Original Article
Decreased expression of microRNA-192 correlates with tumor progression and poor prognosis in patients with colorectal cancer

Baozhen Shan[1,2], Pu Chen[3], Shengbao Li[2], Liu Xu[2], Honggang Yu[1]

[1]Department of Gastroenterology, Renmin Hospital of Wuhan University, Wuhan, China; [2]Department of Gastroenterology, Shiyan Taihe Hospital, Hubei University of Medicine, Hubei, China; [3]Department of School of Humanities and Social Science, Hubei University of Medicine, Hubei, China

Abstract: MicroRNAs, a class of small non-coding RNAs, are involved in pathogenesis of several cancers, including colorectal cancers (CRC). MicroRNA (miR)-192 has been reported to play an important role in controlling biological behaviors, such as cell proliferation and apoptosis. The aim of this study was to investigate the correlations of the tissue expression of miR-192 with clinicopathological characteristics and prognosis in patients with CRC. Colorectal cancer tissues and adjacent tissues were collected from 89 patients with primary CRC during surgery, while normal colorectal tissues were obtained from 30 healthy volunteers during endoscopy. MiR-192 expression of these samples was assessed by quantitative real-time PCR. Kaplan-Meier survival curve was plotted to analyze the association of miR-192 expression with overall survival (OS), and univariate and multivariate logistic regression analysis were performed to further investigate the independent risks for OS. The level of miR-192 was significantly decreased in colorectal cancer tissues compared with that in adjacent tissues ($P<0.001$), and normal tissues ($P<0.001$). Level of miR-192 was found to be negatively correlated with tumor size ($P=0.001$), lymph node metastasis ($P<0.018$), and distant metastasis ($P<0.041$). Patients with high miR-192 level showed higher OS than patients with low miR-192 level ($P=0.014$). Low MiR-192 expression ($P=0.017$), poorly histology ($P=0.035$), T classification (T3/T4) ($P=0.006$), lymph node metastasis ($P<0.001$), distant metastasis ($P=0.013$) and TNM stage (III/IV) ($P=0.003$) were predictive risk factors for OS by univariate analysis. However, only low miR-192 expression ($P=0.033$), lymph node metastasis ($P=0.003$) and distant metastasis ($P=0.043$) were independent risks for OS in patients with CRC by multivariate analysis. Our data indicated that down-regulation of miR-192, which correlates with tumor size, lymph node metastasis, and distant metastasis, was an independent risk for OS in CRC. MiR-192 might be a favorable prognostic biomarker in CRC.

Keywords: MiR-129, CRC, prognosis, OS

Introduction

Colorectal cancer (CRC), which had a low incidence several decades ago, has become one of the leading causes of death from cancer worldwide [1, 2]. Although new approaches to managing CRC have emerged, the main treatment is still surgical resection, combined with radiotherapy and chemotherapy [2]. Besides, the prognosis of CRC is partly dependent on tumor progression [2]. Hence, identifying useful biomarkers to detect tumor progression and predict clinical outcome after curative surgery is urgent needed.

MicroRNAs (miRs), which consist of approximately 22 base pairs, are a family of small non-coding RNA molecules, and regulate target gene expression by binding to 3' untranslated regulations (UTRs) [3, 4]. As reported, miRs are emerging to be involved in various biological behaviors, such as differentiation, proliferation, apoptosis, migration, leading to be key regulators in various pathogenesis and progression of different diseases, especially cancers [5-7]. Moreover, accumulating data have shown that miRs are promising biomarkers of diagnosis, and prognosis in different kinds of cancers. To data, a large number of dysregulated expres-

sions of miRs have been found in CRC, including miR-92, miR-221, miR-222, miR-194, miR-215, and miR-192 [8-10].

Previous studies have reported that miR-192 is significantly decreased in CRC tissues, and involved in cell proliferation, apoptosis, and liver metastasis [11, 12]. In this study, the miR-192 level in colorectal cancer tissues and adjacent tissues of CRC patients was measured, clinicopathological characteristics were collected, and overall survival (OS) was acquired. The aim of this study was to investigate the correlations of the tissue expression of miR-192 with clinicopathological characteristics and OS in patients with CRC.

Materials and methods

Patients and tissue samples

89 patients with primary CRC, who did not receive preoperative radiotherapy or chemotherapy, underwent colorectal surgery at department of General Gastroenterology, Renmin Hospital of Wuhan University and Shiyan Taihe Hospital of Hubei University of Medicine, China, from December 2009 to December 2010, were enrolled in this study. The diagnoses of CRC were based on clinical characteristics, radiological findings and pathological confirmation. Patients with tumors associated with Familial Adenomatous Polyposis or inflammatory bowel disease were excluded in this study. Besides, 30 age and gender matched healthy volunteers, who took endoscopic examination, were also enrolled in this study. All the patients and healthy volunteers provided their written consent, and this study was approved by the Ethics Review Board of both Renmin Hospital and Shiyan Taihe Hospital.

Paired tumor tissues and adjacent tissues (>5 cm from tumor tissues) from CRC patients, as well as normal colonic tissues from healthy volunteers were obtained. Meanwhile, clinicopathological characteristics were collected, including age, gender, histology, tumor location, tumor size, lymphatic invasion, venous invasion, T classification, lymph node metastasis, distant metastasis, TNM stage and OS.

All patients enrolled in this study had been followed up 4 times a year in the first year after surgery, 2 times in the second year, and once a year from third year to the fifth year, and the date of latest follow-up was December 31, 2015. OS was calculated in months from the initial diagnosis to the time of death due to any causes or last data of follow-up.

Analysis of miR-192 expression

Total RNA was extracted from CRC and normal tissues using TRIzol regent (Invitrogen), and a NanoVue spectrophotometer was used to assess the concentration and purity of RNA. RNA was then subjected to reverse transcription with One Step PrimerScript miRNA cDNA Synthesis Kit (Takara) according to the manufacturer's instructions. Quantitative analysis of miR-192 expression was performed using SYBR Premix Ex TaqTM II (Takara), and the miR-192 data were normalized for the expression of U6 and were calculated utilizing the $2^{-\Delta\Delta t}$ method [13]. The primers of miR-129 and U6 were listed as follows: miR-192 (Forward: CTGACCTATGAATTGACAGCCA; Reverse: GCTGTCAACGATACGCTACGT); U6 (Forward: CGCTTCGGCAGCACATATAC; Reverse: TTCACGAATTTGCGTGTCAT).

Statistical analysis

Wilcoxon signed-rank sum test or Wilcoxon rank sum test was performed to compare the level of miR192 between different groups. The chi-square test or Wilcoxon rank sum test was used to measure the miR-192 levels in CRC patients with different characteristics. Kaplan-Meier curve and Log-rank test were established to analyze OS in different groups. The influence of each variable on OS was measured by univariate and multivariate Cox's proportional hazards regression. All statistical analyses were performed using SPSS software, Version 19, and $P<0.05$ was considered as a statistically significant difference.

Results

Patient characteristics

Demographic and clinicopathological characteristics of patients with CRC were shown in **Table 1**. Of all enrolled CRC patients, there were 36 (40%) patients in age ≤60 and 53 (60%) patients in age >60, with 37 (42%) females and 52 (58%) males. As to histology of tumor, 57 (64%) were well/moderately differen-

Table 1. Correlations of tissue miR-192 level with demographic and clinicopathological characteristics of patients with colorectal cancer (n=89)

Parameters		Counts (%)	miR-192 (Median)	p value
Age	≤60	36 (40%)	0.167 (0.079-0.307)	0.947
	>60	53 (60%)	0.187 (0.112-0.221)	
Gender	Female	37 (52%)	0.191 (0.098-0.227)	0.920
	Male	52 (58%)	0.184 (0.087-0.272)	
Histology	Well/moderately	57 (64%)	0.191 (0.098-0.227)	0.171
	Poorly differentiated	32 (36%)	0.184 (0.087-0.272)	
Tumor location	Colon	47 (53%)	0.183 (0.108-0.278)	0.794
	Rectum	42 (47%)	0.179 (0.083-0.223)	
Tumor size	≤50 mm	54 (61%)	0.212 (0.124-0.313)	0.001*
	>50 mm	35 (39%)	0.118 (0.076-0.191)	
Lymphatic invasion	Negative	21 (24%)	0.187 (0.087-0.227)	0.985
	Positive	68 (76%)	0.184 (0.093-0.272)	
Venous invasion	Negative	52 (58%)	0.184 (0.085-0.272)	0.829
	Positive	37 (42%)	0.191 (0.101-0.222)	
T classification	T1/T2	36 (40%)	0.202 (0.133-0.296)	0.141
	T3/T4	53 (60%)	0.156 (0.084-0.219)	
Lymph node metastasis	Negative	45 (51%)	0.211 (0.131-0.311)	0.018*
	Positive	44 (49%)	0.130 (0.080-0.214)	
Distant metastasis	Negative	71 (80%)	0.201 (0.114-0.296)	0.041*
	Positive	18 (20%)	0.128 (0.071-0.192)	
TNM stage	I	21 (24%)	0.214 (0.113-0.300)	0.083
	II	21 (24%)	0.203 (0.109-0.312)	
	III	28 (31%)	0.180 (0.109-0.221)	
	IV	19 (21%)	0.121 (0.060-0.185)	

Data are presented as counts (%) or median (25th-75th), *A p Value <0.05 was considered statistically significant. Significance of the comparison was determined by chi-square test or Wilcoxon rank sum test.
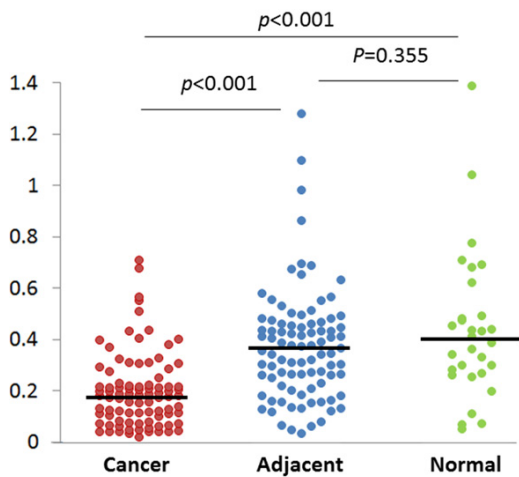

Figure 1. MiR-192 expressions in colorectal cancer tissues, adjacent tissues and normal tissues. The comparison of difference was performed by Wilcoxon signed-rank sum test or Wilcoxon rank sum test.

tiated, 32 (36%) were poorly differentiated. The number of patients with tumor size >50 mm was 35 (39%), and ≤50 mm was 54 (61%), while 47 (53%) tumors were located in colon, and 42 (47%) were in rectum. Besides, patients with positive lymphatic invasion and venous invasion were 68 (76%), and 37 (42%), respectively. As to metastasis, 44 (49%) were positive lymph node metastasis, 18 (20%) were positive distant metastasis. According to T classification, the number of patients with T1/T2 was 36 (40%), and T3/T4 was 53 (60%), while 21 (24%), 21 (24%), 28 (31%), and 19 (21%) subjects were ranked as I, II, III, IV on the basis of TNM stage.

Expression of miR-192 in colorectal cancer tissues, adjacent tissues and normal tissues

To examine the different expression of miR-192 among colorectal cancer tissues, adjacent tissues from CRC patients and normal tissues from healthy donors, qRT-PCR was performed. As shown in **Figure 1**, the level of miR-192 (0.185 (0.097-0.243)) was found to be significantly lower in colorectal cancer tissues than
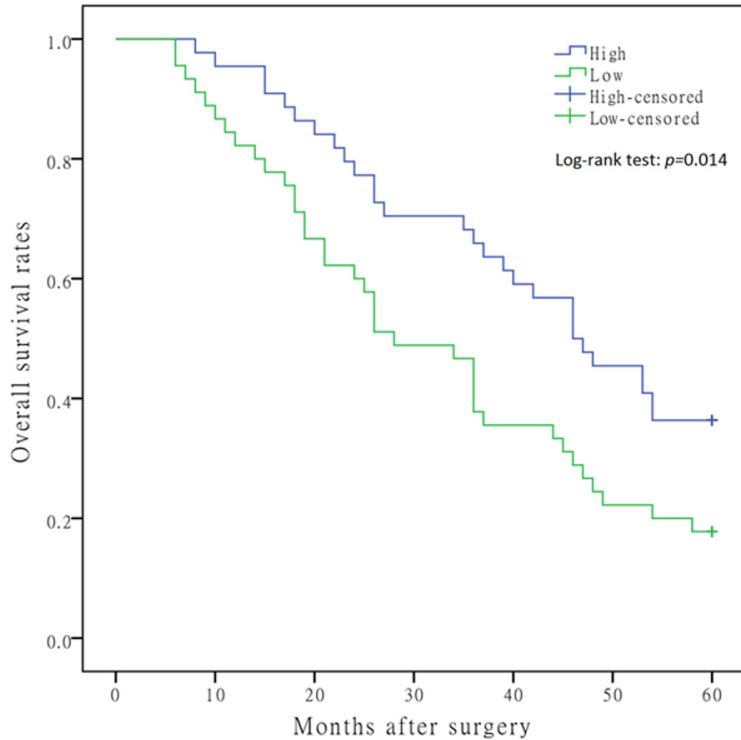
Figure 2. Kaplan-Meier curves for overall survival in patients with CRC divided according to miR-192 expression.

cated that level of miR-192 was negatively correlated with tumor size, lymph node metastasis, and distant metastasis. However, no significant correlations were found between the level of miR-192 and other clinicopathological characteristics (**Table 1**).

Association between tissue miR-192 level and OS

Since OS has been considered as an important indicator of prognosis, we then further investigated whether the level of miR-192 was associated with OS. According to the median value of miR-192 expression in colorectal cancer tissues, we divided CRC patients into high level group (>0.185, n=44) and low level group (≤0.185, n=45). Meanwhile, Kaplan-Meier curve and Log-rank test were performed to analyze the difference of OS in these two groups of CRC patients. We found that groups with high miR-192 level showed significant higher OS than groups with low miR-192 level (**Figure 2**, *P*=0.014), which indicated that miR-192 level was associated with prognosis.

that in paired adjacent tissues (0.370 (0.229-0.467), *P*<0.001) and normal tissues (0.404 (0.271-0.527), *P*<0.001), while there was no statistical difference of miR-192 expressions between adjacent tissues and normal tissues (*P*=0.355).

Correlations of tissue miR-192 level with demographic and clinicopathological characteristics of patients with CRC

To investigate the potential role of miR-192 in the development and progression of CRC, we then measured the miR-192 levels in CRC patients with different characteristics. As shown in **Table 1**, we found that significant lower expressions of miR-192 were found in the groups of larger tumor size (>50 mm, 0.118 (0.076-0.191), *P*=0.001), positive node metastasis (0.130 (0.080-0.214), *P*=0.018), and positive distant metastasis (0.128 (0.071-0.192), *P*=0.041), compared with those in the group of smaller tumor size (≤5 cm, 0.212 (0.124-0.313)), negative node metastasis (0.211 (0.131-0.311)) and negative distant metastasis (0.201 (0.114-0.296)), respectively, which indi-

In order to further investigate the role of miR-192 in prognosis, univariate Cox's and multivate Cox's portional hazards regression model were performed. Low level of miR-192 (*P*=0.017, HR=1.825, 95% CI: 1.114-2.988), poorly differentiated group (histology) (*P*=0.035, HR=1.715, 95% CI: 1.040-2.827), higher grade of T classification (T3/T4) (*P*=0.006, HR=2.064, 95% CI: 1.230-3.464), positive lymph node metastasis (*P*<0.001, HR=2.721, 95% CI: 1.651-4.484), positive distant metastasis (*P*=0.013, HR= 2.035, 95% CI: 1.163-3.560) and advanced TNM stage (III/IV) (*P*=0.003, HR=2.213, 95% CI: 1.292-3.520) were found to be important risky predictors for OS of CRC patients in univariate model (**Table 2**), while only low miR-192 expression (*P*=0.033, HR=1.834, 95% CI: 1.050-3.204), lymph node metastasis positive (*P*=0.003, HR=2.259, 95% CI: 1.308-3.904) and distant metastasis positive (*P*=0.043, HR=1.892, 95% CI: 1.021-3.506) were inde-

Table 2. Univariate analyses of factors for predicting poor prognosis in patients with colorectal cancer

Parameters	HR	95% CI		p value
		Lower	Higher	
MiR-192 (low vs. high)	1.825	1.114	2.988	0.017*
Age (>60 vs. ≤60)	1.244	0.750	2.063	0.393
Gender (male vs. female)	1.347	0.817	2.223	0.239
Histology (poorly vs. moderately/well)	1.715	1.040	2.827	0.035*
Tumor location (rectum vs. colon)	1.274	0.780	2.082	0.333
Tumor size (>50 mm vs. ≤50 mm)	1.538	0.939	2.519	0.087
Lymphatic invasion (positive vs. negative)	1.188	0.666	2.118	0.559
Venous invasion (positive vs. negative)	1.237	0.754	2.030	0.399
T classification (T3/T4 vs. T1/T2)	2.064	1.230	3.464	0.006*
Lymph node metastasis (positive vs. negative)	2.721	1.651	4.484	<0.001*
Distant metastasis (positive vs. negative)	2.035	1.163	3.560	0.013*
TNM stage (III/IV vs. I/II)	2.213	1.292	3.520	0.003*

*A p Value <0.05 was considered statistically significant. Significance was determined by univariate Cox's proportional hazards regression analysis.

Table 3. Multivariate analyses of factors for predicting poor prognosis in patients with colorectal cancer

Parameters	HR	95% CI		p value
		Lower	Higher	
MiR-192 (low vs. high)	1.834	1.050	3.204	0.033*
Histology (poorly vs. moderately/well)	1.422	0.823	2.455	0.207
T classification (T3/T4 vs. T1/T2)	1.302	0.748	2.266	0.351
Lymph node metastasis (positive vs. negative)	2.259	1.308	3.904	0.003*
Distant metastasis (positive vs. negative)	1.892	1.021	3.506	0.043*
TNM stage (III/IV vs. I/II)	1.380	0.799	2.381	0.248

*A p Value <0.05 was considered statistically significant. Significance was determined by multivariate Cox's proportional hazards regression analysis.

pendent risks for OS in patients with CRC in multivariate model (**Table 3**).

Collectively, these data indicated that miR-192 was a favorable prognostic biomarker in CRC.

Discussion

As one of the leading causes of cancer-related death worldwide, CRC has been facing the difficulties in diagnosis, treatment and prognosis [2]. Recently, accumulating studies has focused on identifying molecular biomarkers to improve precision medicine, thus leading to better diagnosis and clinical outcome [14]. Interestingly, miRs, regulating more than 1/3 of human genes and the majority of pathways, are important mediators involved in cancers, through controlling cell proliferation, apoptosis, metastasis and chemoresistence [7, 14]. Furt-

hermore, multiple miRs are considered to be potential biomarkers in various diseases, including cancers [7, 15-17].

To data, several miRs have been reported to be differently expressed in CRC tissue, and correlate with clinicopathological characteristics [17]. For example, miR-21 was found to be significantly increased in tumor tissues in CRC, involved in cell proliferation and invasion, and up-regulation of miR-21 was associated with poor prognosis [18, 19]. Until now, miR-192 has been reported to be decreased in different carcinomas, including CRC [20-23]. Besides, several studies have demonstrated that miR-192 was involved in tumor initiation and progression, through regulating its target genes, such as p53, Zeb2, RBI [12, 23, 24].

Considering that many miRs, including miR-192, have been found to be dysregualted in CRC, and miR-192 is a key mediator in controlling cell proliferation, apoptosis, and liver metastasis in CRC, miR-192 was selected as a candidate biomarker to predict tumor progression and clinical outcome in our study. Firstly, we assessed the different expression of miR-192 in cancer tissues, adjacent-tumor tissues from CRC patients, and normal tissues from healthy donors. We found that the level of miR-192 was markedly decreased in cancer tissues compared with that in adjacent tissues and normal tissues, which is consistent with previous studies [11, 12], while there was no significant difference of miR-192 between adjacent tissues and normal tissues.

Although miR-192 has been found to be decreased in colorectal cancer, it is still unclear

whether tissue miR-192 expression differs in CRC patients with different characteristics. Next, we analyze the correlation between miR-192 tissue level and various clinicopathological characteristics. The data demonstrated that down-regulated expression of miR-192 was associated with larger tumor size, which consistent with previous study [11]. Most importantly, we firstly found that the expression of miR-192 was negatively correlated with lymph node metastasis, distant metastasis, which could be explained by its role in regulating cell invasion [11, 25]. However, no significant correlation of miR-192 expression with other demographic and clinicopathological characteristics, including TNM stage. Previous study showed that miR-192 expression was significantly decreased in stage IV compared with that in stage I or II in CRC patients [12], while no significant difference seen in our study. The discrepancy may be cause by different crowds and analysis methods.

OS has been considered as an important indicator of prognosis, thus we then further investigated whether the level of miR-192 was associated with OS. According to the medium value in CRC tissues, we divided patients into two groups, group of high miR-192 level and group of low miR-192. Patients with high miR-192 level showed higher OS than patients with low miR-192 level measured by Kaplan-Meier curve, which indicated that miR-192 might be a potential biomarker to predict clinical outcome.

In order to further investigate whether miR-192 was an independent risk for OS in CRC patients, univariate Cox's and multivariate Cox's proportional hazards regression analysis were then performed. Interestingly, decreased expression of miR-192 was an independent for OS, which indicated that miR-192 could be a promising molecular useful for prognosis in the future. Besides, as to other variables, poorly differentiated group (histology), higher grade of T classification, positive lymph node metastasis, positive distant metastasis and advanced TNM stage were found to be predictive risks for OS, while only lymph node metastasis and distant metastasis were independent risks for OS in patients with CRC. Lymph node metastasis and distant metastasis have been reported to be independent prognostic factors for CRC patents in previous study [26, 27], which is

consistent with the data in this study, indicating that these two factors actually affect prognosis of CRC patients. However, other characteristics, such as vascular invasion, histology grade were reported to be independent risks in other studies [28, 29], while they were not found to be independent predictive factors for CRC patients in this study. Different subjects, diverse statistical survey may account for the differences.

In summary, this study demonstrated three significant findings as followed: Firstly, the expression level of miR-192 was significant lower in tumor tissues than that in adjacent-tumor tissues from CRC patients and normal tissues from healthy controls; secondly, miR-192 tissue level was negatively correlated with tumor size, lymph node metastasis, and distant metastasis; Thirdly, decreased expression of miR-192 was an independent for OS. All the evidence indicated that decreased expression of microRNA-192 correlates with tumor progression and poor prognosis in patients with CRC, and might be a potential therapeutic target for the treatment of CRC.

Disclosure of conflict of interest

None.

Address correspondence to: Honggang Yu, Department of Gastroenterology, Renmin Hospital of Wuhan University, 238 Jiefang Road, Wuhan 430060, China. Tel: +86-27-88041911; Fax: +86-27-88041911; E-mail: yhgang818@163.com

References

[1] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136: E359-386.

[2] Kuipers EJ, Grady WM, Lieberman D, Seufferlein T, Sung JJ, Boelens PG, van de Velde CJ and Watanabe T. Colorectal cancer. Nat Rev Dis Primers 2015; 1: 15065.

[3] Fabian MR, Sonenberg N and Filipowicz W. Regulation of mRNA translation and stability by microRNAs. Annu Rev Biochem 2010; 79: 351-379.

[4] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.

[5] Volinia S, Galasso M, Costinean S, Tagliavini L, Gamberoni G, Drusco A, Marchesini J, Mascellani N, Sana ME, Abu Jarour R, Desponts C, Teitell M, Baffa R, Aqeilan R, Iorio MV, Taccioli C, Garzon R, Di Leva G, Fabbri M, Catozzi M, Previati M, Ambs S, Palumbo T, Garofalo M, Veronese A, Bottoni A, Gasparini P, Harris CC, Visone R, Pekarsky Y, de la Chapelle A, Bloomston M, Dillhoff M, Rassenti LZ, Kipps TJ, Huebner K, Pichiorri F, Lenze D, Cairo S, Buendia MA, Pineau P, Dejean A, Zanesi N, Rossi S, Calin GA, Liu CG, Palatini J, Negrini M, Vecchione A, Rosenberg A and Croce CM. Reprogramming of miRNA networks in cancer and leukemia. Genome Res 2010; 20: 589-599.

[6] Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136: 215-233.

[7] Di Leva G, Garofalo M and Croce CM. MicroRNAs in cancer. Annu Rev Pathol 2014; 9: 287-314.

[8] 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.

[9] Liu S, Sun X, Wang M, Hou Y, Zhan Y, Jiang Y, Liu Z, Cao X, Chen P, Liu Z, Chen X, Tao Y, Xu C, Mao J, Cheng C, Li C, Hu Y, Wang L, Chin YE, Shi Y, Siebenlist U and Zhang X. A microRNA 221- and 222-mediated feedback loop maintains constitutive activation of NFkappaB and STAT3 in colorectal cancer cells. Gastroenterology 2014; 147: 847-859, e811.

[10] Georges SA, Biery MC, Kim SY, Schelter JM, Guo J, Chang AN, Jackson AL, Carleton MO, Linsley PS, Cleary MA and Chau BN. Coordinated regulation of cell cycle transcripts by p53-Inducible microRNAs, miR-192 and miR-215. Cancer Res 2008; 68: 10105-10112.

[11] Chiang Y, Song Y, Wang Z, Liu Z, Gao P, Liang J, Zhu J, Xing C and Xu H. microRNA-192, -194 and -215 are frequently downregulated in colorectal cancer. Exp Ther Med 2012; 3: 560-566.

[12] Geng L, Chaudhuri A, Talmon G, Wisecarver JL, Are C, Brattain M and Wang J. MicroRNA-192 suppresses liver metastasis of colon cancer. Oncogene 2014; 33: 5332-5340.

[13] 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 2001; 25: 402-408.

[14] Mirnezami R, Nicholson J and Darzi A. Preparing for precision medicine. N Engl J Med 2012; 366: 489-491.

[15] Lewis BP, Burge CB and Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 2005; 120: 15-20.

[16] Calin GA and Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006; 6: 857-866.

[17] Yi R, Li Y, Wang FL, Miao G, Qi RM and Zhao YY. MicroRNAs as diagnostic and prognostic biomarkers in colorectal cancer. World J Gastrointest Oncol 2016; 8: 330-340.

[18] Asangani IA, Rasheed SA, Nikolova DA, Leupold JH, Colburn NH, Post S and Allgayer H. MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. Oncogene 2008; 27: 2128-2136.

[19] Schetter AJ, Leung SY, Sohn JJ, Zanetti KA, Bowman ED, Yanaihara N, Yuen ST, Chan TL, Kwong DL, Au GK, Liu CG, Calin GA, Croce CM and Harris CC. MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. JAMA 2008; 299: 425-436.

[20] Mathe EA, Nguyen GH, Bowman ED, Zhao Y, Budhu A, Schetter AJ, Braun R, Reimers M, Kumamoto K, Hughes D, Altorki NK, Casson AG, Liu CG, Wang XW, Yanaihara N, Hagiwara N, Dannenberg AJ, Miyashita M, Croce CM and Harris CC. MicroRNA expression in squamous cell carcinoma and adenocarcinoma of the esophagus: associations with survival. Clin Cancer Res 2009; 15: 6192-6200.

[21] Jin Z, Selaru FM, Cheng Y, Kan T, Agarwal R, Mori Y, Olaru AV, Yang J, David S, Hamilton JP, Abraham JM, Harmon J, Duncan M, Montgomery EA and Meltzer SJ. MicroRNA-192 and -215 are upregulated in human gastric cancer in vivo and suppress ALCAM expression in vitro. Oncogene 2011; 30: 1577-1585.

[22] Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, Stephens RM, Okamoto A, Yokota J, Tanaka T, Calin GA, Liu CG, Croce CM and Harris CC. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell 2006; 9: 189-198.

[23] Feng S, Cong S, Zhang X, Bao X, Wang W, Li H, Wang Z, Wang G, Xu J, Du B, Qu D, Xiong W, Yin M, Ren X, Wang F, He J and Zhang B. MicroRNA-192 targeting retinoblastoma 1 inhibits cell proliferation and induces cell apoptosis in lung cancer cells. Nucleic Acids Res 2011; 39: 6669-6678.

[24] Ye M, Zhang J, Zhang J, Miao Q, Yao L and Zhang J. Curcumin promotes apoptosis by activating the p53-miR-192-5p/215-XIAP pathway in non-small cell lung cancer. Cancer Lett 2015; 357: 196-205.

[25] Khella HW, Bakhet M, Allo G, Jewett MA, Girgis AH, Latif A, Girgis H, Von Both I, Bjarnason GA

and Yousef GM. miR-192, miR-194 and miR-215: a convergent microRNA network suppressing tumor progression in renal cell carcinoma. Carcinogenesis 2013; 34: 2231-2239.

[26] Zong Z, Zhou T, Rao L, Jiang Z, Li Y, Hou Z, Yang B, Han F and Chen S. Musashi2 as a novel predictive biomarker for liver metastasis and poor prognosis in colorectal cancer. Cancer Med 2016; 5: 623-630.

[27] Hur K, Toiyama Y, Okugawa Y, Ide S, Imaoka H, Boland CR and Goel A. Circulating microRNA-203 predicts prognosis and metastasis in human colorectal cancer. Gut 2015; [Epub ahead of print].

[28] Tian X, Yan L, Zhang D, Guan X, Dong B, Zhao M and Hao C. PTK7 overexpression in colorectal tumors: Clinicopathological correlation and prognosis relevance. Oncol Rep 2016; 36: 1829-1836.

[29] Li ZW, Yang YM, Du LT, Dong Z, Wang LL, Zhang X, Zhou XJ, Zheng GX, Qu AL and Wang CX. Overexpression of miR-223 correlates with tumor metastasis and poor prognosis in patients with colorectal cancer. Med Oncol 2014; 31: 256.