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Original Article

MicroRNA-217 in plasma: a potential biomarker in gastric cancer

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Abstract: Mounting studies indicated that aberrant circulating microRNA expression was associated to gastric cancer (GC). In the present study, we aimed to explore plasma miR-217 expression in GC patients and evaluated its diagnostic value as a promising biomarker for GC screening. The plasma samples were collected from 137 patients with GC and 145 healthy controls. Plasma miR-217 expression was determined through qRT-PCR and further correlated with patients' clinicopathological parameters and follow-up data. Receiver-operating characteristic (ROC) curve analysis was carried out to assess the diagnostic value of plasma miR-217 in GC, and COX regression analysis was performed to find independent risk factors for overall survival (OS) and disease-free survival (DFS) of GC patients. Compared with healthy controls, expression of plasma miR-217 was remarkably lower in GC patients (P<0.001). ROC curve analysis showed that the area under the curve (AUC) of plasma miR-217 was 0.893, and its specificity and sensitivity were 0.832 and 0.813 at a diagnostic threshold of 0.021. Down-regulation of plasma miR-217 was closely associated with larger tumor size (P=0.040), present distant metastasis (P=0.038), poor differentiation status (P=0.016) and advanced TNM stage (P=0.005). GC patients with low plasma miR-217 expression had relatively lower overall survival (OS) rate and disease-free survival (DFS) rate after surgery than those with high plasma miR-217 expression. Moreover, multivariate COX regression analysis demonstrated that plasma miR-217 expression was an independent risk factor for OS and DFS of GC patients. Plasma miR-217 levels were significantly down-regulated in GC patients and it could be considered as a promising non-invasive biomarker for GC.

Keywords: Gastric cancer, noninvasive biomarker, circulating microRNA, diagnosis, prognosis

Introduction

Gastric cancer (GC), featured as one of the most prevailing malignant neoplasms, remains the second cause of cancer-related mortalities around the world [1]. In Asia, especially in China, Japan and South Korea, the number of new cases of GC is increasing and the mortality and occurrence incidence are both highest among the gastrointestinal cancer in these countries [2]. Patients with early stage of GC always have a better prognosis than those with advanced gastric cancer. Their average 5-year survival rates are 15% and 90%, respectively. Despite rapid advances in diagnostic technology of GC, including endoscopy, barium meal examination and iconography, diagnostic efficiency of patients with early-stage GC remains largely unfavorable [3, 4]. Therefore, it is essential for us to find a novel, sensitive and specific method to improve the diagnostic efficiency of GC.

MicroRNAs (miRNAs), originally cloned in C. *elegans* in 2001 [5], are short, endogenous, non-coding RNAs of approximately 21-23 nucleotides in length that play key roles in various cellular processes as negative regulators of target mRNAs [6-8]. Specifically, miRNAs inhibit post-transcriptional translation of mRNAs through binding to their 3'untranslated region (3'-UTR). Emerging evidence demonstrated that aberrant expression of miRNAs might be correlated to a wide variety of malignancies, including breast cancer [9], bladder cancer [10], lung cancer [11] and colorectal cancer [12]. Many studies also revealed that the expression of miRNAs plays an essential role in GC. For example, Zhang *et al.* [13] showed that miR-25 inhibits cell apoptosis of gastric cancer by regulating

Table 1. Demographic characteristics of case group and control group

Characteristics	Case group (n=137)	Control group (n=145)	*P* value
Age (year)	54.3±3.9	53.6±4.4	0.159
Gender			0.716
Male	85 (62.0%)	93 (64.1%)	
Female	52 (38.0%)	52 (35.9%)	
Ethnicity			0.559
Han	129 (94.2%)	134 (92.4%)	
Minority	8 (5.8%)	11 (7.6%)	
Residence			0.594
Urban	61 (44.5%)	60 (41.4%)	
Rural	76 (54.5%)	85 (58.6%)	
History of smoking			0.292
Yes	67 (48.9%)	80 (55.2%)	
No	70 (51.1%)	65 (44.8%)	
History of drinking			0.364
Yes	72 (52.6%)	84 (57.9%)	
No	65 (47.4%)	61 (42.1%)	

Table 2. The sequences of primers used for RT-PCR in this study

Gene name	Primer sequences
Hsa-miR-217	
Forward	5'-CGCTCTACTGCATCAGGAACTGA-3'
Reverse	5'-GTGCAGGGTCCGAGGT-3'
U6	
Forward	5'-CTCGCTTCGGCAGCACA-3'
Reverse	5'-AACGCTTCACGAATTTGCGT-3'

CCNE1 and Myc. Therefore, miRNAs, function as novel biomarkers, may provide a novel method for tumor diagnosis in the future.

Currently, accumulating evidence demonstrated that circulating miRNAs in plasma, serum or urine have been regarded as a novel non-invasive diagnostic method for several cancers [14, 15]. Up-regulation of miR-21 and miR-31 was detected in both cancer tissues and plasma specimens in human pancreatic cancer [16]. Intriguingly, studies have showed that circulating miRNA levels are not always completely consistent with that in corresponding tumor tissue. For example, miR-486 is down-regulated significantly in GC tissue and cell lines but reversely up-regulated in the sera of GC patients [17].

MiR-217 is a member of microRNA family, which has been found to exert an important role in suppressing tumorigenesis. MiR-217 is down-regulated and serves as a tumor suppressor and inhibited the proliferation and invasion of tumor cells in various cancers, including GC [18] and ovarian cancer [19]. Chen *et al.* [1] indicated that miR-217 expression levels were down-regulated in tumor tissues and low expression levels of miR-217 were correlated to tumor phenotypes and unfavorable overall survival in GC patients. However, it is not clear whether miR-217 in plasma had a strong association with GC.

In the present article, we aim to explore the association with the expression level of miR-217 in plasma and GC and its influence to prognostic outcomes of GC patients. In summary, our results will provide a potential therapeutic strategy for GC patients.

Materials and methods

Study subjects

A total of 282 participants, including 137 GC patients and 145 gender- and age-matched healthy individuals, were recruited for plasma samples from Sichuan Provincial People's Hospital (Chengdu, China) between January 2010 and March 2011. All patients were diagnosed as GC in pathology and iconography, and none of them had received chemotherapy or radiotherapy previously. 145 healthy volunteers were enrolled as a control group. Details of the demographic and clinical characteristics of all participants were presented in **Table 1**. There was no remarkable difference of age (*P*=0.159), gender (*P*=0.716), ethnicity (*P*=0.559), residence (*P*=0.594), history of smoking (*P*=0.292) and history of drinking (*P*=0.364) between the two groups. After surgery, all GC patients were allowed to receive FAM plan chemotherapy during the whole research.

Ethics statement

The experimental protocol was approved by the ethics committee of Sichuan Provincial People's Hospital, and the informed consents were obtained from all the participants or their relatives.

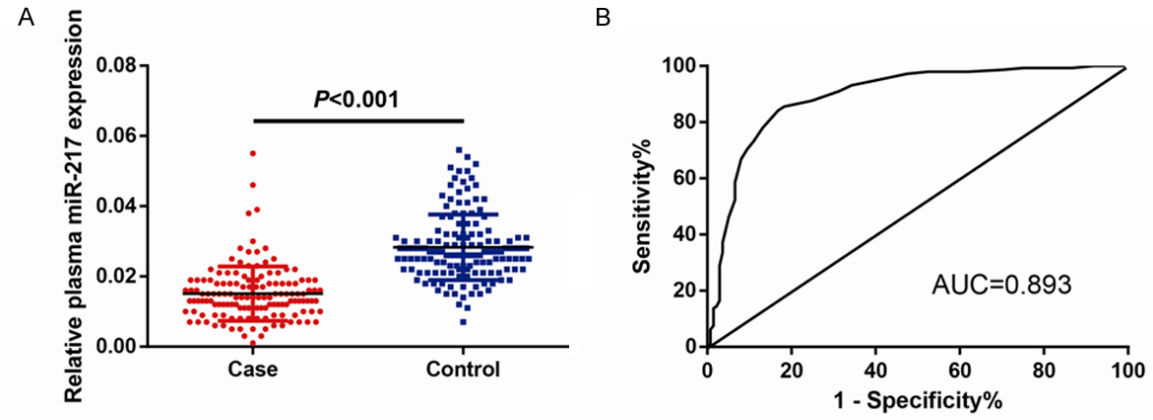

Figure 1. Plasma miR-217 expression was markedly decreased in GC. A. MiR-217 expression in plasma samples (137 GC patients and 145 controls) was determined by qRT-PCR analysis and normalized to U6 expression. B. Receiver-operating characteristic (ROC) curve analysis for detecting the diagnostic value of plasma miR-217 in GC (AUC 0.893 (95% CI, 0.854-0.932); Cutoff value is 0.021; Sensitivity, 0.813; Specificity, 0.832).

RNA isolation from plasma and qRT-PCR

Blood samples, which were stored in the separate vacuum cubes, were centrifuged at 3,000 rpm for 10 min at 4°C. The supernatant was collected and stored at -80°C for qRT-PCR. According to QIAGEN miRNeasy Mini Kit (Qiagen, Valencia, CA, USA), the RNA was extracted from 100 μL of plasma. CDNA of miR-217 was conducted through the instructions of the kit (QIAGEN, Valencia, CA, USA). U6 small RNA was applied as internal control. The sequences of primers used in qRT-PCR were recorded in **Table 2**. The qRT-PCR system was 20 μL, including 10 μL SYBR Premix Ex Taq, 0.4 μL Forward Primer, 0.4 μL Reverse Primer, 0.4 μL ROX Reference Dye II, 2 μL DNA template, and 6.8 μL dH_2O. The protocol of RT-PCR contained 40 cycles (95°C for 30 s, 95°C for 5 s and 60°C for 30 s). MiR-217 relative expression was calculated with the $2^{-\Delta Ct}$ method.

Statistical analysis

All statistical analysis of this research was performed using SPSS 21.0 software (SPSS Inc., Chicago, IL, USA) and presented by Graph PAD prism software 5 (GraphPad Software, Inc., La Jolla, CA, US). Comparisons of the data were conducted using Student's *t*-test or Chi-square test. Receiver-operating characteristic (ROC) curve with Youden's Index correction [20] was plotted to assess the potential value of plasma miRNA-217 for GC diagnosis. Survival durations in the patient groups after surgery were calculated with Kaplan-Meier method and log-rank test. Overall survival (OS) was investigated as the time from cancer diagnosis to death or date of last follow-up, and disease-free survival (DFS) was measured as the time from complete remission to treatment failure including relapse, death, or date at last follow-up. Cox regression analysis was conducted to confirm whether plasma miR-217 expression could be featured as an independent prognostic factor for GC patients. All statistical tests were two-sided and the *P*-value lower than 0.05 was considered as statistically significant.

Results

Plasma miR-217 expression is decreased in GC patients and exerts a diagnostic value

Plasma miR-217 levels in 137 GC patients and 145 healthy controls were detected through qRT-PCR. The results showed that compared with controls, GC patients had significantly lower plasma miR-217 expression (cases vs. controls: 0.015±0.008 vs. 0.028±0.009, *P*<0.001) (**Figure 1A**). ROC curve analyses were performed to evaluate the diagnostic accuracy of plasma miR-217. As shown in **Figure 1B**, the area under the curve (AUC) of ROC curve of plasma miR-217 was 0.893 (95% confidence interval (CI): 0.854-0.932). With an optimal cut-off value (0.021) at which the sum of the sensitivity and specificity was maximal, the sensitivity was 0.813, and the specificity was 0.832 for GC.

Table 3. Relationship between plasma miR-217 expression levels and clinicopathological characteristics of GC patients

Characteristics	Total number	Plasma miR-217 expression		*P* value
		Low (n=72)	High (n=65)	
Age (years)				0.428
<55	71	35 (48.6%)	36 (55.4%)	
≥55	66	37 (51.4%)	29 (44.6%)	
Gender				0.412
Male	85	47 (65.3%)	38 (58.5%)	
Female	52	25 (34.7%)	27 (41.5%)	
History of smoking				0.101
Yes	67	40 (55.6%)	27 (41.5%)	
No	70	32 (44.4%)	38 (58.5%)	
History of drinking				0.279
Yes	72	41 (56.9%)	31 (47.7%)	
No	65	31 (43.1%)	34 (52.3%)	
Tumor size (cm)				0.040
<5	51	21 (29.2%)	30 (46.2%)	
≥5	86	51 (70.8%)	35 (53.8%)	
Lauren type				0.129
Intestinal	88	42 (58.3%)	46 (70.8%)	
Diffuse and mixed	49	30 (41.7%)	19 (29.2%)	
Distant metastasis				0.038
Present	48	31 (43.1%)	17 (26.2%)	
Absent	89	41 (56.9%)	48 (73.8%)	
Lymph node invasion				0.183
Present	102	57 (79.2%)	45 (69.2%)	
Absent	35	15 (20.8%)	20 (30.8%)	
Differentiation status				0.016
Well	16	7 (9.7%)	9 (13.9%)	
Moderate	57	23 (32.0%)	34 (52.3%)	
Poor	64	42 (58.3%)	22 (33.8%)	
TNM stage				0.005
I-II	43	15 (20.8%)	28 (43.1%)	
III-IV	94	57 (79.2%)	37 (56.9%)	

Association between plasma miR-217 expression and clinicopathologic features in GC

The median expression level of plasma miR-217 was used as a cut-off point to divide all the 137 GC patients into two groups: High plasma miR-217 expression group (n=65) and low plasma miR-217 expression group (n=72). The correlations between plasma miR-217 levels and clinicopathological characteristics of GC patients were documented in **Table 3**. The results indicated that down-regulation of plasma miR-217 was closely associated with larger tumor size (*P*=0.040), present distant metastasis (*P*=0.038), poor differentiation status (*P*=0.016) and advanced TNM stage (*P*=0.005), whereas no significantly associated with age, gender, history of smoking, history of drinking, Lauren type and lymph node invasion (all *P*>0.05).

Relationship between plasma miR-217 expression and 5-year survival rates in GC patients

To further detect whether plasma miR-217 levels can predict GC prognosis, we subsequently performed survival analysis. As demonstrated in **Figure 2**, GC patients with low plasma miR-217 expression had relatively lower OS rate and DFS rate after surgery than those with high plasma miR-217 expression (median OS: 25.8 months vs. 47.3 months, *P*=0.029; Median DFS: 19.5 months vs. 38.4 months, *P*=0.027). These results demonstrated that plasma miR-217 could be applied as a potential prognostic indicator for OS and DFS of GC patients.

Multivariate analysis of the prognostic effects of clinicopathologic parameters in GC patients

To further determine whether plasma miR-217 expression could function as a predictor in GC prognosis, we performed logistic regression analysis. Univariate analysis showed that larger tumor size, present distant metastasis, advanced TNM stage and high levels of plasma miR-217 were statistically significant risk factors affecting OS (**Table 4**) and DFS (**Table 5**) of GC patients. Furthermore, multivariate Cox regression analysis enrolling abovementioned significant parameters revealed that plasma miR-217 expression was a prognostic predictor that is independent of other clinicopathological factors.

Discussion

Globally, GC has become the second main reason of cancer-related death among the population [21], and as we know, delayed diagnosis is

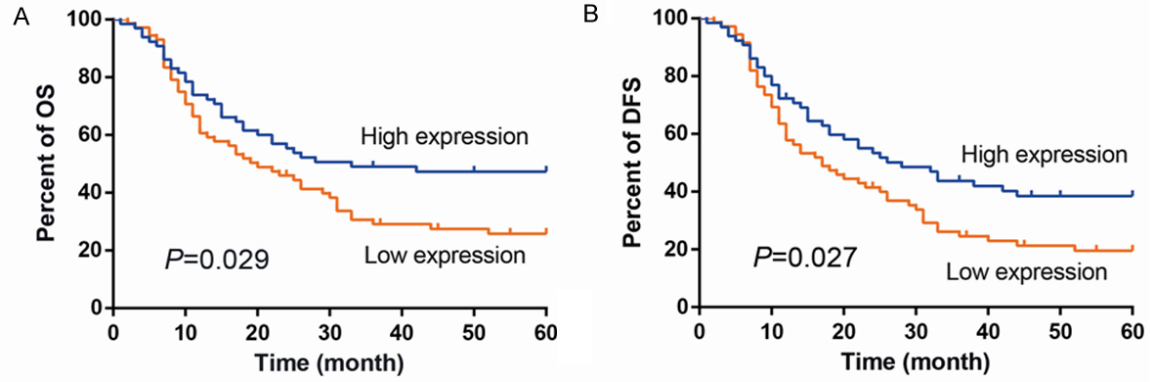

Figure 2. Plasma miR-217 expression was closely associated with 5-year survival rates in GC patients. Kaplan-Meier curves showing (A) the OS and (B) the DFS of 137 GC patients with high or low plasma miR-217 expression. $P<0.05$ (log-rank test) was regarded as statistically significant. OS, overall survival; DFS: Disease-free survival.

Table 4. Univariate and multivariate analysis of factors associated with OS of GC patients

Characteristics	Univariate analysis			Multivariate analysis		
	Risk ratio	95% CI	*P* value	Risk ratio	95% CI	*P* value
Age (years)						
<55 vs. ≥55	1.661	0.826-3.340	0.155			
Gender						
Male vs. Female	1.259	0.615-2.580	0.529			
History of smoking						
Yes vs. No	0.652	0.325-1.307	0.228			
History of drinking						
Yes vs. No	0.583	0.290-1.169	0.128			
Tumor size (cm)						
<5 vs. ≥5	0.417	0.204-0.853	0.017	0.449	0.197-1.023	0.057
Lauren type						
Intestinal vs. Diffuse and mixed	0.635	0.311-1.298	0.213			
Distant metastasis						
Present vs. Absent	0.408	0.188-0.887	0.024	0.440	0.193-1.003	0.051
Lymph node invasion						
Present vs. Absent	0.553	0.254-1.205	0.136			
Differentiation status						
Well vs. Moderate/Poor	0.582	0.289-1.175	0.131			
TNM stage						
I-II vs. III-IV	0.336	0.159-0.709	0.004	0.439	0.200-0.965	0.041
Plasma miR-217 expression						
High vs. Low	0.452	0.223-0.913	0.027	0.457	0.213-0.981	0.045

the main obstacle for treating GC. A wide variety of genetic alterations are found to be involved in tumorigenesis. Accumulating articles have investigated cell-free specific changes to nucleic acids in blood of patients, and have showed the potential value of circulating nucleic acids as new non-invasive tools for cancer diagnosis [22, 23]. Particularly, a number of microRNAs originating from cancer cells have been proven to be stable and easily detected in plasma or serum of patients with several cancers, including breast cancer [24, 25], colorectal cancer [26] and bladder cancer [27]. Lawrie *et al.* originally analyzed the levels of serum miR-155, miR-210 and miR-21 from patients with diffuse large B-cell lymphoma from healthy

Table 5. Univariate and multivariate analysis of factors associated with DFS of GC patients

Characteristics	Univariate analysis			Multivariate analysis		
	Risk ratio	95% CI	*P* value	Risk ratio	95% CI	*P* value
Age (years)						
<55 vs. ≥55	1.665	0.800-3.467	0.173			
Gender						
Male vs. Female	0.907	0.433-1.903	0.797			
History of smoking						
Yes vs. No	0.661	0.319-1.370	0.266			
History of drinking						
Yes vs. No	0.702	0.340-1.449	0.339			
Tumor size (cm)						
<5 vs. ≥5	0.426	0.203-0.894	0.024	0.455	0.200-1.034	0.060
Lauren type						
Intestinal vs. Diffuse and mixed	0.513	0.244-1.078	0.078			
Distant metastasis						
Present vs. Absent	0.251	0.102-0.622	0.003	0.263	0.100-0.687	0.006
Lymph node invasion						
Present vs. Absent	0.705	0.315-1.581	0.396			
Differentiation status						
Well vs. Moderate/Poor	0.568	0.271-1.189	0.133			
TNM stage						
I-II vs. III-IV	0.235	0.108-0.511	<0.001	0.296	0.129-0.678	0.004
Plasma miR-217 expression						
High vs. Low	0.464	0.222-0.968	0.041	0.429	0.187-0.982	0.045

controls and observed higher levels in patients than controls [28]. However, as a matter of fact, only relatively few miRNAs which are abundantly expressed in tumor cells could be easily detected in circulation [29], and approximately 30% of the released miRNAs could not reflect the expression patterns of the specific cancer cells [30]. These reports lay emphasis on the importance of discovering novel circulating microRNAs as predictors for cancers.

MiR-217 is found to be up-regulated in a wide variety of human cancers, including breast cancer [31, 32], pancreatic cancer [33] and lung cancer [34]. Wang *et al.* revealed that miR-217 was greatly down-regulated in human GC cell lines and tissues, and miR-217 directly targeted GPC5 to suppress GC cell proliferation and invasion [18]. In the present research, it was found that the expression of plasma miR-217 in GC patients was remarkably higher than that in healthy controls, which yielded an AUC of 0.893. At a cut-value of 0.021 for plasma miR-217, the sensitivity was 0.813 and specificity was 0.832. These results indicated that plasma miR-217 could serve as a reliable marker for the detection of GC. To the best of our knowledge, this might be the first research to reveal the potential function of plasma miR-217 in detection of GC.

Tumor growth and metastasis are critical factors for the identification of tumor phenotypes. It was also found that the aberrant expression of plasma miR-217 was significantly correlated to tumor size, distant metastasis, differentiation status, and TNM stage, which indicated that miR-217 might play a crucial role in the pathogenesis of GC from the clinical perspective. Other studies have suggested that miR-217 could inhibit tumor progression, metastasis and induce resistance to anti-tumor therapy through regulating various targets, including Wnt5a [35], IGF1R [19], and PTEN signaling [32].

Another intriguing finding of the present study is that miR-217 expression in plasma also functions as a prognostic biomarker for GC patients. Our results are consistent with previous studies

that have revealed the potential of tissue miR-217 as a prognostic marker in GC [1]. However, our findings revealed that low expression levels of plasma (rather than tissue) miR-217 indicated an unfavorable prognosis in GC patients. Furthermore, the multivariable Cox proportional hazards model illustrated that plasma miR-217 expression was an independent prognostic variable for OS and DFS of GC patients. Moreover, plasma levels of miR-217 might not only diagnose neoplasia but also predict metastases or cancer recurrence with relatively higher accuracy.

Our mechanistic studies are currently ongoing to decipher the role of miR-217 in onset of GC and its target gene. This clinical study lays the solid foundation towards investigating the underlying molecular mechanisms and genetic influences engaged in GC pathogenesis. Since miRNAs are important regulators of gene expression, it will be intriguing to detect the gene targets of miR-217 that influence GC. We acknowledged that our study has several limitations. First, the recruited participants were recruited from limited institutions, and it is unclear whether the sample can represent the general patient population with GC. Second, the methods of postoperative treatments and managements were not considered in the multivariate Cox proportional hazards regression analyses, which might bring some bias into the results. Third, the results of the study may have been more persuasive if the sample size had been increased. Fourth, we did not consider the expression of circulating miR-217 in other types of cancers; Therefore it might be challenging to distinguish if dysregulated circulating miR-217 expression is specifically related to GC itself.

Collectively, our results suggested that plasma miR-217 could be regarded as a noninvasive biomarker for screening, stage predicting and monitoring GC. Furthermore, if possible, more sensitive biomarkers in plasma miRNAs ought to be investigated for translation into the clinical setting of GC in the near future.

Disclosure of conflict of interest

None.

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References

[1] Chen DL, Zhang DS, Lu YX, Chen LZ, Zeng ZL, He MM, Wang FH, Li YH, Zhang HZ, Pelicano H, Zhang W and Xu RH. MicroRNA-217 inhibits tumor progression and metastasis by downregulating EZH2 and predicts favorable prognosis in gastric cancer. Oncotarget 2015; 6: 10868-10879.

[2] Sugano K. Screening of gastric cancer in Asia. Best Pract Res Clin Gastroenterol 2015; 29: 895-905.

[3] Qiu X, Zhang J, Shi W, Liu S, Kang M, Chu H, Wu D, Tong N, Gong W, Tao G, Zhao Q, Qiang F, Zhu H, Wu Q, Wang M and Zhang Z. Circulating MicroRNA-26a in plasma and its potential diagnostic value in gastric cancer. PLoS One 2016; 11: e0151345.

[4] Imdahl A. [Early diagnosis is crucial for prognosis in gastric cancer]. MMW Fortschr Med 2008; 150: 27-28.

[5] Lau NC, Lim LP, Weinstein EG and Bartel DP. An abundant class of tiny RNAs with probable regulatory roles in Caenorhabditis elegans. Science 2001; 294: 858-862.

[6] Zhou X, Wang J, Sun H, Qi Y, Xu W, Luo D, Jin X, Li C, Chen W, Lin Z, Li F, Zhang R and Li G. MicroRNA-99a regulates early chondrogenic differentiation of rat mesenchymal stem cells by targeting the BMPR2 gene. Cell Tissue Res 2016; 366: 143-53.

[7] Li Y, Jin L, Chen D, Liu J, Su Z, Yang S, Gui Y, Mao X, Nie G and Lai Y. Tumor suppressive miR-196a is associated with cellular migration, proliferation and apoptosis in renal cell carcinoma. Mol Med Rep 2016; 14: 560-566.

[8] Shah R, Tanriverdi K, Levy D, Larson M, Gerstein M, Mick E, Rozowsky J, Kitchen R, Murthy V, Mikalev E and Freedman JE. Discordant expression of circulating microRNA from cellular and extracellular sources. PLoS One 2016; 11: e0153691.

[9] Emmadi R, Canestrari E, Arbieva ZH, Mu W, Dai Y, Frasor J and Wiley E. Correlative analysis of miRNA expression and Oncotype Dx recurrence score in estrogen receptor positive breast carcinomas. PLoS One 2015; 10: e0145346.

[10] Zhao X, He W, Li J, Huang S, Wan X, Luo H and Wu D. MiRNA-125b inhibits proliferation and migration by targeting SphK1 in bladder cancer. Am J Transl Res 2015; 7: 2346-2354.

[11] Zhao FY, Han J, Chen XW, Wang J, Wang XD, Sun JG and Chen ZT. MiR-223 enhances the sensitivity of non-small cell lung cancer cells to erlotinib by targeting the insulin-like growth factor-1 receptor. Int J Mol Med 2016; 38: 183-191.

[12] Liu C, Zhang A, Cheng L and Gao Y. MiR410 regulates apoptosis by targeting Bak1 in human colorectal cancer cells. Mol Med Rep 2016; 14: 467-473.
[13] Zhang Y, Peng Z, Zhao Y and Chen L. MicroRNA-25 inhibits cell apoptosis of human gastric adenocarcinoma cell line AGS via regulating CCNE1 and MYC. Med Sci Monit 2016; 22: 1415-1420.
[14] Joyce DP, Kerin MJ and Dwyer RM. Exosome-encapsulated microRNAs as circulating biomarkers for breast cancer. Int J Cancer 2016; 139: 1443-1448.
[15] Zekri AR, Youssef AS, Lotfy MM, Gabr R, Ahmed OS, Nassar A, Hussein N, Omran D, Medhat E, Eid S, Hussein MM, Ismail MY, Alenzi FQ and Bahnassy AA. Circulating serum miRNAs as diagnostic markers for colorectal cancer. PLoS One 2016; 11: e0154130.
[16] Ali S, Dubaybo H, Brand RE and Sarkar FH. Differential expression of MicroRNAs in tissues and plasma co-exists as a biomarker for pancreatic cancer. J Cancer Sci Ther 2015; 7: 336-346.
[17] Zhu C, Ren C, Han J, Ding Y, Du J, Dai N, Dai J, Ma H, Hu Z, Shen H, Xu Y and Jin G. A five-microRNA panel in plasma was identified as potential biomarker for early detection of gastric cancer. Br J Cancer 2014; 110: 2291-2299.
[18] Wang H, Dong X, Gu X, Qin R, Jia H and Gao J. The MicroRNA-217 functions as a potential tumor suppressor in gastric cancer by targeting GPC5. PLoS One 2015; 10: e0125474.
[19] Li J, Li D and Zhang W. Tumor suppressor role of miR-217 in human epithelial ovarian cancer by targeting IGF1R. Oncol Rep 2016; 35: 1671-1679.
[20] Ruopp MD, Perkins NJ, Whitcomb BW and Schisterman EF. Youden Index and optimal cut-point estimated from observations affected by a lower limit of detection. Biom J 2008; 50: 419-430.
[21] Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.
[22] Diehl F, Schmidt K, Choti MA, Romans K, Goodman S, Li M, Thornton K, Agrawal N, Sokoll L, Szabo SA, Kinzler KW, Vogelstein B and Diaz LA Jr. Circulating mutant DNA to assess tumor dynamics. Nat Med 2008; 14: 985-990.
[23] Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB and Tewari M. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A 2008; 105: 10513-10518.
[24] Zhang L, Xu Y, Jin X, Wang Z, Wu Y, Zhao D, Chen G, Li D, Wang X, Cao H, Xie Y and Liang Z. A circulating miRNA signature as a diagnostic biomarker for non-invasive early detection of breast cancer. Breast Cancer Res Treat 2015; 154: 423-434.
[25] Kodahl AR, Lyng MB, Binder H, Cold S, Gravgaard K, Knoop AS and Ditzel HJ. Novel circulating microRNA signature as a potential non-invasive multi-marker test in ER-positive early-stage breast cancer: a case control study. Mol Oncol 2014; 8: 874-883.
[26] Hur K, Toiyama Y, Okugawa Y, Ide S, Imaoka H, Boland CR and Goel A. Circulating microRNA-203 predicts prognosis and metastasis in human colorectal cancer. Gut 2015; [Epub ahead of print].
[27] Motawi TK, Rizk SM, Ibrahim TM and Ibrahim IA. Circulating microRNAs, miR-92a, miR-100 and miR-143, as non-invasive biomarkers for bladder cancer diagnosis. Cell Biochem Funct 2016; 34: 142-148.
[28] Lawrie CH, Gal S, Dunlop HM, Pushkaran B, Liggins AP, Pulford K, Banham AH, Pezzella F, Boultwood J, Wainscoat JS, Hatton CS and Harris AL. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br J Haematol 2008; 141: 672-675.
[29] Chen TS, Lai RC, Lee MM, Choo AB, Lee CN and Lim SK. Mesenchymal stem cell secretes microparticles enriched in pre-microRNAs. Nucleic Acids Res 2010; 38: 215-224.
[30] Pigati L, Yaddanapudi SC, Iyengar R, Kim DJ, Hearn SA, Danforth D, Hastings ML and Duelli DM. Selective release of microRNA species from normal and malignant mammary epithelial cells. PLoS One 2010; 5: e13515.
[31] Zhang Q, Yuan Y, Cui J, Xiao T and Jiang D. MiR-217 promotes tumor proliferation in breast cancer via targeting DACH1. J Cancer 2015; 6: 184-191.
[32] Zhang AX, Lu FQ, Yang YP, Ren XY, Li ZF and Zhang W. MicroRNA-217 overexpression induces drug resistance and invasion of breast cancer cells by targeting PTEN signaling. Cell Biol Int 2015; [Epub ahead of print].
[33] Hong TH and Park IY. MicroRNA expression profiling of diagnostic needle aspirates from surgical pancreatic cancer specimens. Ann Surg Treat Res 2014; 87: 290-297.
[34] Guo J, Feng Z, Huang Z, Wang H and Lu W. MicroRNA-217 functions as a tumour suppressor gene and correlates with cell resistance to cisplatin in lung cancer. Mol Cells 2014; 37: 664-671.
[35] Wei R, Deng Z and Su J. MiR-217 targeting Wnt5a in osteosarcoma functions as a potential tumor suppressor. Biomed Pharmacother 2015; 72: 158-164.