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Expression levels of miR-205 and miR-506 in colon cancer tissues and their relationships with clinicopathological features

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Abstract. The study intended to investigate the expression levels of micro ribonucleic acid (miR)-205 and miR-506 in colon cancer tissues and their relationships with clinicopathological features. The expression levels of miR-205 and miR-506 in colon cancer tissues and para-carcinoma normal colonic mucosa tissues were detected via fluorescence reverse transcription quantitative polymerase chain reaction (RT-qPCR), and the expression levels of the two miRNAs in plasma of colon cancer patients and healthy control population were also detected. Moreover, the relationships of the two miRNAs with clinicopathological features of patients with colon cancer were analyzed. The expression levels of the two miRNAs in colon cancer tissues were higher than those in para-carcinoma normal colonic mucosa tissues, and also significantly higher in plasma of the colon cancer patients than those in the healthy control population. The differences were statistically significant ($P<0.05$). The expression level of miR-205 was associated with tumor-node-metastasis (TNM) staging and lymph node metastasis, while the expression level of miR-506 was associated with lymph node metastasis. The differences were statistically significant ($P<0.05$). The expression levels of miR-205 in the colon cancer tissues and plasma in patients had no significant correlation ($r=0.467$, $P=0.081$). There was a positive correlation between the expression levels of miR-506 in the colon cancer tissues and plasma in patients ($r=0.599$, $P=0.038$). The expression levels of miR-205 and miR-506 are upregulated in the colon cancer patients, both of which may be closely related to the occurrence and development of colon cancer, and may become potential tumor markers as well as relevant therapeutic targets.

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Introduction

At present, colon cancer is the most common malignant tumor of the digestive tract around the world. According to a literature report (1,2), statistical studies released by the American Cancer Society in 2015 pointed out that the incidence and mortality rates of colon cancer show increasing trends year by year in male and female tumor patients in the United States, both of which rank third. Nowadays, the incidence rate of colon cancer in China is also increased year by year due to the changes in lifestyle and influence of aging of the population, seriously threatening human health and safety. Despite the progress in diagnosis and treatment levels in recent years, the 5-year survival rate does not exceed 50% (3,4). The occurrence and development of colon cancer is a multi-stage, multi-step and multi-factor process. Therefore, further investigating the pathogenesis of colon cancer and finding out the molecular biomarker for early diagnosis of colon cancer have very important significance in clinical research.

Micro ribonucleic acid (miRNA or miR) is a kind of recently-discovered non-coding RNA with approximately 21-25 nucleotides in length. After transcription, miRNA exerts its function of regulating the gene expression through enhancing the degradation and inhibiting the translation of mRNA (5). In recent years, it has been found in research on miRNA that it plays an important role in occurrence, development, proliferation and invasion of tumor as well as regulation of drug resistance of tumor cells (6-8). In this experiment, the expression levels of miR-205 and miR-506 in tissues and plasma of patients with colon cancer were detected, and the relationships of the expression levels of miR-205 and miR-506 in colon cancer patients with clinicopathological features were also detected.

Materials and methods

Specimen collection. In this experiment, samples from 166 patients with colon cancer treated in Weifang People's Hospital (Weifang, China) from May 2008 to July 2012 were collected. Colon cancer tissues and normal fresh tissues at 20 mm near the cancer resected during operation were taken and immediately stored in liquid nitrogen, and then they were

transferred into a refrigerator at -80˚C for standby application. The plasma of patients before operation and the plasma of 100 healthy subjects receiving physical examination in our hospital were taken as the control group. There were no statistically significant differences in age and sex between them. Colon cancer tissues were diagnosed and staged according to the seventh edition of tumor-node-metastasis (TNM) staging criteria of the American Joint Committee on Cancer (AJCC). None of the patients with colon cancer received chemotherapy, radiotherapy and special treatment for cancer before operation. This study was approved by the Ethics Committee of Weifang People's Hospital (Weifang, China), and all patients and their families were informed and signed the consent.

Plasma treatment. After 5 ml plasma specimen of patients and 5 ml plasma specimen of the control group were placed into the anticoagulant tube containing ethylene diamine tetraacetic acid (EDTA; Beyotime Institute of Biotechnology, Shanghai, China), they were let stand at room temperature for 30 min and centrifuged at 2,300 x g for 10 min at 4˚C. The supernatant was transferred into an RNase-free tube (Sangon Biotech, Shanghai, China) by using a pipettor and centrifuged at 9,600 x g for 15 min at 4˚C. Then the supernatant was taken and placed into the RNase-free tube, after which the supernatant was stored in a refrigerator at -80˚C. Clinical data of patients are shown in Table I.

Reagents and equipment. TRIzol reagent used for RNA extraction was purchased from Shanghai Pufei Biotechnology Co., Ltd. (Shanghai, China); mirVana™ PARIS™ kit and TaqMan MicroRNA assay were purchased from ABI (Foster City, CA, USA); NanoDrop 2000 ultraviolet spectrophotometer was purchased from Thermo Fisher Scientific, Inc. (Waltham, MA, USA); Countess II FL Automated Cell Counter fluorescence reverse transcription quantitative polymerase chain reaction (RT-qPCR) instrument was purchased from ABI.

Methods

Total RNA extraction. Total RNA was extracted from colon cancer tissues and para-carcinoma tissues by using TRIzol reagent (one-step extraction method), and RNA was extracted from the centrifuged plasma by using the mirVana™ PARIS™ kit in strict accordance with the manufacturer's instructions. The concentration and purity of extracted RNA were detected by using NanoDrop 2000 ultraviolet spectrophotometer. The integrity of the extracted RNA was detected via agarose gel electrophoresis.

Reverse transcription reaction. The reverse transcription primers of miR-205 and miR-506 extracted were designed, and special circular structures were made for the experiments. The primer sequences were designed and synthesized by Takara Biotech (Beijing) Co., Ltd. (Beijing, China). In this experiment, U6 was used as an internal reference gene. A total of 15 μl reverse transcription system included 0.5 μl reverse transcription primer, 0.5 μl reverse transcriptase, 2.0 μl buffer solution and 2 μl RNA; diethylpyrocarbonate (DEPC)-treated water was added if the volume was insufficient. Reaction conditions: 37˚C for 10 min, and 95˚C for 5 min; after synthesis, complementary DNA (cDNA) was stored at 4˚C.

Detection of miR-205 and miR-506 via RT-qPCR. A total of 20 μl RT-qPCR system was prepared by using the TaqMan probe method according to the manufacturer's instructions: 1 μl TaqMan MicroRNA assay (20*); 1.33 μl cDNA (diluted at 1:15); 10 μl TaqMan 2X Universal PCR Master Mix II (Beyotime Institute of Biotechnology); finally, DEPC-treated water was added to make up for the volume to 20 μl. PCR amplification was performed by using the Countess II FL Automated Cell Counter RT-qPCR instrument. Reaction conditions: 95˚C for 5 min, 95˚C for 20 sec, and 60˚C for 45 sec; a total of 45 cycles. The cycle threshold (Cq) values were calculated by the $2^{-\Delta\Delta Cq}$ method (9) (Table II).

Statistical methods. In this study, Statistical Product and Service Solutions (SPSS) 17.0 software package (SPSS, Inc., Chicago, IL, USA) was used for data processing, and GraphPad Prism 5 software (GraphPad Software, Inc., La Jolla, CA, USA) was used for image processing. Enumeration data are presented as percentage (%), and the Chi-square test was used for comparison between the two groups. Measurement data are presented as mean ± standard deviation (mean ± SD), and the least significant difference (LSD) method was used for intragroup comparisons. Spearman analysis was used for correlation analysis. $P<0.05$ suggested that the difference was statistically significant.

Results

Expression levels of miR-205 and miR-506 in colon cancer tissues and para-carcinoma tissues. The results of RT-qPCR of miR-205 and miR-506 showed that the expression levels of them in colon cancer tissues were significantly increased compared with those in para-carcinoma tissues, and the differences were statistically significant ($P<0.05$) (Fig. 1).

Differential expression levels of miR-205 and miR-506 in plasma of colon cancer patients and healthy control group. The expression levels of miR-205 and miR-506 in plasma of colon cancer patients were significantly increased compared with those in the control group, and the differences were statistically significant ($P<0.05$) (Fig. 2).

Associations of miR-205 and miR-506 expression levels with clinicopathological features of colon cancer patients. Association analyses of clinicopathological features with miR-205 and miR-506 showed that associations of them with sex and age had no statistically significant differences ($P>0.05$). The expression level of miR-205 was associated with TNM staging and lymph node metastasis ($P<0.05$), while the expression level of miR-506 was associated with lymph node metastasis ($P<0.05$) (Table I).

Correlations of the expression levels of the two miRNAs in plasma and colon cancer tissues. The experimental results showed that the expression levels of miR-205 in colon cancer tissues and plasma in patients had no significant correlation ($r=0.467$, $P=0.081$). There was a positive correlation between the expression levels of miR-506 in colon cancer tissues and plasma in patients, and the difference was statistically significant ($r=0.599$, $P=0.038$) (Table III).

Table I. Associations of miR-205 and miR-506 expression levels with clinicopathological data of colon cancer patients.

Clinical feature	n	miR-205 expression level	χ^2	P-value	miR-506 expression level	χ^2	P-value
Age (years)							
≥55	84	1.894±0.447	0.219	0.812	3.417±1.267	1.307	0.264
<55	82	1.836±0.514			3.157±1.474		
Sex							
Male	97	1.819±0.681	1.684	0.627	3.387±1.678	0.354	0.437
Female	69	1.864±0.544			3.574±1.461		
Smoking (years)							
≥10	88	2.074±0.374	2.146	0.061	3.671±1.257	1.814	0.296
<10	78	1.964±0.416			3.174±1.651		
Lymph node metastasis							
Yes	100	2.341±0.241	4.227	0.037	3.754±1.058	4.519	0.031
No	66	1.841±0.539			3.271±1.487		
Degrees of differentiation							
Low differentiation	25	1.851±0.684	0.624	0.731	3.341±1.424	0.874	0.481
Moderate differentiation	79	1.944±0.473			3.697±1.198		
High differentiation	62	2.181±0.314			3.864±0.874		
TNM staging							
I	13	1.830±0.846	4.847	0.029	3.074±1.384	2.234	0.056
II	87	1.924±0.681			3.438±1.108		
III+IV	66	2.324±0.437			3.733±0.798		

Table II. Primer sequences.

Gene	Forward primers	Reverse primers
miRNA-205	5'-AATCCTTCATTCCACCGG-3'	5'-GTGCAGGGTCCGAGGT-3'
miRNA-506	5'-TGCGGTAAGGCACCCTTCTGAGTAC-3'	5'-CCAGTGCAGGGTCCGAGGT-3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-AACGCTTCACGAATTTGCGT-3'

Table III. Correlations of the expression levels of the two miRNAs in plasma and tissues of colon cancer patients.

Group	miRNA-205 expression level	r	P-value	miR-506 expression level	r	P-value
Plasma of patients	2.579±1.655	0.467	0.081	4.541±1.419	0.599	0.038
Tissues of patients	1.806±0.424			3.147±0.441		

Discussion

Colon cancer is a kind of malignant tumor of the digestive tract with relatively high incidence and mortality rates, seriously endangering human health and safety. It is estimated that in 2012, there were approximately 1.4 million new cases of colon cancer and 690,000 deaths in the world (10). miRNA is a kind of endogenous non-coding microRNA with a length of 17-25 nucleotides. Incomplete matching in non-coding region of 3'-untranslated region (UTR) of its target genes blocks the mRNA translation of target genes, which is closely related to cell apoptosis, proliferation, metastasis, differentiation and other vital activities, as well as the occurrence, development and progression of tumors. More and more evidence has shown that the occurrence of colon cancer is a multi-target and multi-step process (11-13). Some miRNAs play an important role in the pathological process of colon cancer. Different miRNAs, such as miR-141 (14),

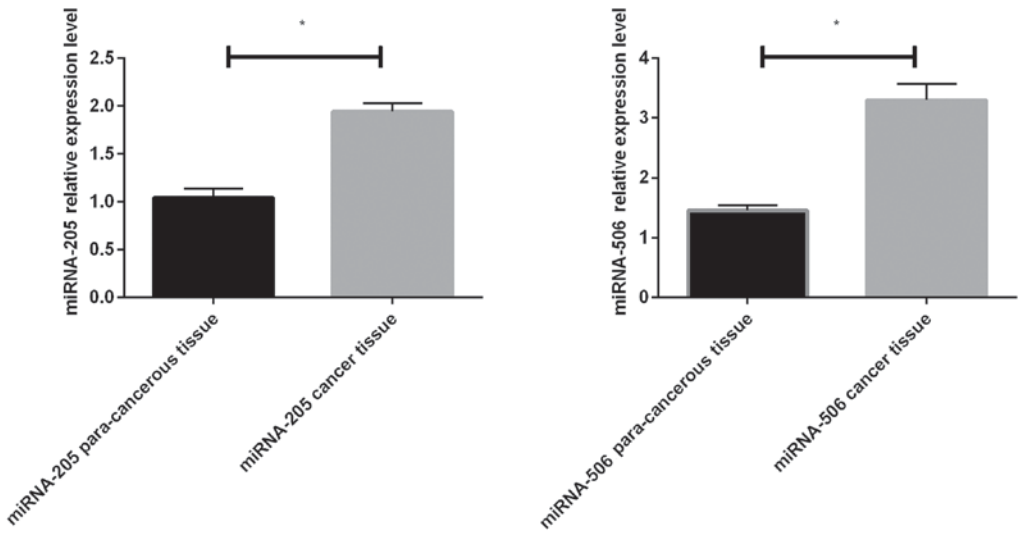

Figure 1. Expression levels of miR-205 and miR-506 in colon cancer tissues and para-carcinoma tissues. In the figure, the comparisons of the expression levels of miR-205 and miR-506 in colon cancer tissues and para-carcinoma tissues are shown: 1.105±0.094 vs. 1.806±0.424; 1.484±0.273 vs. 3.147±0.441; in statistical analyses, *P<0.05.

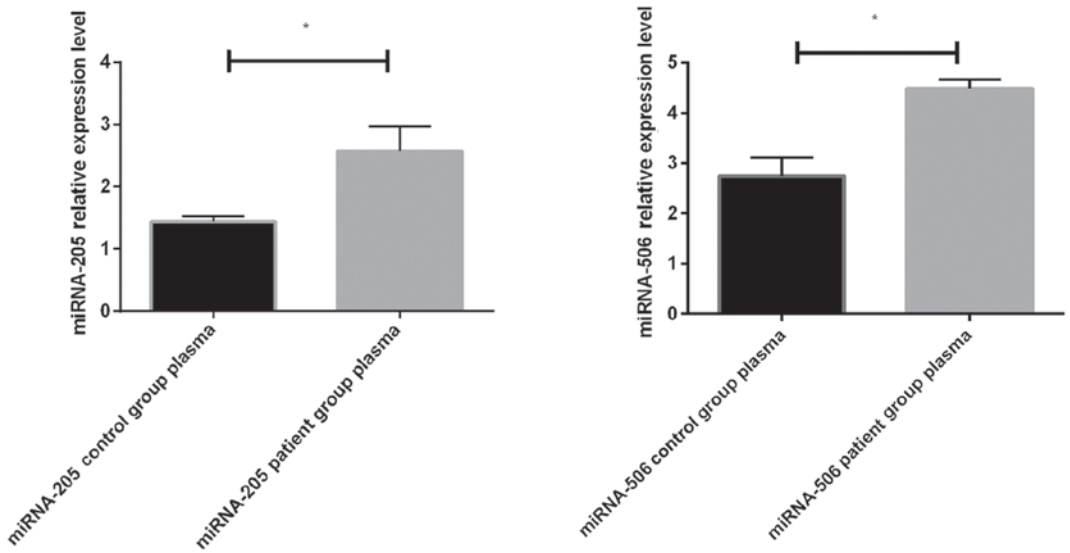

Figure 2. Differential expression levels of miR-205 and miR-506 in plasma of colon cancer patients and healthy control group. Comparisons of miR-205 and miR-506 in plasma in patient group and control group are shown: 1.341±0.127 vs. 2.579±1.655; 1.574±0.304 vs. 4.541±1.419; in statistical analyses, *P<0.05.

miR-145 (15), miR-231 (16) and miR-215 (17), have differential expression levels in tissues.

In this experiment, the RT-qPCR technique was used to detect the expression levels of miR-205 and miR-506 in colon cancer tissues and para-carcinoma tissues. The differential expression levels of them in the plasma of colon cancer patients and healthy subjects were investigated, and the correlations of their expression levels in tissues and plasma of colon cancer patients were analyzed.

This study showed that the expression levels of miR-205 and miR-506 in colon cancer tissues were higher than those of para-carcinoma tissues (P<0.05), indicating that the two miRNAs are closely related to the occurrence of colon cancer and may participate in the pathological process of colon cancer as proto-oncogenes. The associations of miR-205 and miR-506 with clinicopathological data were further analyzed, and the results showed that the expression level of miR-205 was related to TNM staging and lymph node metastasis, and the expression level of miR-506 was related to lymph node metastasis. The expression of miR-205 was positively associated with TNM staging of patients with colon cancer, and the higher the staging was, the higher the expression level of miR-205 would be. de Carvalho *et al* (18) pointed out in his study that miR-205 has comparatively good sensitivity, specificity and accuracy in differentiating the metastasis of patients with head and neck squamous carcinoma, indicating that miR-205 may participate in the metastasis of cancer cells to a large extent, so it may serve as a potential biological target for whether tumor metastasis occurs. This study showed that miR-205 was highly expressed in colon cancer patients with lymph node metastasis, which promoted cell metastasis in colon cancer patients, indicating that miRNA is expressed

identically in different tissues. Besides, miR-506 is located in chromosome Xq27.3. Studies have shown that miR-506 can be used as a target for a variety of target genes, silence the target gene expression levels and influence the tumor proliferation, invasion, metastasis and other processes (19). In the study of Zhang *et al* (20), the expression level of miR-506 is downregulated in colon cancer tissues, and it is negatively associated with tumor size, lymph node metastasis and TNM staging. However, the results in this experiment were opposite to the above conclusions, which may be caused by the differences in regions and races.

At present, colonoscope is mainly used in the primary screening of colon cancer, which is the most effective screening tool. However, it has certain dangers to the patients, and its cost is high, so it is difficult to be widely used in clinical practice. In particular, the sensitivity of occult blood test in detection is low, and patients are strictly required to control the diet and life to a certain degree, so a kind of more effective and convenient biomarker is urgently needed for the detection of colon cancer. miRNA can be detected in serum, plasma and body fluids (21), suggesting that plasma miRNA can be used as a biomarker for detection. Many studies indicated that (22,23) specific miRNA exists in the patient's tumor tissues and plasma. This experiment showed that the expression levels of miR-205 and miR-506 in plasma were higher than those in the normal control group, so miR-205 and miR-506 may be used as potential molecular markers for screening colon cancer. However, whether the two miRNAs can become tumor markers requires the big-data test to verify the results.

With the improvement of living standards and changes in the dietary structure, colon cancer, as a malignant tumor of the digestive tract, has become one of the main factors threatening human health. Therefore, improving the survival rate and the quality of life of patients is a problem to be solved urgently.

In conclusion, the differential expression levels of miR-205 and miR-506 in colon cancer and para-carcinoma tissues of patients in this study suggest that they play an important role in the occurrence and development of colon cancer, and it was found that the expression levels of miR-205 and miR-506 in tissues of colon cancer patients were associated with the clinicopathological indexes. Moreover, changes in the miRNA expression level in plasma can be used as potential tumor markers. As therapeutic targets, miR-205 and miR-506 may act as new treatment means for colon cancer.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

NJ was responsible for the extraction of total RNA. LY and JS were responsible for the reverse transcription reaction. ZC and WM performed PCR. NJ edited and WM revised the manuscript. NJ was responsible for the data collection; LY was responsible for the data analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Weifang People's Hospital (Weifang, China), and all patients and their families were informed and signed the consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2015. CA Cancer J Clin 5: 7-30, 2015.
2. Huang Z, Huang D, Ni S, Peng Z, Sheng W and Du X: Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer. Int J Cancer 127: 118-126, 2010.
3. Terzić J, Grivennikov S, Karin E and Karin M: Inflammation and colon cancer. Gastroenterology 138: 2101-2114.e5, 2010.
4. Buunen M, Veldkamp R, Hop WC, Kuhry E, Jeekel J, Haglind E, Påhlman L, Cuesta MA, Msika S, Morino M, *et al*; Colon Cancer Laparoscopic or Open Resection Study Group: Survival after laparoscopic surgery versus open surgery for colon cancer: Long-term outcome of a randomised clinical trial. Lancet Oncol 10: 44-52, 2009.
5. Schetter AJ, Leung SY, Sohn JJ, Zanetti KA, Bowman ED, Yanaihara N, Yuen ST, Chan TL, Kwong DL, Au GK, *et al*: MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. JAMA 299: 425-436, 2008.
6. Shen Z, Zhan G, Ye D, Ren Y, Cheng L, Wu Z and Guo J: MicroRNA-34a affects the occurrence of laryngeal squamous cell carcinoma by targeting the antiapoptotic gene survivin. Med Oncol 29: 2473-2480, 2012.
7. Xiao CZ, Wei W, Guo ZX, Zhang MY, Zhang YF, Wang JH, Shi M, Wang HY and Guo RP: MicroRNA-34c-3p promotes cell proliferation and invasion in hepatocellular carcinoma by regulation of NCKAP1 expression. J Cancer Res Clin Oncol 143: 263-273, 2017.
8. Fattore L, Sacconi A, Mancini R and Ciliberto G: MicroRNA-driven deregulation of cytokine expression helps development of drug resistance in metastatic melanoma. Cytokine Growth Factor Rev 36: 39-48, 2017.
9. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
10. Douaiher J, Ravipati A, Grams B, Chowdhury S, Alatise O and Are C: Colorectal cancer-global burden, trends, and geographical variations. J Surg Oncol 115: 619-630, 2017.
11. Munteanu CR, Magalhães AL, Uriarte E and González-Díaz H: Multi-target QPDR classification model for human breast and colon cancer-related proteins using star graph topological indices. J Theor Biol 257: 303-311, 2009.
12. Ha M and Kim VN: Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 15: 509-524, 2014.
13. Inui M, Martello G and Piccolo S: MicroRNA control of signal transduction. Nat Rev Mol Cell Biol 11: 252-263, 2010.
14. Cheng H, Zhang L, Cogdell DE, Zheng H, Schetter AJ, Nykter M, Harris CC, Chen K, Hamilton SR and Zhang W: Circulating plasma MiR-141 is a novel biomarker for metastatic colon cancer and predicts poor prognosis. PLoS One 6: e17745, 2011.

15. Yu Y, Nangia-Makker P, Farhana L, Rajendra SG, Levi E and Majumdar AP: miR-21 and miR-145 cooperation in regulation of colon cancer stem cells. Mol Cancer 14: 98, 2015.
16. Cekaite L, Rantala JK, Bruun J, Guriby M, Agesen TH, Danielsen SA, Lind GE, Nesbakken A, Kallioniemi O, Lothe RA, *et al*: MiR-9, -31, and -182 deregulation promote proliferation and tumor cell survival in colon cancer. Neoplasia 14: 868-879, 2012.
17. Karaayvaz M, Pal T, Song B, Zhang C, Georgakopoulos P, Mehmood S, Burke S, Shroyer K and Ju J: Prognostic significance of miR-215 in colon cancer. Clin Colorectal Cancer 10: 340-347, 2011.
18. de Carvalho AC, Scapulatempo-Neto C, Maia DC, Evangelista AF, Morini MA, Carvalho AL and Vettore AL: Accuracy of microRNAs as markers for the detection of neck lymph node metastases in patients with head and neck squamous cell carcinoma. BMC Med 13: 108, 2015.
19. Ruiz AJ and Russell SJ: MicroRNAs and oncolytic viruses. Curr Opin Virol 13: 40-48, 2015.
20. Zhang Y, Lin C, Liao G, Liu S, Ding J, Tang F, Wang Z, Liang X, Li B, Wei Y, *et al*: MicroRNA-506 suppresses tumor proliferation and metastasis in colon cancer by directly targeting the oncogene EZH2. Oncotarget 6: 32586-32601, 2015.
21. Tölle A, Jung M, Rabenhorst S, Kilic E, Jung K and Weikert S: Identification of microRNAs in blood and urine as tumour markers for the detection of urinary bladder cancer. Oncol Rep 30: 1949-1956, 2013.
22. Yaman Agaoglu F, Kovancilar M, Dizdar Y, Darendeliler E, Holdenrieder S, Dalay N and Gezer U: Investigation of miR-21, miR-141, and miR-221 in blood circulation of patients with prostate cancer. Tumour Biol 32: 583-588, 2011.
23. Schwarzenbach H, Milde-Langosch K, Steinbach B, Müller V and Pantel K: Diagnostic potential of PTEN-targeting miR-214 in the blood of breast cancer patients. Breast Cancer Res Treat 134: 933-941, 2012.