Expression and Clinical Significance of Serum Exosomal miR-375 in Patients with Gastric Cancer

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To explore exosomal miR-375 expression in gastric cancer patients and its relationship with patient prognosis. A total of 53 patients diagnosed with gastric cancer in our hospital from May 2014 to May 2016 were included as the gastric cancer group, and 46 healthy women who came to our hospital for physical examination during the same period were enrolled as the healthy group. Exosomal miR-375 expression level was detected using qRT-PCR, and the diagnostic performance and prognostic significance of exosomal miR-375 in gastric cancer were explored. The gastric cancer group showed increased exosomal miR-375 expression than the healthy group ($P< 0.05$); Kaplan–Meier survival analysis exhibited that serum exosomal miR-375 has an AUC of 0.778, sensitivity of 69.57%, and specificity of 75.47%, whereas Cox regression analysis showed that the miR-375 expression in exosomes was an independent risk factor affecting the prognosis of gastric cancer patients ($P< 0.05$). Patient with gastric cancer showed upregulated miR-375 expression in serum exosomes. Serum exosomal miR-375 was found to has positive sensitivity and specificity in the diagnosis of gastric cancer, which may be associated with poor prognosis of gastric cancer patients.

Keywords: Serum Exosomal miR-375, Gastric Cancer

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Gastric cancer (GC) is the second leading cause of cancer-related mortality and the fourth most frequently diagnosed malignant tumor. Its incidence rate in the middle-aged and young population increases every year [1,2]. Early symptoms of GC are similar to common stomach diseases, which has a subtle onset and a longer course. The occurred of Metastasis might happened during diagnosis, and it has low effectivity rate of eradication therapy. As a result, the morbidity and mortality rates remain high, making it a global health challenge [3,4].

Exosomes belongs to small vesicles with diameter between 30–200 nm released from cells and enclosed by lipid bilayer membranes [5,6], which can wrap and deliver miRNA, DNA, and proteins. They are widely present in various body fluids, such as blood, saliva, urine, and ascites [7]. Several studies have shown that exosomes can reflect the pathologic condition of the cells from which they are derived; hence, in recent years, they have been regarded as a new type of diagnostic marker with great potential and have been used in disease diagnosis [8,9]. Previous studies have shown that

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tumor-secreted exosomal miRNAs were abnormally expressed. Cancer patients showed upregulated expression level of cancer-promoting miRNA in the serum exosomes [10,11], and the exosomes are crucial to has the GC's occurrence and development. STEC et al. [12] found that after exposing gastric cancer GC1415 cells to exosomes of their own origin *in vitro* for 24 h, and then transplanting them into immunodeficient mice, the tumor formation rate was significantly accelerated and accompanied by abundant formation of blood vessels. Furthermore, NING et al. [13] reported that GC cell-derived exosomes can promote pericyte proliferation and migration and induce their differentiation toward tumor-associated fibroblasts. miRNA regulates gene expression by inhibiting mRNA stability and interfering with translation [14]. REN et al. [15] reported that GC cells showed increased exosome level compared with the normal gastric epithelial cells, and sequencing assay indicated that the expression levels of miR-100 and miR-148 in the exosomes of GC cells were higher than that of normal gastric epithelial cells. However, there has been no study on miR-375 expression in the exosomes from GC cells.

Therefore, this study comparatively analyzed the clinical significance of serum exosomal miR-375 expression in patients with advanced GC, and provided a reference for potential biomarker selection.

MATERIALS AND METHODS

General Information

A total of 53 patients aged 31–63 years, with an average age of 49.15 ± 10.28 years, which were diagnosed with GC in our hospital from May 2014 to May 2016, were selected (GC group). Then, 46 healthy women during the same period aged 27–59 years, with an average age of 48.87 ± 9.71 years, were selected as the healthy group. Inclusion criteria: patients with GC who were diagnosed by pathologic examination, and their blood specimens were collected before surgery; patients with complete medical records and follow-up data; and patients who did not undergo chemotherapy, radiotherapy, and immunotherapy before enrollment. This study was approved by the Ethics Committee of this hospital, and the patients and their families were informed and signed a written informed consent document. The exclusion criteria in the study includes patients with history of other cancer, patients who have combined respiratory, cardiovascular and cerebrovascular, neurological systemic, and gastrointestinal diseases; patients with severe liver and kidney dysfunction; patients with physical disability and intolerance to surgery; patients who were transferred from other hospital and treated in other hospitals; patients who were pregnant or lactating; patients with a history of mental illness; patients with a history of prescription drug and contraceptive use within the past 3 months; and patients in their menstrual period.

Main Instruments and Reagents

The total RNA extraction kit (Beijing Solable Technology Co., Ltd., R1200-100); PCR instrument (Applied Biosystems, USA, 100073); reverse-transcription kit (Wuhan Purity Biotechnology Co., Ltd., China, CD102539GM); UV spectrophotometer (ThermoFisher, USA, Multiskan Sky); reverse-transcription kit (Beijing Baierdi Biotechnology Co., Ltd., China, DEM201-20T); and exosome extraction kit (Beijing Pulilai Gene Technology Co., Ltd., C1270) are cited with the corresponding manufacturers. Both miR-375 and internal reference (U6) primers were synthesized by Shanghai Shengong Biological Engineering Co., Ltd. (Table 1).

Table 1.
Primer sequences of exosomal miR-375 and U6.

Gene	Forward primer	Reverse primer
Exosomal miR-375	5'-AGTTTGTTCGTTCGGCTC-3 '	5'- GTGCAGGGTCCGAGGT -3 '
U6	5'-CTCGCTTCGGCAGCACA-3 '	5'-AACGCTTCACGAATTTGCGT-3 '

qRT-PCR Detection

Peripheral blood (5 ml) was collected from the patients in the morning, and miR-375 expression in the tissues and peripheral blood exosomes were detected using qRT-PCR. The serum exosomes were extracted using the exosome extraction kit, and RNA was extracted using the TRIzol method. Total RNA purity and concentration were measured using ultraviolet spectrophotometer and agarose gel electrophoresis, followed by reverse-transcription. Subsequently, a PCR amplification experiment was performed with cDNA obtained through reverse-transcription, with U6 as the internal reference. Then, a 20-μL PCR reaction system was prepared with PCR Premix (10 μl), upstream primer (10×) (2 μl), downstream primer (10×) (2 μl), and deionized distilled water. Amplification was performed on a real-time quantitative PCR instrument. The reaction conditions were 95 °C for 3 min, 95 °C for 15 s, and 60 °C for 45 s, for a total of 35 cycles. Three replicate wells were set for each sample, and the experiment was performed tree times. The relative miRNA expression was calculated using the 2-ΔΔCt method.

Follow-up

Patients were followed up by telephone, social media, etc.; and during the 1–2 year postoperative period, follow-up was performed every 3 months. During the 2–5 year postoperative period, patients were followed up every 6 months until March 2019 (culmination). The overall survival (OS) is the time from the first day after surgery until the last follow-up day or death.

Statistical Methods

SPSS23.0 (China Shanghai Kabe Information Technology Co., Ltd.) was used to analyze data. Measurement data (mean ± SD) were examined by independent sample *t*-tests. The count data (n,%) was examined using χ^2 test. The receiver operating characteristic (ROC) curve was used to evaluate the diagnostic efficacy of exosomal miR-375 for GC. Kaplan–Meier was used to establish the survival curve for high and low expression of exosomal miR-375 groups. The evaluation of difference between the two survival curves was done by using Log-rank test, and univariate and multivariate analyses of the independent factors affecting GC prognosis were performed using the Cox regression model. $P< 0.05$ indicated a statistically significant difference.

RESULTS

Baseline Data

The baseline data between both groups were not statistically significant in terms of gender, age, height, weight, exercise habits, alcohol consumption, smoking history, and place of residence ($P> 0.05$,Table 2).

Expression of Exosomal miR-375 in the Gastric Cancer and Healthy Groups

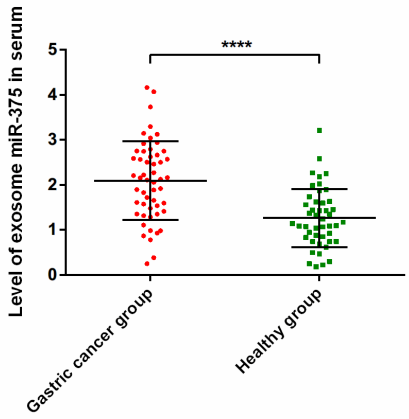
The qRT-PCR results showed that the GC group showed increased exosomal miR-375 expression level compared with the healthy group ($P< 0.05$, Figure 1).

Table 2.
Clinical data of the two patient groups.

Factor	Gastric cancer group (n = 53)	Health group (n = 46)		t/χ^2	P
Gender				0.6	0.4384
Male	34 (64.15)	26 (56.52)			
Female	19 (35.85)	20 (43.48)			
Weight (kg)	48.16 ± 3.58	48.77 ± 3.23	0.885	0.3786	
Age (year)	49.15 ± 10.28	48.87 ± 9.71	0.332	0.7717	
History of alcoholism			1.03	0.3103	
Yes	11 (20.75)	6 (13.04)			
No	42 (79.25)	40 (86.96)			

Place of residence			0.172	0.6783
Urban	31 (58.49)	25 (54.35)		
Rural	22 (41.51)	21 (45.65)		
Exercise habits			2.598	0.107
Yes	22 (41.51)	12 (26.09)		
No	31 (58.49)	34 (73.91)		
Smoking history			2.439	0.1183
Yes	15 (28.3)	7 (15.22)		
No	38 (71.7)	39 (84.78)		
PLT (× 109 / L)	155.67 ± 16.38	153.51 ± 18.69	0.897	0.3721
ALT (U / L)	21.78 ± 8.23	22.68 ± 9.16	0.515	0.6078
RBC (× 1012 / L)	4.53 ± 0.27	4.41 ± 0.46	1.607	0.1112
AST (U / L)	19.08 ± 7.56	18.73 ± 7.15	0.236	0.8143
Hb(g/dl)	14.37 ± 0.88	14.68 ± 0.67	1.32	0.19

Figure 1.
Exosomal miR-375 expression in both gastric cancer and healthy group.



The exosomal miR-375 expression level in the gastric cancer group was significantly higher than that in healthy group ($P< 0.05$).

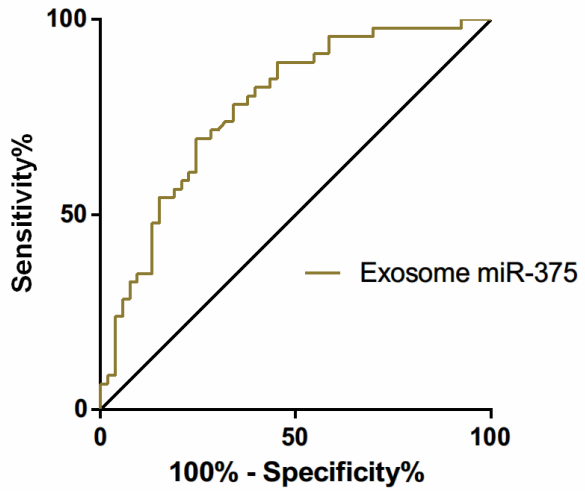
Diagnostic Performance of Serum Exosomal miR-375 in Gastric Cancer

The ROC curve of serum exosomal miR-375 expression for diagnosing GC was plotted. The AUC of serum exosomal miR-375 in the diagnosis of GC was 0.778 (95% confidence interval (CI): 0.687–0.869), with 1.470 cut-off value, 69.57% diagnostic sensitivity, and 75.47% specificity (Table 3, Figure 2).

Table 3.
Diagnostic value of serum exosomal miR-375 for gastric cancer.

Diagnostic indicators	AUC	95% CI	Standard error	Cutoff	Sensitivity (%)	Specificity (%)
Exosomal miR-375	0.778	0.687 ~ 0.869	0.046	1.470	69.57	75.47

Figure 2.
The diagnostic value of serum exosomal miR-375 in gastric cancer.

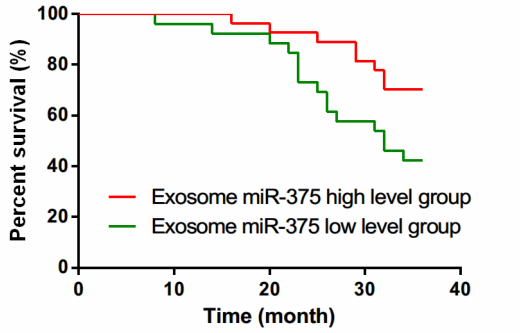


It showed cut-off value of 1.470, diagnostic sensitivity of 69.57%, and specificity of 75.47%.

Survival Rate of Patients with Gastric Cancer

The GC patients were divided into 27 cases (high expression group, ≥2.111) and 26 cases (low expression group, <2.111), according to the median value of exosomal miR-375 expression level. The patients were followed up for 3 years by telephone, review, letter, and other follow-up methods. Furthermore, 17 patients in the high expression group survived for 3 years, and the survival rate (OS) was 62.96%. Also, 12 patients in the low expression group survived for 3 years, and the 3-year OS was 46.15%. The high expression group showed increased 3-year OS compared with the low expression group (P< 0.05) (Figure 3).

Figure 3.
Survival of gastric cancer patients.



The high expression group showed survival rate (OS) of 62.96%, which is higher than the 46.15% in the low expression group (P< 0.05).

Univariate Analysis of Survival Rate

Differences were observed in age, Borrmann type, history of *Helicobacter pylori* infection, degree of differentiation, and exosomal miR-375 expression (P< 0.05, Table 4).

Table 4.

Univariate analysis of patient survival.

Factor	Survival group (n = 29)	Death group (n = 24)	t/χ^2	P
Gender				
Male	18 (62.07)	16 (66.67)	0.121	0.728
Female	11 (37.93)	8 (33.33)		
Age (year)			9.596	0.002
≤ 60	23 (79.31)	9 (37.5)		
> 60	6 (20.69)	15 (62.5)		
Smoking history			0.74	0.460
Yes	7 (24.14)	8 (33.33)		
No	22 (75.86)	16 (66.67)		
Exercise habits			0.291	0.590
Yes	13 (44.83)	9 (37.5)		
No	16 (55.17)	15 (62.5)		
Place of residence			1.208	0.272
Urban	15 (51.72)	16 (66.67)		
Rural	14 (48.28)	8 (33.33)		
History of alcoholism			1.887	0.170
Yes	4 (13.79)	7 (29.17)		
No	25 (86.21)	17 (70.83)		
Pathological type			2.914	0.572
Tubular adenocarcinoma	6 (20.69)	5 (20.83)		
Papillary adenocarcinoma	7 (24.14)	6 (25)		
Mucinous adenocarcinoma	5 (17.24)	3 (12.5)		
Signet ring cell carcinoma	5 (17.24)	8 (33.33)		
Undifferentiated carcinoma	6 (20.69)	2 (8.33)		
History of gastrointestinal ulcer			0.959	0.328
No	13 (44.83)	14 (58.33)		
Yes	16 (55.17)	10 (41.67)		
Borrmann type			5.443	0.020
Ⅰ + Ⅱ	19 (65.52)	8 (33.33)		
Ⅲ + Ⅳ	10 (34.48)	16 (66.67)		
History of Helicobacter pylori infection			10.58	0.001
No	10 (34.48)	19 (79.17)		
Yes	19 (65.52)	5 (20.83)		
Differentiation			6.944	0.031
Differentiated	10 (34.48)	17 (70.83)		
Poorly differentiated	11 (37.93)	4 (16.67)		
Undifferentiated	8 (27.59)	3 (12.5)		
Exosomal miR-375 expression			3.187	0.001
High expression (≥ 2.111)	9 (31.03)	18 (75)		
Low expression (< 2.111)	20 (68.97)	6 (25)		
PLT (× 109 / L)	154.39 ± 15.27	155.86 ± 14.23	0.36	0.721
ALT (U / L)	22.54 ± 7.29	21.19 ± 9.36	0.59	0.558
RBC (× 1012 / L)	4.39 ± 0.21	4.48 ± 0.51	0.867	0.390
AST (U / L)	17.56 ± 7.07	18.14 ± 7.61	0.287	0.775
Hb(g/dl)	14.37 ± 0.92	14.61 ± 0.73	1.036	0.305

Multivariate Analysis of Survival Rate

Cox proportional hazards model showed that the degree of differentiation, age, Borrmann type, and exosomal miR-375 expression were independent risk factors for GC prognosis (P< 0.05,Tables 5 and 6).

Table 5.
Assignment table.

	Assignment
Age (years)	≥60 = 1, <60 = 0
Differentiation	Undifferentiated = 0 , poorly differentiated type = 1, differentiated type = 2
Borrmann type	Ⅰ + Ⅱ = 1, Ⅲ + Ⅳ = 0
Exosomal miR-375 expression	≥2.111 = 1, << 2.111
Survival	Death = 1, survival = 0

Table 6.
Multi-factorial analysis affecting gastric cancer prognosis.

Factor	B	SE	Waldχ^2	OR (95% CI)	P
Degree of differentiation (differentiated, poorly differentiated, undifferentiated)	0.427	0.041	7.317	1.613 (1.211-2.172)	0.004
Age (≤ 60 years old,> 60 years old)	0.651	0.037	4.173	1.072 (0.012-1.148)	0.045
Borrmann type(Ⅰ+Ⅱ, Ⅲ+Ⅳ)	0.236	0.173	4.812	1.215(1.049-1.876)	0.005
Exosomal miR-375 expression (≥ 2.111 , <2.111)	1.231	0.326	5.624	1.816(0.913-2.113)	0.003

DISCUSSION

GC is a common malignant tumor. *H. pylori* infection is a prevalent risk factor for GC, but its specific mechanism of action is still unclear [16,17]. Gastric resection and lymphadenectomy are the only potential radical cures for GC, it's difficult to make desired therapeutic effect when GC developed into middle and advanced stages [18,19]. As such, GC biomarkers with high diagnostic performance are of great significance for improving patient prognosis and increasing OS rate [2].

Exosomes are nanovesicles containing biological factors and proteins secreted by various cells, which can be separated and extracted from biological fluids, including serum, urine, and from accumulated fluids in bodily cavities, such as in ascites. [20-22]. Several studies have found that exosomes are rich in miRNA, and exosomes can selectively load miRNAs in the cell cytoplasm [23]. The exosomes secreted by tumor cells contain special miRNAs with unique composition ratios, which is determined by the type of cells derived from exosomes [24]. When exosomes are released, they can enter the cell by fusing with the membrane of the recipient cell. They transfer bioactive molecules, such as miRNAs, to other cells, thereby participating in local and distant inter-cell information exchange and regulating the proliferation, invasion, metastasis, and resistance of tumor cells [25,26]. Huang et al. [27] found that miR-1290 and miR-375 in exosomes can be used as prognostic indicators for castration-resistant prostate cancer, whereas Meng [28] et al. found that miR-373, miR-200a, miR-200b, and miR-200c show high diagnostic performance and prognosis determination in ovarian cancer. This demonstrated that exosomal miRNA plays an important role in cancer diagnosis.

Moreover, miR-375 is located between the gene regions of cryba2 and Ccdc108 on the 2q35 region of the human chromosome. In recent years, relevant studies have found that miR-375 is abnormally expressed in various tumors, including liver cancer and prostate cancer, and is involved in the disease process [29,30]. However, there is no study centering on miR-375 expression in serum exosomes of GC patients. This study showed that serum exosomal miR-375 expression levels in GC

patients were significantly higher than those in the healthy group.Tsukamoto et al. [31] used gene chips to screen 22 pairs of GC and adjacent tissues. The results showed that GC tissues has lower miR-375 expression than the normal tissues, but miR-375 expression in exosomes was not explored.

This study also found that age, Borrmann type, history of *H. pylori* infection, degree of differentiation, and exosomal miR-375 expression were correlated with survival rate (all $P< 0.05$), and degree of differentiation, age, type of Borrmann, and miR-375 expression in exosomes were independent prognostic factors of GC. Patients with age > 60 years, undifferentiated tumor, and serum exosomal miR-375 ≥ 2.111 have poor prognosis. Di et al. [32] found that survival rate was correlated with tumor differentiation degree (P = 0.0228), suggesting that the latter is a factor that affects the prognosis. The study by Llanos et al. [33] pointed out that under the premise of radical cure, the prognosis of GC in young people is far better than that in elderly patients. Li et al. [34] conducted a retrospective study of 3,966 patients with GC and concluded that Borrmann type is an independent factor to judge GC prognosis. The aforementioned findings support our views that age, Borrmann type, and degree of differentiation are correlated with GC prognosis. Patients with these risk factors should be given more attention in clinic[35].

Although this study confirmed the diagnostic value of exosomal miR-375 for GC and its relationship with prognosis, there are still some shortcomings. First, for this retrospective study, the data obtained is sometimes inevitably interfered by subjective factors; and second, the specific mechanism of exosomal miR-375 is also unclear. Therefore, we recommend further in-depth investigation to verify the results of our research.

REFERENCES

1. Jemal A, Center M M, DeSantis C and Ward E M. Global patterns of cancer incidence and mortality rates and trends. Cancer Epidemiol Biomarkers Prev 2010; 19: 1893-1907.
2. Van Cutsem E, Sagaert X, Topal B, Haustermans K and Prenen H. Gastric cancer. Lancet 2016; 388: 2654-2664.
3. Smyth E C, Verheij M, Allum W, Cunningham D, Cervantes A and Arnold D. Gastric cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2016; 27: v38-v49.
4. Zong L, Abe M, Seto Y and Ji J. The challenge of screening for early gastric cancer in China. Lancet 2016; 388: 2606.
5. Lai R C, Yeo R W and Lim S K. Mesenchymal stem cell exosomes. Semin Cell Dev Biol 2015; 40: 82-88.
6. Pegtel D M and Gould S J. Exosomes. Annu Rev Biochem 2019; 88: 487-514.
7. Cocucci E and Meldolesi J. Ectosomes and exosomes: shedding the confusion between extracellular vesicles. Trends Cell Biol 2015; 25: 364-372.
8. Melo S A, Luecke L B, Kahlert C, Fernandez A F, Gammon S T, Kaye J, et al. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. Nature 2015; 523: 177-182.
9. Lin J, Li J, Huang B, Liu J, Chen X, Chen X M, et al. Exosomes: novel biomarkers for clinical diagnosis. ScientificWorldJournal 2015; 2015: 657086.
10. Kalluri R. The biology and function of exosomes in cancer. J Clin Invest 2016; 126: 1208-1215.
11. Pitt J M, André F, Amigorena S, Soria J C, Eggermont A, Kroemer G, et al. Dendritic cell-derived exosomes for cancer therapy. J Clin Invest 2016; 126: 1224-1232.
12. Stec M, Szatanek R, Baj-Krzyworzeka M, Baran J, Zembala M, Barbasz J, et al. Interactions of tumour-derived micro(nano)vesicles with human gastric cancer cells. J Transl Med 2015; 13: 376.
13. Ning X, Zhang H, Wang C and Song X. Exosomes Released by Gastric Cancer Cells Induce Transition of Pericytes Into Cancer-Associated Fibroblasts. Med Sci Monit 2018; 24: 2350-2359.
14. Reddy and Kaladhar B. MicroRNA (miRNA) in cancer. Cancer Cell Int 2015; 15:
15. Ren J, Zhou Q, Li H, Li J, Pang L, Su L, et al. Characterization of exosomal RNAs derived from human gastric cancer cells by deep sequencing. Tumour Biol 2017; 39: 1010428317695012.
16. Graham D Y. Helicobacter pylori update: gastric cancer, reliable therapy, and possible benefits. Gastroenterology 2015; 148: 719-731.e713.
17. Ajani J A, D'Amico T A, Almhanna K, Bentrem D J, Chao J, Das P, et al. Gastric Cancer, Version 3.2016, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 2016; 14: 1286-1312.
18. Wagner A D, Syn N L, Moehler M, Grothe W, Yong W P, Tai B C, et al. Chemotherapy for advanced gastric cancer. Cochrane Database Syst Rev 2017; 8: Cd004064.
19. Ono H, Yao K, Fujishiro M, Oda I, Nimura S, Yahagi N, et al. Guidelines for endoscopic submucosal dissection and endoscopic mucosal resection for early gastric cancer. Dig Endosc 2016; 28: 3-15.
20. Vlassov A V, Magdaleno S, Setterquist R and Conrad R. Exosomes: current knowledge of their composition, biological functions, and diagnostic and therapeutic potentials. Biochim Biophys Acta 2012; 1820: 940-948.
21. Fernandez-Mercado M, Manterola L, Larrea E,

Goicoechea I, Arestin M, Armesto M, et al. The circulating transcriptome as a source of non-invasive cancer biomarkers: concepts and controversies of non-coding and coding RNA in body fluids. J Cell Mol Med 2015; 19: 2307-2323.
22. Li X J, Ren Z J, Tang J H and Yu Q. Exosomal MicroRNA MiR-1246 Promotes Cell Proliferation, Invasion and Drug Resistance by Targeting CCNG2 in Breast Cancer. Cell Physiol Biochem 2017; 44: 1741-1748.
23. Niu J, Xue A, Chi Y, Xue J, Wang W, Zhao Z, et al. Induction of miRNA-181a by genotoxic treatments promotes chemotherapeutic resistance and metastasis in breast cancer. Oncogene 2016; 35: 1302-1313.
24. Villarroya-Beltri C, Gutiérrez-Vázquez C, Sánchez-Cabo F, Pérez-Hernández D, Vázquez J, Martin-Cofreces N, et al. Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs. Nat Commun 2013; 4: 2980.
25. Xie S Y, Li Y J, Wang P Y, Jiao F, Zhang S and Zhang W J. miRNA-regulated expression of oncogenes and tumor suppressor genes in the cisplatin-inhibited growth of K562 cells. Oncol Rep 2010; 23: 1693-1700.
26. Wang T, Xu H, Qi M, Yan S and Tian X. miRNA dysregulation and the risk of metastasis and invasion in papillary thyroid cancer: a systematic review and meta-analysis. Oncotarget 2018; 9: 5473-5479.
27. Huang X, Yuan T, Liang M, Du M, Xia S, Dittmar R, et al. Exosomal miR-1290 and miR-375 as prognostic markers in castration-resistant prostate cancer. Eur Urol 2015; 67: 33-41.
28. Meng X, Müller V, Milde-Langosch K, Trillsch F, Pantel K and Schwarzenbach H. Diagnostic and prognostic relevance of circulating exosomal miR-373, miR-200a, miR-200b and miR-200c in patients with epithelial ovarian cancer. Oncotarget 2016; 7: 16923-16935.
29. Li L M, Hu Z B, Zhou Z X, Chen X, Liu F Y, Zhang J F, et al. Serum microRNA profiles serve as novel biomarkers for HBV infection and diagnosis of HBV-positive hepatocarcinoma. Cancer Res 2010; 70: 9798-9807.
30. Wach S, Nolte E, Szczyrba J, Stöhr R, Hartmann A, Ørntoft T, et al. MicroRNA profiles of prostate carcinoma detected by multiplatform microRNA screening. Int J Cancer 2012; 130: 611-621.
31. [31] Tsukamoto Y, Nakada C, Noguchi T, Tanigawa M, Nguyen L T, Uchida T, et al. MicroRNA-375 is downregulated in gastric carcinomas and regulates cell survival by targeting PDK1 and 14-3-3zeta. Cancer Res 2010; 70: 2339-2349.
32. Di Martino N, Izzo G, Cosenza A, Cerullo G, Torelli F, Monaco L, et al. Surgical therapy of adenocarcinoma of the esophagogastric junction: analysis of prognostic factors. Hepatogastroenterology 2005; 52: 1110-1115.
33. Llanos O, Butte J M, Crovari F, Duarte I and Guzmán S. Survival of young patients after gastrectomy for gastric cancer. World J Surg 2006; 30: 17-20.
34. Li C, Oh S J, Kim S, Hyung W J, Yan M, Zhu Z G, et al. Macroscopic Borrmann type as a simple prognostic indicator in patients with advanced gastric cancer. Oncology 2009; 77: 197-204.
35. Hao Wang , Xiao-Meng Zhang , Go Tomiyoshi, et al . Association of serum levels of antibodies against M MP1, CBX1, and CBX5 with cerebral infarction. On cotarget, 2017, 9(5): 5600-5613.Doi: 10.18632/onc otarget.23789