and disease-specific survival in GC patients [33]. miR-17-5p/20a were reported to be significantly associated with the differentiation status and TNM stages of GC. Higher expression levels of miR-17-5p/20a were significantly correlated with poor overall survival of GC patients [34]. miR19a/b has been found to be upregulated in metastatic GC, which promoted cell migration, invasion and metastasis, by regulating the tumor suppressor MXD1 [35]. In addition, miR-92a was revealed to be an independent predictor of overall survival in GC patients [36].

In the present study, we found that four members of the exosomal miR-17-92 cluster including miR-17, miR-18, miR-19a and miR-92 were significantly upregulated in the serum samples of GC patients. In addition, the elevated exosomal miR-17-92 expressions were closely correlated with tumor size, tumor depth, lymph node metastasis, distant metastasis and TNM stage of GC patients. ROC curves are widely used to assess diagnostic performance. We constructed the ROC curve to define the diagnostic effectiveness of exosomal miR-17-92. ROC curve revealed that serum exosomal miR-17, miR-18, miR-19a and miR-92 level may distinguish GC patients from healthy controls. The AUC for serum exosomal miR-17, miR-18, miR-19a and miR-92 was 0.750, 0.736, 0.700, 0.689, respectively. The AUC for the newly combined panel consisting of miR-17, miR-18, miR-19a and miR-92 was 0.808, which showed much higher clinical diagnostic value for GC than any of the four alone or any pair. Moreover, the AUC for the newly developed panel consisting of the two traditional tumor biomarkers, CEA and CA19-9, and the four miR-17-92 cluster members showed the greatest powerful clinical diagnostic value for GC, with AUC of 0.881. Consistently, it is reported that combined detection of serum exosomal miR-92a-3p, CEA and CA19-9 were more sensitive in GC diagnosis [28].

In this study, combination of serum traditional biomarkers (CEA and CA19-9) and serum exosomal miRNAs (miR-17, miR-18, miR-19a and miR-92) reached the highest diagnostic power for the early detection of GC. However, the number of GC patients enrolled in this study was limited. A much larger cohort of GC patients are needed to further verify the diagnostic potential of the exosomal miR-17-92 cluster in GC.

Conclusions

In summary, the expression of exosomal miR-17-92 cluster was significantly upregulated in the serum samples of GC patients. The elevated exosomal miR-17-92 expressions were closely correlated with tumor size, tumor depth, lymph node metastasis, distant metastasis and TNM stage of GC patients. The combined panel consisting of miR-17, miR-18, miR-19a and miR-92 showed much higher clinical diagnostic value for GC than any of the four alone or any pair. More importantly, the newly developed panel consisting of CEA, CA19-9 and the four miR-17-92 cluster members showed the greatest powerful clinical diagnostic value for GC. In conclusion, our findings revealed that circulating exosomal miR-17-92 cluster may be used as a novel potential non-invasive biomarker to improve the diagnostic efficiency in GC.

Declarations

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Authors' contribution

WCC and JJX designed the research. FL, YH and DBL performed the research and analyzed the data. YH, DBL and JZ collected the samples and performed the research. FL and WCC wrote the paper. All authors have read and approved the final version of this manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the Ethics Committee of The First Affiliated Hospital of Soochow University.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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Tables

Table 1. Primer sequences used in qRT-PCR analysis.

Gene	Sequence（5'-3'）
miR-16	CAGCACGTAAATATTGGCGA
miR-17	GCAAAGTGCTTACAGTGCAGGTAG
miR-18	CCGGTAAGGTGCATCTAGT
miR-19a	TGTGCAAATCTATGCAAAACTG
miR-19b	GCATCCCAGTGTGCAAATCC
miR-20	GGTAAAGTGCTTATAGTGCAGGTAG
miR-92	ATTGCACTTGTCCCGGCCTGT

Table 2. The correlation between the expression levels of serum exosomal miR-17-92 cluster and clinical pathological parameters in gastric cancer patients.

Variable	No. of patients	miR-17 expression	P value	miR-18 expression	P value	miR-19a expression	P value	miR-92 expression	P value
	(N=72)								
Age(years)									
<60	30	14.083(11.920-16.247)	0.188	10.297(8.681-11.913)	0.629	11.383(9.945-12.822)	0.117	31.007(26.953-35.060)	0.34
≥60	42	16.179(13.968-18.389)		10.793(9.473-12.112)		9.864(8.585-11.144)		28.633(25.534-31.733)	
Gender									
male	39	16.021(14.083-17.958)	0.323	10.659(9.408-11.910)	0.876	10.255(8.832-11.677)	0.643	28.936(25.170-32.701)	0.544
female	33	14.460(11.867-17.054)		10.500(8.822-12.178)		10.703(9.369-12.036)		30.433(27.359-33.508)	
Tumor size(cm)									
<5	46	13.600(11.773-15.427)	0.003**	9.774(8.547-11.001)	0.03*	10.602(9.456-11.748)	0.772	29.585(26.414-32.756)	0.968
≥5	26	18.323(15.718-20.928)		12.023(10.348-13.699)		10.312(8.523-12.101)		29.688(25.697-33.680)	
Histological grade									
well-moderately	32	15.075(12.525-17.625)	0.794	10.463(8.935-11.990)	0.827	9.725(8.216-11.234)	0.149	28.931(25.705-32.158)	0.615
poorly	40	15.490(13.468-17.512)		10.685(9.306-12.064)		11.115(9.877-12.353)		30.175(26.531-33.819)	
Tumor depth									
T1-T2	35	13.613(11.290-15.972)	0.036*	9.946(8.494-11.397)	0.216	9.197(5.529-10.457)	0.007**	27.006(23.425-30.586)	0.036*
T3-T4	37	16.889(14.852-18.926)		11.192(9.778-12.606)		11.727(10.378-13.076)		32.097(28.851-35.344)	
Lymph node metastasis									
absent	24	15.121(11.663-18.574)	0.883	9.938(8.007-11.868)	0.364	9.071(7.624-10.518)	0.033*	27.521(23.749-31.293)	0.225
present	48	15.398(13.720-17.076)		10.910(9.722-12.098)		11.21(9.999-12.422)		30.673(27.509-33.837)	
Venous invasion									
negative	51	15.448(12.109-18.786)	0.908	10.282(9.005-11.560)	0.348	10.682(9.474-11.890)	0.511	29.159(26.302-32.016)	0.557
positive	21	15.247(13.452-17.042)		11.324(9.762-12.886)		10.048(8.494-11.602)		30.748(25.785-35.710)	
Distant metastasis									
M0	58	14.666(12.849-16.843)	0.038*	9.767(8.702-10.832)	0.001**	10.410(9.358-11.463)	0.713	28.129(25.532-30.727)	0.012*
M1	14	17.957(15.331-20.583)		13.979(12.033-15.924)		10.857(8.344-13.370)		35.807(29.936-41.679)	
TNM stage									
I-II	34	13.215(11.328-15.101)	0.01*	8.959(7.465-10.453)	0.002**	9.003(7.860-10.146)	0.002**	25.676(22.035-29.318)	0.002**

III-IV	38	17.176(14.842-19.511)	12.042(10.825-13.259)	11.834(10.442-13.226)	33.153(30.205-36.100)

* $P<0.05$, ** $P<0.01$

Figures

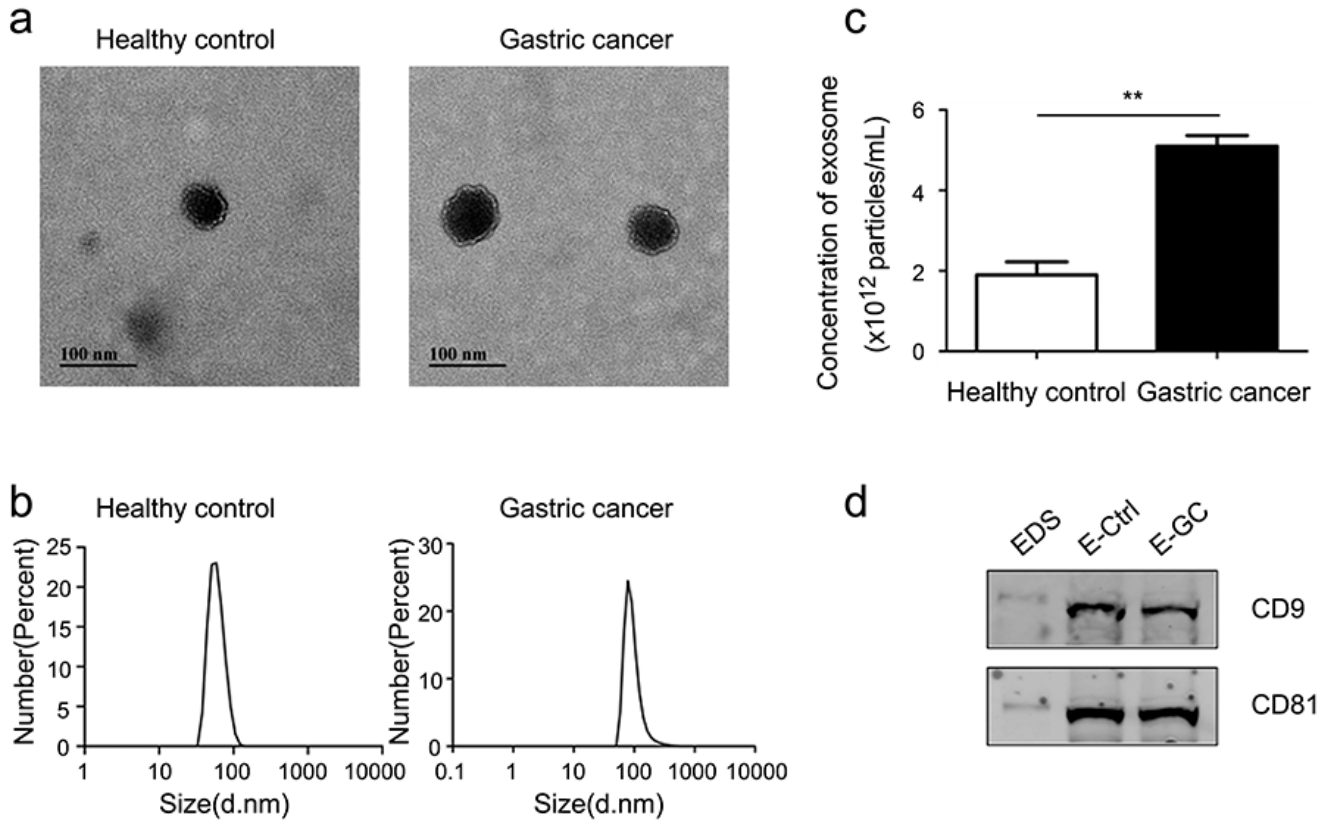

Figure 1

Characterization of exosomes derived from the serum of gastric cancer patients and healthy controls. a Transmission electron microscopy (TEM) images of serum exosomes. b The size distribution of serum exosomes examined by the NTA characterization system (**P<0.01). c The concentrations of exosomes derived from gastric cancer patients and healthy volunteers. d Western blot analysis of exosomal protein markers including CD9 and CD81 in the exosome-depleted supernatant (EDS), exosomes from the serum of gastric cancer patients (E-GC) and healthy controls (E-Ctrl).

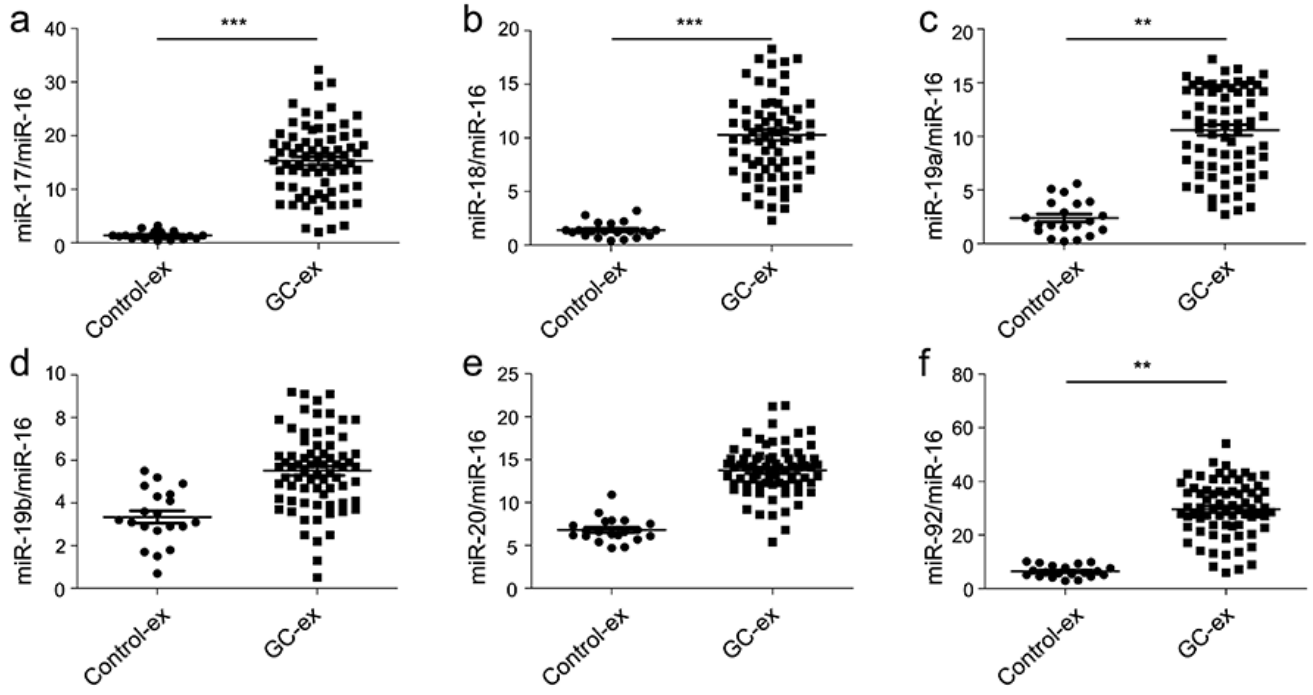

Figure 2

The expression levels of exosomal miR-17-92 cluster in serum samples of gastric cancer patients and healthy controls. a Expression level of miR-17 b Expression level of miR-18 c Expression level of miR-19a d Expression level of miR-19b e Expression level of miR-20 f Expression level of miR-92 (**P<0.01, ***P<0.001).

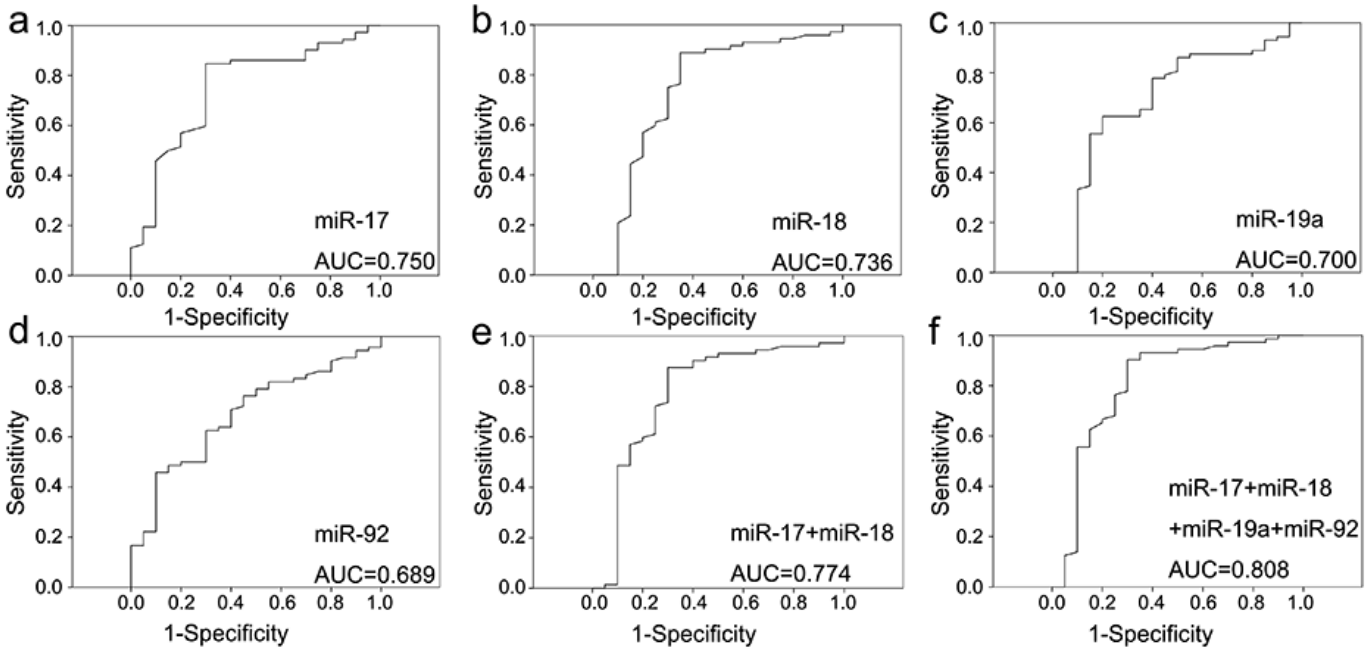

Figure 3

ROC curve analysis using serum exosomal miR-17-92 cluster for the discrimination of gastric cancer patients from healthy controls. a ROC curve for miR-17 b ROC curve for miR-18 c ROC curve for miR-19a d ROC curve for miR-92 e ROC curve for miR-17 + miR-18 f ROC curve for miR-17 + miR-18 + miR-19a + miR-92.

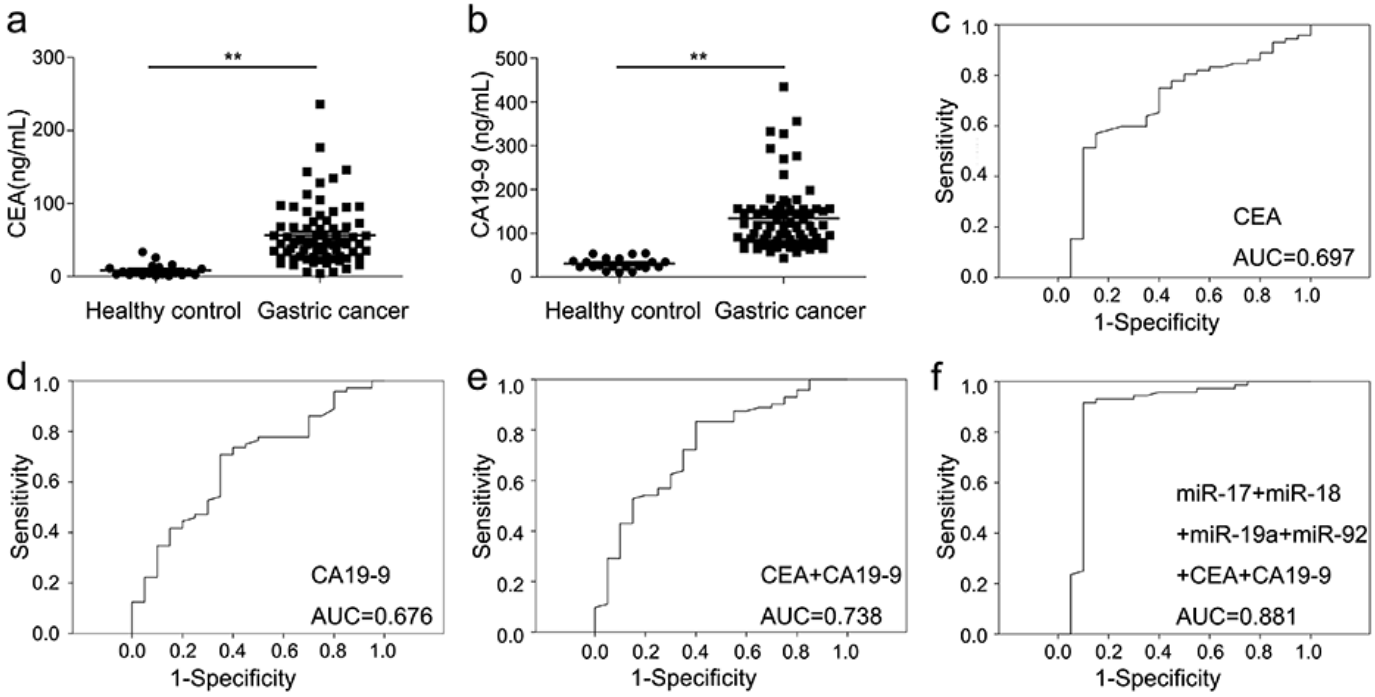

Figure 4

The expression levels of CEA and CA19-9 in serum samples of gastric cancer patients and healthy controls, and their clinical diagnostic values alone or along with serum exosomal miRNAs. a Expression level of CEA b Expression level of CA19-9 c ROC curve for CEA d ROC curve for CA19-9 e ROC curve for CEA + CA19-9 f ROC curve for miR-17 + miR-18 + miR-19a + miR-92 + CEA + CA19-9.