ORIGINAL ARTICLE

Correlation between miR-21 and miR-145 and the incidence and prognosis of colorectal cancer

Qing Liu, Weili Yang, Yongsheng Luo, Shuangcheng Hu, Linchang Zhu

Department of Gastroenterology, the Second People's Hospital of Futian District, Shenzhen 518049, P.R. China

Summary

Purpose: *The purpose of this study was to investigate the expression of microRNA (miR)-21 and miR-145 in serum and tumor tissues of patients with colorectal cancer (CRC), and to explore the correlation between the expression of these miRs and the clinicopathological parameters and prognosis of CRC patients.*

Methods: *Serum specimens, frozen tumor tissue and adjacent normal tissue of 50 CRC patients who were hospitalized in our hospital from February 2009 to February 2011 were collected, along with serum specimens of 30 healthy people (control). The expression levels of miR-21 and miR-145 in serum, tumor tissues and adjacent normal tissues were detected by quantitative real time polymerase chain reaction (qRT-PCR). The correlation between the expression of the two miRs in serum was analyzed by Spearman method. The relationship between the expression of miR-21 and miR-145 in serum and the pathological parameters and prognosis of patients with CRC were analyzed using clinical data.*

Results: *The expression level of miR-21 in CRC tissue was significantly higher than that in adjacent normal tissues, while the expression of miR-145 in CRC tissue was signifi-*

cantly lower than that in adjacent normal tissues. The expression level of miR-21 in the serum of CRC patients was significantly higher compared with healthy people, while the expression of miR-145 in the serum of CRC patients was significantly lower than that in healthy people. The expression of miR-21 and miR-145 in the serum was positively correlated with their expression in tumor tissue. High expression level of miR-21 in the serum was correlated with tumor size, grade of differentiation, invasion, metastasis and clinical stage, and low expression level of miR-145 in the serum was correlated with tumor size, grade of differentiation, invasion, metastasis and clinical stage. The 5-year overall survival (OS) was 52% (26/50). Single factor survival analysis showed that miR-21 and miR-145 were the influencing factors of OS of patients with CRC.

Conclusions: *High expression of miR-21 and low expression of miR-145 are closely related to the development and progression of CRC, especially with the grade of differentiation, invasion, metastasis and clinical stage. MiR-21 and miR-145 in the serum can be used as markers for early screening of CRC and indicators for prognosis prediction.*

Key words: *colorectal cancer, miR-21, miR-145, prognosis*

Introduction

As a common malignant tumor of the digestive tract, the incidence and mortality of CRC are relatively high. Clinical data showed that CRC affected about 1 million new patients and caused 500,000 deaths in 2016 worldwide [1]. In recent years, with economic development and improvement of people's living standards in China, the incidence and mortality of CRC was steadily in-creased, seriously affecting the health of people [2]. Early diagnosis of CRC is difficult, and metastasis and recurrence rates are high. Current studies mainly focus on finding early diagnostic markers and new targets for treatment [3].

MicroRNAs (miRs) are endogenous single-stranded small molecule RNAs having no a coding function but can regulate the expression of target

genes at post-transcriptional level by specifically binding to the 3'UTR region of the target mRNA [4]. It has been reported that miRs play important roles in the regulation of various biological functions and diseases, and the abnormal expression of some miRs are closely related to the proliferation, differentiation and apoptosis of tumor cells [5,6]. As a member of miR family, miR-21 is highly expressed in a variety of tumors and plays a key role in the proliferation, invasion and apoptosis of tumor cells. So miR-21 can be used as a prognostic indicator for multiple tumors [7-9].MiR-145 is located on chromosome 5, and it has been shown that its expression was inhibited in a variety of tumors and was thought to be a tumor suppressor gene that regulated the proliferation and invasion of tumor cells by inhibiting the expression of target genes [10-12].

However, the correlation between the expression of miR-21 and miR-145 in the serum and tumor tissues of patients with CRC and the relationship with pathological parameters and prognosis of CRC haven't been reported yet. Therefore, in this study, quantitative real time polymerase chain reaction (qRT-PCR) was used to detect the expression of miR-21 and miR-145 in serum and tumor tissues of CRC patients, while the correlation between the expression of miR-21 and miR-145 was also explored. Combined with clinical data analysis, the effects of miR-21 and miR-145 on the pathological parameters and prognosis of patients with CRC were studied.

Methods

Patients

A total of 50 patients with CRC treated-followed up in our hospital from February 2009 to February 2011 were included in this study. There were 29 males and 21 females with an average age of 52.3±8.7 years. All patients were subjected to surgical treatment and chemotherapy for the first time. A total of 30 healthy people including 17 males and 13 females were selected as control group, and their average age was 54.6±9.5 years. No significant differences in gender and age were found between CRC patients and the normal control group. Fasting peripheral venous blood (5 ml) was taken from patients and healthy people in the morning just before surgery. Blood samples were kept at room temperature for 30 min, followed by centrifugation at 3000 r/min for 10 min to collect the supernatant, which was kept at -80°C. Tumor tissue and adjacent normal tissue collected from CRC patients during surgery were kept in liquid nitrogen.

This study was approved by the ethics committee of our institute and all patients or their family members signed informed consent. Trizol kit, reverse tran-

scription kit and qRT-PCR kit were purchased from Invitrogen (Carlsbad, CA, USA), and primer synthesis was purchased from TaKaRa (Dalian, China).

QRT-PCR to detect the expression of miR-21 and miR-145 in serum and tissue samples of patients

Trizol reagent was used to extract total RNA from serum (100 μl), tumor tissue (100 mg) and adjacent normal tissue (100 mg) according to the manufacturer's instructions. Absorbance value of each RNA sample as measured and only RNA samples with a ratio of A260/A280 between 1.8 and 2.0 were used for consequent studies. Reverse transcription was performed to synthesize cDNA to serve as template for PCR reaction. PCR was performed according to the instructions of qRT-PCR kit. All primers used in PCR are listed in Table 1. U6 RNA was used as endogenous control. PCR reaction conditions were: 94°C for 10 min, followed by 45 cycles at 95°C for 15 s and 60°C for 30 s. The relative expression of the target gene was calculated using the following formula: ΔCt (target gene) = Ct (target gene) -Ct (control gene).

Table 1. QRT-PCR PCR primer sequences

Genes	Primer sequences
miR-21	Forward: 5'-ACACTCCAGCTGGGTAGCTTATCAGACTG-3'
	Reverse: 5'-TGGTGTCGTGGAGTCG-3'
miR-145	Forward: 5'-CAGCATACATGATTCCTTGTA-3'
	Reverse: 5'-CTTTGGTGTTTGAGATGTTTGG-3'
U6	Forward: 5'-GCTTCGGCAGCACATATACTAAAAT-3'
	Reverse: 5'-CGCTTCACGAATTTGCGTGTCAT-3'

Analysis of the correlation between the expression of miR-21 and miR-145 in serum and the pathological parameters and prognosis of CRC patients

With the average expression level in serum of healthy controls as baseline (1), the average expression level of miR-21 was 2.34, and the average expression level of miR-145 was 1.72. Patients with significantly higher expression level than healthy controls were classified as high expression group, while patients with significantly lower expression level than healthy controls were classified as low expression group. Chi square test was performed to analyze the correlation between the expression of miR-21 and miR-145 and the pathological parameters of the patients. Patients were followed up once a month for 5 years after surgery. No patients were missed.

Statistics

Data were processed using SPSS 17.0 software. Count data between groups were compared using x^2 test. Measurement data were expressed as mean ± stan-

dard deviation and t-test was used for comparisons between two groups. Correlation analysis was performed by Pearson's correlation analysis. Single factor survival analysis was performed using Kaplan-Meier method and $p < 0.05$ was considered to be statistically significant.

Results

Expression of miR-21 and miR-145 in serum detected by qRT-PCR

As shown in Figure 1, the expression level of miR-21 in the serum of CRC patients was significantly higher than in healthy controls, and the expression level of miR-145 in the serum of CRC patients was significantly decreased compared with healthy controls ($p < 0.01$).

Expression of miR-21 and miR-145 in tumor tissue detected by qRT-PCR

As shown in Figure 2, compared with adjacent healthy tissue, the expression level of miR-21 was significantly increased and the expression level of miR-145 was significantly decreased in tumor tissue ($p < 0.01$).
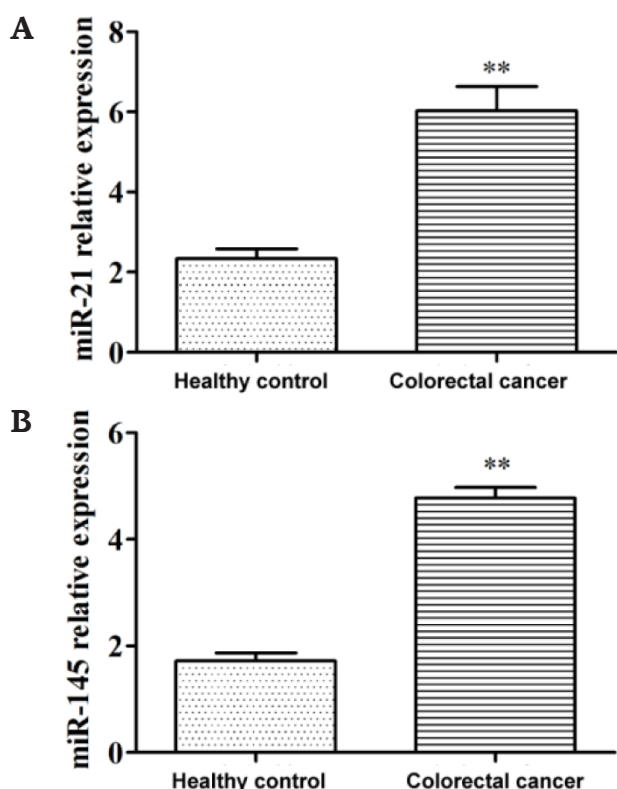

Figure 1. Expression of miR-21 and miR-145 in serum detected by qRT-PCR **(A)** Expression of miR-21 in serum; **(B)** Expression of miR-145 in serum. The expression level of miR-21 was significantly increased and the expression level of miR-145 was significantly decreased in serum compared with healthy control. **compared with healthy control, $p < 0.01$

Correlation between the expression of miR-21 and miR-145 in serum and their expression in tumor tissue of CRC patients

The correlation between the expression of miR-21 and miR-145 in the serum and their expression in tumor tissue of CRC patients was analyzed by Pearson's correlation analysis. As shown in Table 2, the expression of miR-21 and miR-145 was significantly positively correlated with their expression in tumor tissue ($p < 0.01$).
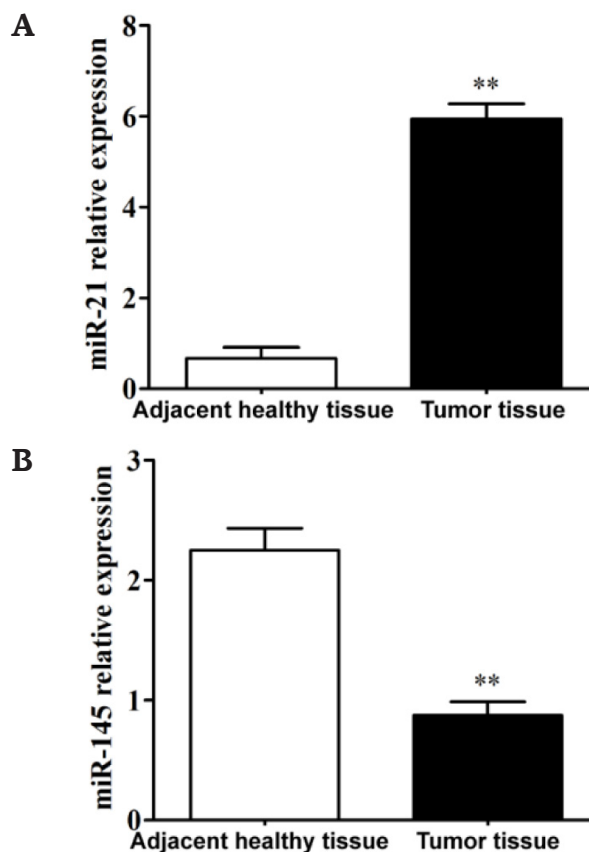

Figure 2. Expression of miR-21 and miR-145 in tumor tissue and adjacent healthy tissue detected by qRT-PCR. **(A)** Expression of miR-21 in tumor tissue and adjacent healthy tissue; **(B)** Expression of miR-145 in tumor tissue and adjacent healthy tissue. The expression level of miR-21 was significantly increased and the expression level of miR-145 was significantly decreased in tumor tissue compared with the adjacent healthy tissue. **compared with adjacent healthy tissue, $p < 0.01$

Table 2. Correlation between the expression of miR-21 and miR-145 in serum and their expression tumor tissue of patients with colorectal cancer

Serum	Tumor tissue	r	p value
miR-21	miR-21	0.763	<0.01
miR-145	miR-145	0.558	<0.01

Correlation between the expression of miR-21 and miR-145 in serum and clinicopathological parameters of CRC patients

Compared with healthy control, miR-21 was highly expressed in the serum of 76.0% (38/50) of CRC patients, and miR-145 was lowly expressed in the serum of 70.0% (35/50) of CRC patients. The correlation between the expression of miR-21 and miR-145 in the serum and clinicopathological parameters of patients is listed in Table 3. Chi square test showed that high expression level of miR-21 and low expression level of miR-145 were significantly associated with tumor size, grade of differentiation, invasion, metastasis and clinical stage.

Survival and prognosis analysis

During 5-year follow up, 26 patients survived and 24 died. The 5-year OS rate was 52% (26/50) and the mortality rate was 48% (24/50). Kaplan-Meier survival analysis is shown in Figures 3A and B. The prognosis was poor for patients with high expression of miR-21 and low expression of miR-145. As shown in Table 4, single factor survival analysis showed that miR-21 and miR-145 can significantly affect the OS rate of CRC patients (p<0.05).
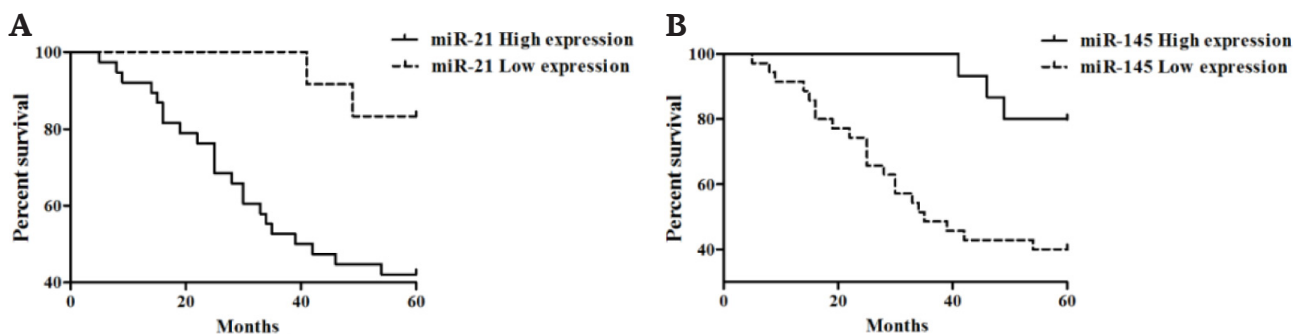

Figure 3. Kaplan-Meier survival curves plotted based on the expression levels of miR-21 and miR-145. **(A)** Kaplan-Meier survival curves of patients with high and low expression levels of miR-21 (p<0.05); **(B)** Kaplan-Meier survival curves of patients with high and low expression levels of miR-145 (p<0.05).

Table 3. Correlation between the expression of miR-21 and miR-145 in serum and clinicopathological parameters of patients with colorectal cancer

Clinicopathological parameters	Cases, n	miR-21			miR-145		
		High cases (%)	x^2	p value	Low cases (%)	x^2	p value
Gender			0.23	>0.05		0.06	>0.05
Male	28	22 (78.6)			20 (71.4)		
Female	22	16 (72.7)			15 (68.2)		
Age, years			0.01	>0.05		0.01	>0.05
≥50	35	27 (77.1)			24 (68.6)		
<50	15	11 (73.3)			11 (73.3)		
Tumor size (cm)			4.02	<0.05		0.68	>0.05
≥5	31	27 (87.1)			23 (74.2)		
<5	19	11 (57.9)			12 (63.2)		
Differentiation			8.86	<0.01		9.97	<0.01
Low	23	13 (56.5)			11 (47.8)		
Medium/high	27	25 (92.6)			24 (88.9)		
Metastasis			5.72	<0.05		6.46	<0.05
Yes	33	29 (87.9)			27 (81.8)		
No	17	9 (52.9)			8 (47.1)		
Clinical stage			7.22	<0.01		7.47	<0.01
I-II	19	10 (52.6)			9 (47.4)		
III-IV	31	28 (90.3)			26(83.9)		

Table 4. Correlation between the expression of miR-21 and miR-145 and the overall survival rate of patients with colorectal cancer analyzed by single factor survival analysis

Groups		Cases	5-year survival cases	5-year overall survival rate (%)	Wald (log-rank)	p value
miR-21	High	38	16	42.1	6.03	<0.05
	Low	12	10	83.3		
miR-145	High	15	12	80.0	7.30	<0.05
	Low	35	14	40.0		

Discussion

In recent years, with the improvement of living standards and changes in diet, the incidence of CRC in China follows an increasing trend [13]. Clinical treatments of CRC mainly include surgery, chemotherapy and radiotherapy. Some CRC patients are diagnosed in advanced stage, therefore early diagnosis of this disease is of paramount importance for an effective treatment [14].

Studies have shown that a variety of miRs were differentially expressed in normal tissue and tumor tissue, and those miRs may play a role of either tumor suppressor gene or oncogene in the development of tumor. Those miRs can regulate tumor cell proliferation, differentiation and invasion by regulating the expression of various growth factors or transcription factors [15].

As a non-coding small molecule RNA, miR-21 is closely related to the proliferation, differentiation and apoptosis of tumor cells [16-18]. A study found that miR-21 was highly expressed in hepatocellular carcinoma tissue and played an important role in tumor cell invasion and metastasis [19]. Studies have also found that miR-21 was highly expressed in breast cancer [20], gastric cancer [21] and other malignant tumors [22], and the high expression level of miR-21 was related to poor prognosis [23]. It has been reported that the expression level of miR-21 was closely correlated with grade of differentiation, and high expression level of miR-21 could contribute to increased grade of tumor differentiation of patients [24]. Michael et al. found that the expression level of miR-145 in CRC was significantly lower than that in adjacent tissues [25]. Studies have also found that the expression level of miR-145 was significantly decreased in colon cancer cell lines, and the overexpression of miR-145 significantly inhibited the growth and proliferation of tumor cells. So, miR-145 may be a new target for the treatment of CRC tumors [26,27].

In order to investigate the expression of miR-21 and miR-145 in the serum and tumor tissues of CRC patients and the effects on patient pathological parameters and prognosis, qRT-PCR was performed to detect the expression of miR-21 and miR-145 in patient serum and tumor tissue. The results showed that, compared with adjacent healthy tissue, the expression of miR-21 was significantly increased and the expression of miR-145 was significantly decreased in tumor tissue. The expression level of miR-21 in the serum of patients with CRC was significantly higher than that of healthy controls, while the expression of serum level of miR-145 of the patients was significantly lower compared with healthy controls. In addition, the expression of serum miR-21 and miR-145 was significantly positively correlated with their expression in tumor tissue. Further analysis with clinical data showed that high expression level of miR-21 and low expression level of miR-145 were associated with tumor size, grade of differentiation, invasion, metastasis and clinical stage. Single factor Kaplan-Meier survival analysis showed that both miR-21 and miR-145 could significantly affect the OS of CRC patients. The results showed that miR-21 overexpression was a good indicator for poor prognosis, while miR-145 overexpression was a good indicator for good prognosis.

Studies have confirmed that miR-21 is highly expressed in colon cancer tissues and is closely correlated to clinical stage and patient prognosis [28,29], while miR-145 was significantly downregulated in colon cancer, breast cancer, gastric cancer and other tumors and it was found to be able to inhibit the proliferation of tumor cells [30-33]. This study further confirmed that miR-21 was upregulated and miR-145 was downregulated in CRC tissues. In addition, the expression of miR-21 and miR-145 in serum was positively correlated with their expression in tumor, and the expression of miR-21 and miR-145 was correlated to the pathological parameters and prognosis of patients.

In summary, high expression level of miR-21 and low expression level of miR-145 are closely related to the occurrence and development of CRC, especially with the grade of tumor differentiation, invasion, metastasis and clinical stage. The

use of tumor markers in peripheral venous blood for tumor detection has the following advantages over tissue specimen testing: minimal invasion, high repeatability and easy testing, so these miRs are promising markers for tumor diagnosis. This study proved that miR-21 and miR-145 in serum of patients can be used as a reference index to guide the diagnosis and prognosis prediction of CRC patients.

Acknowledgements

Conflict of interests

The authors declare no conflict of interests.

References

1. Siegel RL, Miller KD, Fedewa SA et al. Colorectal cancer statistics, 2017. CA Cancer J Clin 2017;67:177-93.

2. Chen W, Zheng R, Zhang S, Zhao P, Zeng H, Zou X. Report of cancer incidence and mortality in China, 2010. Ann Transl Med 2014;2:61.

3. Cunningham D, Atkin W, Lenz HJ et al. Colorectal cancer. Lancet 2010;375:1030-47.

4. Panera N, Gnani D, Crudele A, Ceccarelli S, Nobili V, Alisi A. MicroRNAs as controlled systems and controllers in non-alcoholic fatty liver disease. World J Gastroenterol 2014;20:15079-86.

5. Bartel DP. MicroRNAs: Target recognition and regulatory functions. Cell 2009;136:215-33.

6. Siomi H, Siomi MC. On the road to reading the RNA-interference code. Nature 2009;457:396-404.

7. Li SJ, Zhou J, Zhang L et al. The effect of miR-21 on SWOZ2 glioma cells and its biological mechanism. JBUON 2017;22:468-73.

8. Gao W, Xu J, Liu L, Shen H, Zeng H, Shu Y. A systematic-analysis of predicted miR-21 targets identifies a signature for lung cancer. Biomed Pharmacother 2012;66:21-28.

9. Kumarswamy R, Volkmann I, Thum T. Regulation and function of miRNA-21 in health and disease. RNA Biol 2011;8:706-13.

10. Gregersen LH, Jacobsen AB, Frankel LB, Wen J, Krogh A, Lund AH. MicroRNA-145 targets YES and STAT1 in colon cancer cells. PLoS One 2010;5:e8836.

11. Xu Q, Liu LZ, Qian X et al. MiR-145 directly targets p70S6K1 in cancer cells to inhibit tumor growth and angiogenesis. Nucleic Acids Res 2012;40:761-74.

12. Zhang Y, Wen X, Hu XL, Cheng LZ, Yu JY, Wei ZB. Downregulation of miR-145-5p correlates with poor prognosis in gastric cancer. Eur Rev Med Pharmacol Sci 2016;20:3026-30.

13. Chen W, Zheng R, Baade PD et al. Cancer statistics in China, 2015. CA Cancer J Clin 2016;66:115-32.

14. Gill S. Adjuvant therapy for resected high-risk colon cancer: Current standards and controversies. Indian J Med Paediatr Oncol 2014;35:197-202.

15. Zhu Z, Zhang X, Wang G, Zheng H. Role of MicroRNAs in hepatocellular carcinoma. Hepat Mon 2014;14:e18672.

16. Nana-Sinkam SP, Fabbri M, Croce CM. MicroRNAs in cancer: Personalizing diagnosis and therapy. Ann N Y Acad Sci 2010;1210:25-33.

17. Lagendijk AK, Moulton JD, Bakkers J. Revealing details: Whole mount microRNA in situ hybridization protocol for zebrafish embryos and adult tissues. Biol Open 2012;1:566-9.

18. Scott GK, Goga A, Bhaumik D, Berger CE, Sullivan CS, Benz CC. Coordinate suppression of ERBB2 and ERBB3 by enforced expression of micro-RNA miR-125a or miR-125b. J Biol Chem 2007;282:1479-86.

19. Zhong M, Ma X, Sun C, Chen L. MicroRNAs reduce tumor growth and contribute to enhanced cytotoxicity induced by gefitinib in non-small cell lung cancer. Chem Biol Interact 2010;184:431-8.

20. Estatabadi MJD, Farhangi B, Montazeri M, Sistani RN, Sadeghizadeh M. Up-regulation of MIR-21 decreases chemotherapeutic effect of dendrosomal curcumin in breast cancer cells. Iran J Basic Med Sci 2017;20:350-9.

21. Arias Sisa LA, Cuspoca Ozduz AF, Bernal Gomez BM. Deregulation of microRNAs in gastric cancer: upregulation by miR-21 and miR-106. Rev Gastroenterol Peru 2017;37:65-70.

22. Montazi AA, Shahabipour K, Khatibi S, Johnston TP, Pirro M, Sahemkar A. Curcumin as a MicroRNA Regulator in Cancer: A Review. Rev Physiol Biochem Pharmacol 2016;171:1-38.

23. Zoller M. Tetraspanins: Push and pull in suppressing and promoting metastasis. Nat Rev Cancer 2009;9:40-55.

24. Romanska HM, Berditchevski F. Tetraspanins in human epithelial malignancies. J Pathol 2011;223:4-14.

25. Michael MZ, O' CS, van Holst PN, Young GP, James RJ. Reduced accumulation of specific microRNAs in colorectal neoplasia. Mol Cancer Res 2003;1:882-91.

26. Akao Y, Nakagawa Y, Naoe T. MicroRNA-143 and -145 in colon cancer. DNA Cell Biol 2007;26:311-320.

27. Motoyama K, Inoue H, Takatsuno Y et al. Over- and under-expressed microRNAs in human colorectal cancer. Int J Oncol 2009;34:1069-75.

28. Basati G, Emami RA, Abdi S, Mirzaei A. Elevated level of microRNA-21 in the serum of patients with colorectal cancer. Med Oncol 2014;31:205.

29. Masuda S, Izpisua BJ. Serum miR-21 as a diagnostic and prognostic biomarker in colorectal cancer. J Natl Cancer Inst 2014;106:t457.

30. Gotte M, Mohr C, Koo CY et al. MiR-145-dependent targeting of junctional adhesion molecule a and modulation of fascin expression are associated with reduced breast cancer cell motility and invasiveness. Oncogene 2010;29:6569-80.

31. Zhang J, Guo H, Zhang H et al. Putative tumor suppressor miR-145 inhibits colon cancer cell growth by targeting oncogene Friend leukemia virus integration 1 gene. Cancer 2011;117:86-95.

32. Takagi T, Iio A, Nakagawa Y, Naoe T, Tanigawa N, Akao Y. Decreased expression of microRNA-143 and -145 in human gastric cancers. Oncology (Basel) 2009;77:12-21.

33. Chiyomaru T, Enokida H, Tatarano S et al. MiR-145 and miR-133a function as tumour suppressors and directly regulate FSCN1 expression in bladder cancer. Br J Cancer 2010;102:883-91.