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Original Article

Serum expression of miRNA-103, a potential diagnostic and prognostic biomarker for colorectal cancer

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Abstract: Objectives: To investigate the potential clinical utility of miR-103 as noninvasive diagnostic, prognostic and metastasis-predictive biomarker in patients with colorectal cancer (CRC). Methods: 108 patients scheduled to undergo surgery in Department of Gastrointestinal Surgery, Ningbo NO. 2 hospital from July, 2009 to September, 2015 were enrolled in this study. Blood samples were collected from all the 108 CRC patients and 53 volunteers for the evaluation of miR-103. The association between serum expressions of miR-103 with clinicopathological characteristics and overall survival rate in CRC patients were analyzed. Results: The expressions of miR-103 were significantly elevated in the serum of CRC patients compared with the matched healthy controls ($P<0.01$). The patients with TNM stage of II-IV were all with significantly higher expressions of miR-103 than those patients with TNM stage I ($P<0.01$). Receiver operating characteristic (ROC) curve analysis suggested that serum miR-103 was a useful biomarker for discriminating CRC from healthy controls ($P<0.001$), with the areas under the ROC curve (AUC) of 0.883, 95% confidence interval (CI) of 0.833 to 0.933. High expressions of miR-103 was a significant impact factor for overall survival rate of CRC patients by the Kaplan-Meier analysis with log-rank test. Conclusions: The serum expression of miR-103 might be used as a robust diagnostic, prognostic and metastasis predictive biomarker in patients with CRC.

Keywords: Colorectal cancer, serum miRNA-103, noninvasive biomarker, diagnosis, prognosis

Introduction

Colorectal cancer (CRC) has been reported as a major cause of cancer mortality worldwide with considerable health burden [1]. The overall incidence of CRC is as high as 5% in the general population and the 5-year survival rate is 40%-60% [2]. The prognosis mostly relies upon the morphology and histopathology stage of the tumor [3]. The diagnostic values are relatively limited due to the high costs, lack of sensitivity and inconvenience, resulting in the diagnosis at an advanced stage and poor prognosis [4]. Early stage diagnosis of CRC by noninvasive approaches could reduce the mortality and improve the prognosis [5]. MicroRNAs (miRNAs) are single-stranded, non-coding RNA strands of 19-25 nucleotides that regulate the translation of specific protein coding genes in mammals [6]. Recent studies have revealed that miRNAs played important roles in a variety of biological and pathological processes [7]. miRNAs were also reported to be involved in many key cellular processes, including cell cycle, proliferation, apoptosis, metastasis, metabolism, gene regulation and cancer development [8-10]. Moreover, many researchers have focused themselves on searching for the association between miRNA expressions and CRC. A small fraction of the differentially expressed miRNAs may be of clinical utility as potential diagnostic, prognostic biomarkers or even therapeutic targets for CRC [11]. Previous studies have revealed that miR-103 is one of the most significantly up-regulated miRNAs in colorectal cancers and the inhibition of miR-103 could apparently repress the proliferation of colorectal cancer cells [11, 12]. However, the diagnostic and prognostic values of serum miR-103 expression in CRC still remains unclear to date. The aim of this study was to evaluate the clinical significance of serum miR-103 expression in CRC.

Table 1. Clinical characteristics of patients with colorectal cancer and control participates in this study

	Patients (n=108)	Control (n=53)	*P*-value
Age (year)	51.8±13.5	49.7±11.0	0.327
Gender			
Male	63 (44.8%)	30 (37.0%)	
Female	45 (55.2%)	23 (63.0%)	0.835
BMI (kg/m^2)	22.5±2.8	22.4±3.5	0.845
Location of primary tumor			
Colon	67	—	—
Rectum	41	—	—
Tumor size (cm)			
≥5	38	—	—
<5	70	—	—
Tumor differentiation			
Poorly	29	—	—
Moderately	45	—	—
Well	34	—	—
TNM stage			
I	17	—	—
II	33	—	—
III	37	—	—
IV	21	—	—
Distant metastasis			
Yes	27	—	—
No	81	—	—
Lymph node metastasis			
Yes	57	—	—
No	51	—	—

Material and methods

Patients and samples

This study was approved by the Medical Institutional Ethics Committee of Zhejiang province. One hundred and eight patients scheduled to undergo surgery in Department of Gastrointestinal Surgery, Ningbo NO. 2 hospital from July, 2009 to September, 2015 were enrolled in this study.

The detailed inclusion criteria were that patients confirmed with CRC pathologicallyand (1) without history of other cancers; (2) no history of preoperative treatment (radiotherapy or chemotherapy); (3) can persist the follow-up; (4) with written informed consent. Exclusion criteria were patients with (1) history of other surgery within 6 months; (2) incomplete clinical information or hardly follow-up. In addition, total 53 healthy volunteers were also enrolled in the present study as control. Blood samples were collected from all the 108 CRC patients on the day of admission and from the 53 healthy volunteers in the clinic. The fast peripheral venous blood (5 ml) was drawn and placed at room temperature for 1 h. And then the obtained samples were centrifuged (1000 g, 10 min, 4°C) to spin down the blood cells. All the serum samples were than stored at -80°C for further use. The overall survival (OS) time was calculated from the date of surgery to death. All the CRC patients were followed up every 3-6 months until September, 2015 with a median period of 61 months (11-73 months). The detailed clinical and pathologic characteristics of the CRC patientsare were summarized in **Table 1**.

RNA isolation and quantitative real-time PCR

The miRNeasy™ RNA isolation kit (Qiagen, Valencia, CA, USA) was used for the isolation of miRNA from serum samples according to the manufacturer's instructions. The miRNA expression was detected and quantified by using TaqMan miR real-time quantitative reverse-transcription PCR (qRT-PCR) (AppliedBiosystems, Foster City, California, USA). The $2^{-\Delta\Delta Ct}$ method according to the manufacturer's guidelines was used for the analysis of relative miRNA expression, with miR-16 as endogenous controls.

Statistical analysis

Student's t-test was used for the evaluation of differences between groups. The correlations analysis between serum miR-103 expression and clinicopathologic features of CRC patients were performed by using Fisher's exact or Chi-square test. Kaplan-Meier method, log-rank test and multivariate Cox regression analysis were used for the survival analysis. The prediction of cut-off values was analyzed by using receiver operating characteristic (ROC) curve. Bilateral probability and $P<0.05$ was accepted as statistically significant.

Results

Serum expressions of miR-103 in healthy controls and CRC patients

We examined the expressions of miR-103 in the serum sample obtained from the 108 CRC

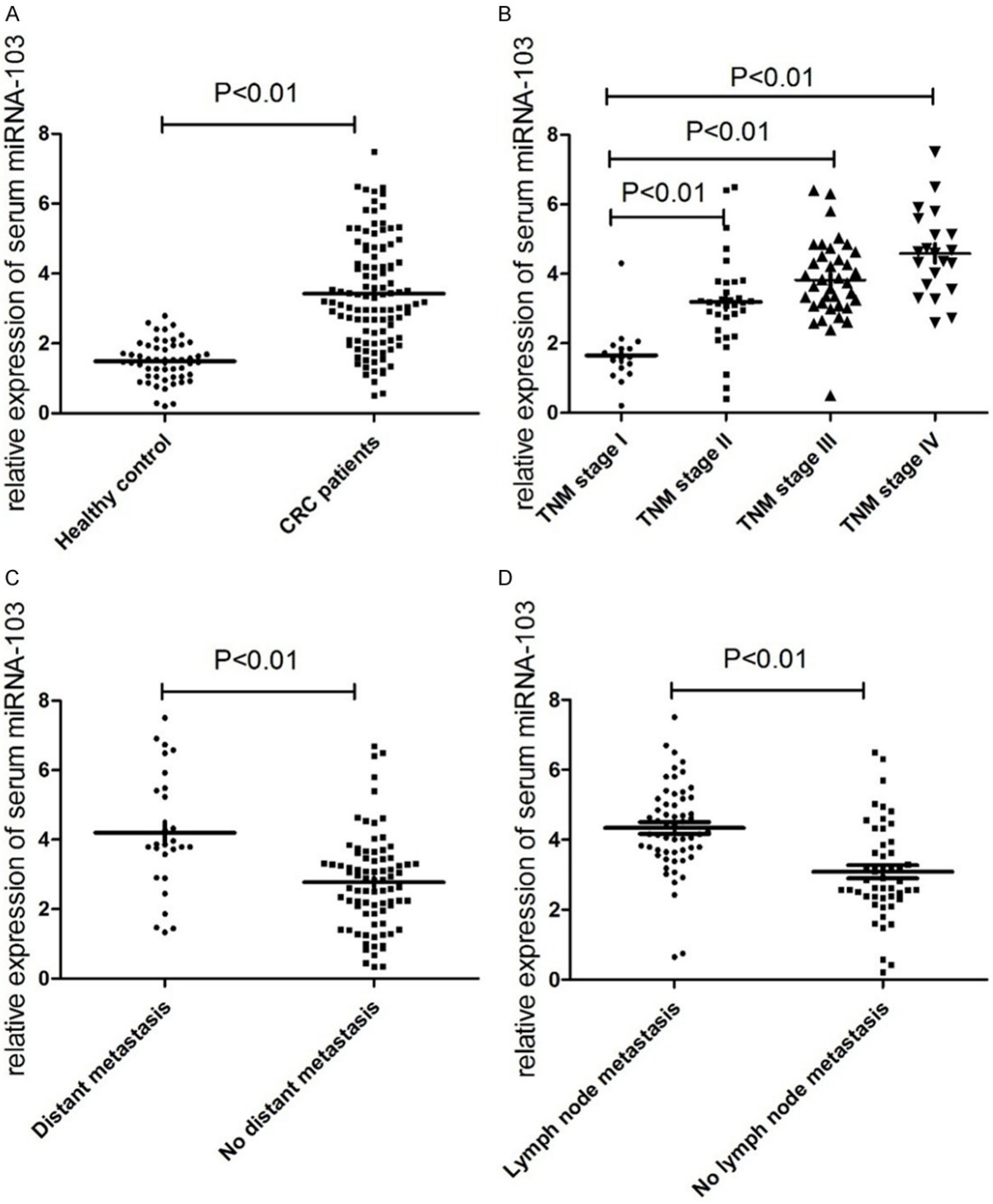

Figure 1. The serum expressions of miR-103 and clinicopathologic features of CRC patients. A. Serum miR-103 expressions in healthy controls and CRC patients. Serum expressions of miR-103 were significantly elevated in CRC patients in comparison with the matched healthy controls (P<0.01). B. Serum miR-103 expressions in different TNM stages. The patients with TNM stage of II, III and IV were all with significantly higher expressions of miR-103 than those patients with TNM stage I (P<0.01). C. Serum miR-103 expressions and distant metastasis. The serum miR-103 expressions in patients with distant metastasis were significantly higher than those without distant metastasis (P<0.01). D. Serum miR-103 expressions and lymph node metastasis. Compared with those who didn't develop lymph node metastasis, the serum expressions of miR-103 in patients with metastasis were increased (P<0.01).

patients and 53 healthy controls by RT-qPCR. As shown in **Figure 1A**, the expressions of miR-103 were significantly elevated in the serum of CRC patients compared with the healthy con-

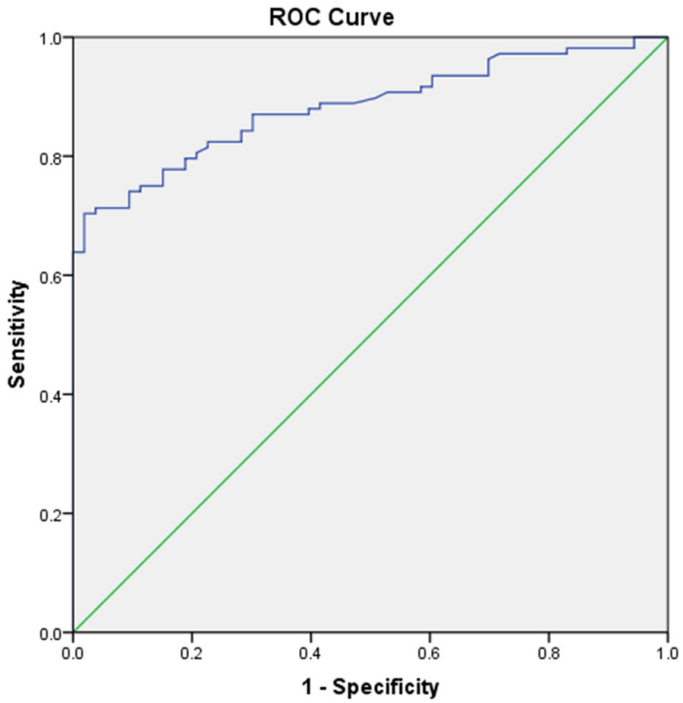

Figure 2. The analysis of diagnostic value of serum miR-103 for CRC by receiver operating characteristics (ROC) curve analysis. The areas under the ROC curve (AUC) was 0.883, and 95% confidence interval was 0.833 to 0.933 (P<0.001).

trols (P<0.01). The patients with TNM stage of II, III and IV were all with significantly higher expressions of miR-103 than those patients with TNM stage I (**Figure 1B**, P<0.01). In addition, we also attempted to investigate the association between serum miR-103 levels and tumor metastasis. The results showed that the serum miR-103 expressions in patients with distant or lymph node metastasis were significantly higher than those without metastasis (P<0.01).

After the basis of observtions of different serum miR-103 levels in CRC patients and healthy controls, we subsequently focused on validating and further exploring the diagnostic value of serum miR-103 for CRC. As supported by the results of ROC curve analysis (**Figure 2**), serum miR-103 may be a useful biomarker for discriminating CRC from healthy controls (P<0.001), with the areas under the ROC curve (AUC) of 0.883, 95% confidence interval (CI) of 0.833 to 0.933. Moreover, the cut-off value of serum miR-103 level was 2.085 with a sensitivity of 77.8% and a specificity of 84.9%.

Serum expressions of miR-103 and clinicopathological characteristics in CRC patients

Patients were divided into two groups according to the detected serum miR-103 expressions and the obtained cut-off value, high expression group (above the cut-off value) and low expression group (less than the cut-off value). Then we investigated the associations between serum miR-103 expressions and clinicopathological characteristics of CRC patients. The results of **Table 2** showed that the serum expressions of miR-103 was statistically correlated with tumor size, tumor differentiation, TNM stage, the presence of distant metastasis or lymph node metastasis (P<0.05).

Serum expressions of miR-103 and overall survival rate

The associations between serum expressions of miR-103 and overall survival rate in CRC patients were presented in **Figure 3**. High expressions of miR-103 was a significant impact factor for overall survival rate of CRC patients by the Kaplan-Meier analysis with log-rank test. To evaluate the independent prognostic parameters for the prognosis of CRC patients, multivariate analysis with Cox regression analysis was utilized. The results suggested that serum miR-103 level was an independent prognosis factor for the overall survival as well as the other clinicopathological characteristics including tumor differentiation, TNM stage, distant metastasis and lymph node metastasis (shown in **Table 3**).

Discussion

Along with the progress in the diagnosis and prognosis of CRC, exploring ideal biomarkers to improve early detection screening and prognostic methods is still needed. miRNAs were easy to measure and proved to have a potential strong association with clinical outcomes [13]. Deregulation of miRNA expression has been widely demonstrated in CRC [14] and many other types of cancers [15]. The aberrant expression of certain miRNA has a close corre-

Table 2. The serum expression of miR-103 and clinicopathological characteristics in patients with colorectal cancer

Parameters	n	Relative expression of serum miR-103		*P*-value
		High	Low	
Age (year)				
≥60	49	20	29	
<60	59	28	31	0.478
Gender				
Male	63	27	36	
Female	45	21	24	0.695
Location of primary tumor				
Colon	67	31	36	
Rectum	41	17	24	0.626
Tumor size (cm)				
≥5	38	29	9	
<5	70	19	51	<0.01
Tumor differentiation				
Poorly	29	19	10	
Moderately	45	22	23	
Well	34	7	27	0.001
TNM stage				
I	17	3	14	
II	33	10	23	
III	37	18	19	
IV	21	17	4	<0.01
Distant metastasis				
Yes	27	19	8	
No	81	29	52	0.002
Lymph node metastasis				
Yes	57	33	24	
No	51	15	36	0.003

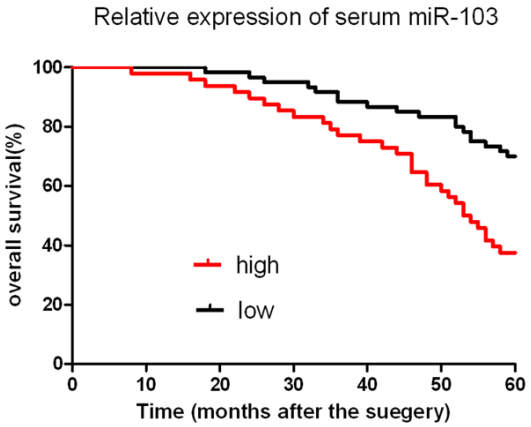

Figure 3. The overall survival and serum expressions of miR-103 by analysis of Kaplan-Meier survival curves. The overall survival rates of patients with high expressions of miR-103 were lower than those with low expressions of miR-103 (P<0.001).

lation with tumorigenesis because of its location at fragile sites or cancer-associated regions of the genome [16]. It has been revealed by previous studies that circulating miRNAs are not only derived from tumor and blood cells, but also from cancer or other tissue cells affected by the disease [17]. The association between tumors and serum miRNA expressions was widely reported [18], which strongly suggested the application of serum miRNAs as potential molecular biomarkers inthe early diagnosis and prognosis of tumors [19].

This study aimed to investigate the potential clinical utility of miR-103 to serve as noninvasive diagnostic, prognostic and metastasis-predictive biomarker in CRC patients. To this end, we performed independent validation experiments by using a cohort of serum sample from 108 CRC patients and 53 healthy controls. The results strongly provided the evidence that serum expressions of miR-103 in patients with CRC were significantly higher than in healthy controls. Furthermore, expressions of miR-103 were much higher in patients with TNM stage II-IV than those with TNM stage I. In addition, serum miR-103 level was a useful noninvasive diagnostic biomarker for the discrimination between CRC cases and healthy control subjects, with a sensitivity of 77.8% and specificity of 84.9% at the cut-off value of 2.085 with Youden Index correction [20] by ROC curve analysis. Moreover, high serum miR-103 levels were also significantly associated with tumor size, tumor differentiation, clinical TNM stage, the presence of distant metastasis or lymph node metastasis. From **Figure 3**, the results of Kaplan-Meier analysis with the log-rank test revealed that high serum expression of miR-103 was significantly correlated with a lower overall survival rate. As shown in **Table 3**, the multivariate analysis indicated that serum miR-103 could serve as an independent prognostic biomarker in patients with CRC. Previous studies have demonstrated that miR-103 is universally up-regulated in colorectal cancer cells and carcinoma tissues. The increased miR-103 expression might contribute to tumor malignant phenotype and tumor development and miR-103 might be a new potential therapeutic target for colorectal carcinoma treatment [12]. Previous studies have also shown that miR-103 is involved in various biological and pathologi-

Table 3. Multivariate analysis of overall survival in 108 patients with colorectal cancer by Cox regression analysis

	Overall survival		
Parameter	HR	95% CI	*P* value
Age	0.824	0.472-1.432	0.528
Gender	1.063	0.585-1.921	0.840
Location of primary tumor	1.145	0.554-2.354	0.711
Tumor size	1.547	0.769-3.012	0.191
Tumor differentiation	2.314	1.242-4.289	<0.001
TNM stage	5.842	1.643-18.892	0.007
Distant metastasis	31.798	14.543-85.653	<0.001
Lymph node metastasis	21.231	7.685-68.334	<0.001
Relative miRNA-103 expression	5.757	3.059-11.816	<0.001

cal processes, including the modulation of glucose homeostasis and insulin sensitivity [21]. miR-103 was also significantly down-regulated in heart failure (HF) and it might be a useful biomarker for the diagnosis of HF by a recent report [22]. miR-103 was able to promote the growth and invasion of endometrial cancer cell lines by down-regulating the expression of the tumor suppressor gene tissue inhibitor of metalloproteinase 3 (TIMP-3) [23]. Recent studies have revealed that miR-103 was over-expressed in colorectal cancer as an oncogenic miRNA by targeting *DAPK*, *KLF4* and *PER3* [20], however, the exact role of miR-103 in the growth and metastasis of CRC still remains unknown [24].

In conclusion, this present study demonstrated thatthe serum expression of miR-103 might be used as a robust diagnostic, prognostic and metastasis predictive biomarker in patients with CRC.

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Disclosure of conflict of interest

None.

Authors' contribution

LG Mao, WY Feng and XM Xu participated in the conception and design, data collection, statistical analysis and wrote the manuscript. YM Yu and XM Xu participated in the conception and design and data collection.

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References

[1] Parkin DM, Bray F, Ferlay J and Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005; 55: 74-108.

[2] Weitz J, Koch M, Debus J, Hohler T, Galle PR and Buchler MW. Colorectal cancer. Lancet 2005; 365: 153-165.

[3] Liefers GJ and Tollenaar RA. Cancer genetics and their application to individualised medicine. Eur J Cancer 2002; 38: 872-879.

[4] Walsh JM and Terdiman JP. Colorectal cancer screening: scientific review. JAMA 2003; 289: 1288-1296.

[5] Narayanan V, Peppelenbosch MP and Konstantinov SR. Human fecal microbiome-based biomarkers for colorectal cancer. Cancer Prev Res (Phila) 2014; 7: 1108-1111.

[6] Krol J, Loedige I and Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet 2010; 11: 597-610.

[7] Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR and Golub TR. MicroRNA expression profiles classify human cancers. Nature 2005; 435: 834-838.

[8] Ambros V. The functions of animal microRNAs. Nature 2004; 431: 350-355.

[9] Winter J, Jung S, Keller S, Gregory RI and Diederichs S. Many roads to maturity: microRNA biogenesis pathways and their regulation. Nat Cell Biol 2009; 11: 228-234.

[10] Alvarez-Garcia I and Miska EA. MicroRNA functions in animal development and human disease. Development 2005; 132: 4653-4662.

[11] Ma Y, Zhang P, Yang J, Liu Z, Yang Z and Qin H. Candidate microRNA biomarkers in human colorectal cancer: systematic review profiling studies and experimental validation. Int J Cancer 2012; 130: 2077-2087.

[12] Geng L, Sun B, Gao B, Wang Z, Quan C, Wei F and Fang XD. MicroRNA-103 promotes colorectal cancer by targeting tumor suppressor DICER and PTEN. Int J Mol Sci 2014; 15: 8458-8472.

[13] Ng EK, Chong WW, Jin H, Lam EK, Shin VY, Yu J, Poon TC, Ng SS and Sung JJ. Differential expression of microRNAs in plasma of patients

with colorectal cancer: a potential marker for colorectal cancer screening. Gut 2009; 58: 1375-1381.
[14] Akao Y, Nakagawa Y and Naoe T. MicroRNA-143 and -145 in colon cancer. DNA Cell Biol 2007; 26: 311-320.
[15] Porkka KP, Pfeiffer MJ, Waltering KK, Vessella RL, Tammela TL and Visakorpi T. MicroRNA expression profiling in prostate cancer. Cancer Res 2007; 67: 6130-6135.
[16] Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M and Croce CM. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci U S A 2004; 101: 2999-3004.
[17] Mo MH, Chen L, Fu Y, Wang W and Fu SW. Cell-free Circulating miRNA Biomarkers in Cancer. J Cancer 2012; 3: 432-448.
[18] Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo X, Li Q, Li X, Wang W, Zhang Y, Wang J, Jiang X, Xiang Y, Xu C, Zheng P, Zhang J, Li R, Zhang H, Shang X, Gong T, Ning G, Wang J, Zen K, Zhang J and Zhang CY. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res 2008; 18: 997-1006.
[19] Taylor DD and Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecol Oncol 2008; 110: 13-21.
[20] Hong Z, Feng Z, Sai Z and Tao S. PER3, a novel target of miR-103, plays a suppressive role in colorectal cancer in vitro. BMB Rep 2014; 47: 500-505.
[21] Trajkovski M, Hausser J, Soutschek J, Bhat B, Akin A, Zavolan M, Heim MH and Stoffel M. MicroRNAs 103 and 107 regulate insulin sensitivity. Nature 2011; 474: 649-653.
[22] Ellis KL, Cameron VA, Troughton RW, Frampton CM, Ellmers LJ and Richards AM. Circulating microRNAs as candidate markers to distinguish heart failure in breathless patients. Eur J Heart Fail 2013; 15: 1138-1147.
[23] Yu D, Zhou H, Xun Q, Xu X, Ling J and Hu Y. microRNA-103 regulates the growth and invasion of endometrial cancer cells through the down-regulation of tissue inhibitor of metalloproteinase 3. Oncol Lett 2012; 3: 1221-1226.
[24] Chen HY, Lin YM, Chung HC, Lang YD, Lin CJ, Huang J, Wang WC, Lin FM, Chen Z, Huang HD, Shyy JY, Liang JT and Chen RH. miR-103/107 promote metastasis of colorectal cancer by targeting the metastasis suppressors DAPK and KLF4. Cancer Res 2012; 72: 3631-3641.