RESEARCH ARTICLE

MicroRNAs as Promising Biomarkers for Tumor-staging: Evaluation of MiR21 MiR155 MiR29a and MiR92a in Predicting Tumor Stage of Rectal Cancer

Yun Yang[1], Wei Peng[2], Tian Tang[3], Lin Xia[1], Xiao-dong Wang[1], Bao-feng Duan[4], Ye Shu[1]*

Abstract

Objective: In this study, tumor-stage predictive abilities of miR21, miR155, miR29a and miR92a were evaluated in rectal cancer (RC). Methods: Expression of miR21, miR155, miR29a and miR92a was detected and quantitated in tumor tissue and in adjacent normal tissue from 40 patients by TaqMan MicroRNA assay. Results: Significant overexpression of miR21, miR155, miR29a and miR92a was observed in RC tissues. While high expression of miR21, miR155 and miR29a in N1-2 and C-D stages presented a potential correlation with N and Duke stages, partial correlation analysis suggested that only miR155 rather than miR21 and miR29a played a greater influencing role. Receiver operating characteristics (ROC) curve analysis showed that miR155 could discriminate N0 from N1-2 with 85.0% sensitivity and 85.0% specificity, N2 from N0-1 with 90.0% sensitivity and 96.7% specificity, and C-D stage from A-B stage with 81.0% sensitivity and 84.2% specificity. Conclusions: Increase in expression of miR155 might represent a novel predictor for RC N and Dukes staging.

Keywords: Oncogenic microRNAs - rectal cancer - N stage - Dukes stage - miR21 - miR155 - miR29a - miR92a

Asian Pac J Cancer Prev, **15 (13)**, 5175-5180

Introduction

Rectal cancer (RC) is one of the most common malignant tumors. Its morbidity ranks the third place and mortality ranks the second place among all malignant tumors. Unfortunately, 50% of RC patients have already had regional or distant metastases at the time of diagnosis (Figueredo et al., 2008). So earlier diagnosing and tumor staging of RC are the key basis to choose treatment options, and directly affect patients' prognosis and overall survival. Biochemical tumor markers such as CEA and imaging examination like CT and MRI are used for early detecting and progression monitoring. However, due to their wide variation in accuracy, they are not the "golden standard" for RC preoperative diagnosis, even less so for tumor staging (Karantanas et al., 2007; Nishiumi et al., 2012). Therefore the search for novel markers indicating RC tumor stage is needed.

MicroRNAs are noncoding small RNAs (18–25 nucleotides) that play regulating roles in cell differentiation, cell cycle progression and apoptosis (Esquela-Kerscher et al., 2006). They can be developed into microRNA-induced silencing complex (miRISC) that combines with specific 3'-untranslated-regions (3'-UTR) on mRNAs to induce mRNA degradation and inhibit protein translation (Lai et al., 2002; He et al., 2004). Therefore, microRNAs are considered as "regulators" in numerous biological events including genesis and development of carcinoma. Although not every mechanism and function of microRNAs is fully understood, certain studies suggest that abnormal expression of microRNAs is associated with a variety of tumors (Chiang et al., 2012; Iwaya et al., 2012; Hashimoto et al., 2013; Song et al., 2013). Thus it is possible that some microRNAs may have biological and clinical correlation with rectal cancer.

MiR-21, miR-155, miR-29a and miR-92a are these kinds of oncogenic microRNAs, which have been found overexpressing in several types of human malignant solid tumors (Gironella et al., 2007; Yan et al., 2008; Gebeshuber et al., 2009; Shigoka et al., 2010). But these previous studies didn't link the microRNA expression to tumor stage quantitatively. So this study aims to further explore the quantitative relationship between expression of these microRNAs and tumor stages of RC, and evaluate their potential abilities to predict tumor stage.

[1]Department of Gastrointestinal Surgery, West China Hospital, [2]West China Medical School, Department of Hepatic Surgery and Hepatic Transplantation Center, West China Hospital, [3]West China Medical School, Department of Gynecology and Obstetrics, West China Women's and Children's Hospital, [4]Department of Oncology, West China Hospital, Sichuan University, Chengdu, China
**For correspondence: sy999222@163.com*

Materials and Methods

Patients and Tissue Samples

From August to October, 2012, 40 newly biopsy-proven RC patients were consecutively recruited into this study at the gastrointestinal surgery center of West China Hospital, according to the following inclusive criteria: no familial adenomatous polyposis or hereditary nonpolyposis CRC; no preoperative neoadjuvant therapy; no intestinal obstruction, perforation and any other acute abdomen conditions. From the inclusive patients, samples of tumor tissue and normal tissue (>5cm proximal to tumor site) were collected and frozen in liquid nitrogen right after surgical resection. Same well-trained laboratory technician tested CEA 3 days preoperatively, and same pathologist staged tumors according to the tumor-node-metastasis (TNM) staging system postoperatively. No treatment was administered in these 3 days. Also, same technical group detected expressions of microRNAs in normal and tumor tissue samples. The tissue samples were labeled such as to ensure that they were blind to the normal and tumor tissue identifications. The ethics committee of West China Hospital approved the study protocol (No. 2012-149 approved at June 25th 2012), and an informed consent agreement was obtained from every participant.

TaqMan MicroRNA Assay

Using Trizol (ABI, USA), total RNAs which contain microRNAs were isolated from tumor and normal tissue samples of the 40 patients with primary RC. MiR21, miR155, miR29a, miR92a, U6 snRNA and U47-specific probes were synthesized by ABI. The whole procedure was followed according to the TaqMan MicroRNA assay protocol (4364031 Rev. E, 01/2011) of ABI. The geometric average of two housekeeping genes, U6 snRNA and U47, was used to normalize the expression of microRNAs (Vandesompele et al., 2002). In brief, reverse transcriptase reaction was performed using Taqman MicroRNA Reverse Transcription Kit (ABI, USA). Each 25ul-reaction contained 5ng of total RNAs, 9.6ul 5×RT primer (1.6ul/gene), 0.3ul RNase Inhibitor (20U/uL), 2.5ul 10×RT buffer, 1.6ul MultiScribe Reverse Transcriptase and 0.25ul dNTPs (100mM). The reaction samples were incubated in PCR System for 30 min at 16°C, 30 min at 42°C and 5 min at 85°C, and then kept at 4°C. TaqMan qPCR was performed using Taqman Universal PCR Master Mix (ABI, USA). The 3 replicates reaction (10ul/reaction) mix included 2.4ul template, 1.6ul 20×Taqman MicroRNA Assay Mix and 16ul 2×Taqman Universal PCR buffer, UNG. The reaction samples were added into 384-well optical plates. And the PCR was run on 7900 HT Sequence Detection System (ABI, USA) in the conditions of 50° C for 2min, 95 ° C for 10 min, followed by 40 cycles at 95 ° C for 15 s and 60 ° C for 1min. Relative quantification of microRNA expression was calculated by the $2^{-\Delta Ct}$ method, where $2^{-\Delta Ct} = 2^{\sqrt{(CtU6 \times CtU47)} - Ct_{(target\ microRNA)}}$.

Statistical Analysis

Expression levels of microRNAs were compared by paired-samples T-test or independent sample T-test according to the methods of grouping. The bivariate and partial correlations between microRNAs and tumor stage were analyzed by Spearman's and Pearson's correlation test. Receiver-operating characteristics (ROC) curves were established to evaluate the prognostic value of microRNA in differentiating tumor stage. *P* values less than 0.05 were considered statistically significant with 95% 2-sided confidence. All statistical analysis was performed with IBM SPSS 20.0 software (IBM SPSS, Inc., Chicago).

Table 1. Patient Population

Characteristics	Patients, n=40
Gender	
Male	23
Female	17
Age	
Mean±SD	57.5±11.951
Median	56.5
Histological differentiation	
Moderately differentiated	26
Poorly differentiated	14
T stage	
T1	2
T2	10
T3	28
T4	0
N stage	
N0	20
N1	10
N2	10
M stage	
M0	36
M1	4
Dukes stage	
A	7
B	12
C	17
D	4

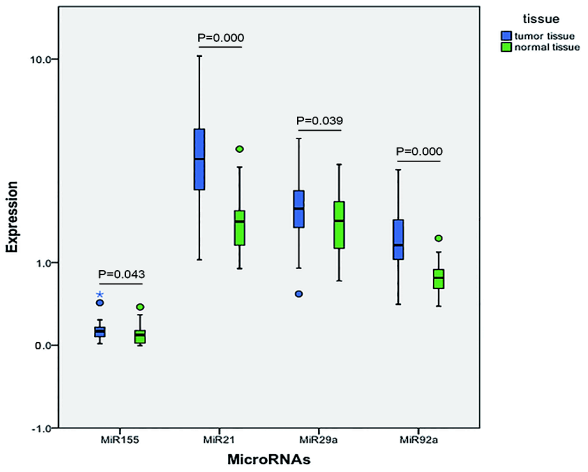

Figure 1. Expression of Four MicroRNAs in Tumor and Normal Tissues. Expression levels of microRNAs were compared by paired-samples T-test between tumor and normal tissues. The mean (±SD) expression level of miR21 was apparently higher in tumor tissue than in corresponding normal tissue (4.122±1.973 vs. 1.825±0.661, *P*=0.000). So were miR155 (0.137±0.095 vs. 0.093±0.091, *P*=0.043), miR29a (2.220±0.834 vs. 1.863±0.730, *P*=0.039) and miR92a (1.437±0.581 vs. 0.761±0.241, *P*=0.000)

Table 2. Correlations Between Clinicopathological Parameters and MicroRNAs Expression in Tumor Tissue

Varieties	Patient	CEA (ng/ml) mean±SD	*P* value	MiR21 mean±SD	*P* value	MiR155 mean±SD	*P* value	MiR29a mean±SD	*P* value	MiR92a mean±SD	*P* value
Histological differentiation											
Moderately differentiated	26	12.988±25.700	0.31	4.031±2.141	0.697	0.142±0.076	0.653	2.255±0.804	0.729	1.459±0.629	0.749
Poorly differentiated	14	29.015±72.026		4.290±1.677		0.127±0.125		2.157±0.914		1.396±0.499	
T stage											
T1, T2	12	7.298±12.734	0.326	4.199±2.283	0.873	0.158±0.122	0.365	2.320±0.790	0.627	1.276±0.515	0.255
T3, T4	28	23.440±55.216		4.088±1.869		0.128±0.081		2.178±0.862		1.506±0.603	
N stage											
N0	20	5.051±5.458	0.075	3.391±1.171	0.019*	0.09±0.038	0.001*	1.928±0.757	0.024*	1.364±0.484	0.429
N1, N2	20	32.145±64.230		4.852±2.344		0.183±0.111		2.514±0.820		1.511±0.669	
M stage											
M0	36	10.769±22.129	0.3	4.175±2.033	0.611	0.138±0.097	0.719	2.135±0.759	0.051	1.390±0.591	0.127
M1	4	89.05±125.284		3.638±1.420		0.120±0.084		2.988±1.200		1.860±0.214	
Dukes stage											
A, B	19	4.057±3.260	0.057	3.386±1.203	0.021*	0.091±0.039	0.002*	1.876±0.740	0.011*	1.347±0.491	0.356
C, D	21	31.753±62.629		4.787±2.304		0.178±0.111		2.532±0.804		1.519±0.653	

*$P<0.05$

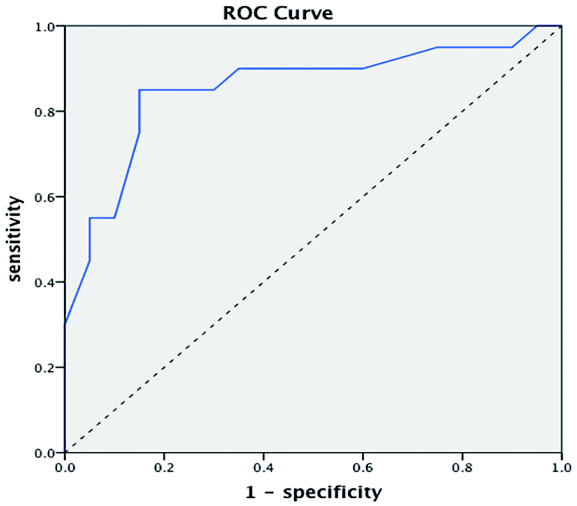

Figure 2. N1-2 Stage was Distinguished from N0 Stage by MiR155. In Receiver Operating Characteristics (ROC) curve analysis, when N0 stage was differentiated from N1-2 stage by miR155 with an AUC of 0.855 (95% CI: 0.730-0.980), the best cutoff value was 0.125 with 85.0% sensitivity and 85.0% specificity

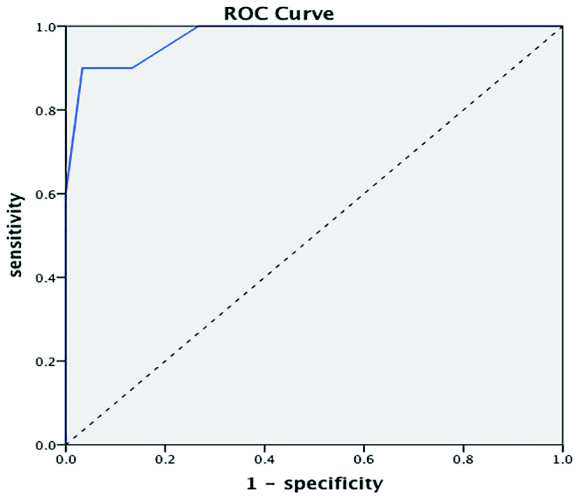

Figure 3. N2 Stage was Distinguished from N0-1 Stage by MiR155. In Receiver Operating Characteristics (ROC) curve analysis, when N2 stage was differentiated from N0-1 stage by miR155 with an AUC of 0.975 (95% CI: 0.930-1.000), the best cutoff value was 0.165 with 90% sensitivity and 96.7% specificity

Results

Patient Characteristics

From August to October 2012, a total of 40 RC patients were recruited into this study (Table 1). There should be no significant differences about baseline information between RC tissues and normal tissues, because each pair of RC and normal tissues was obtained from a same patient.

Expression of MicroRNAs in Tumor and Normal Tissue

Between the tumor tissue and normal tissue, the statistical difference of expression was observed in miR21 miR155 miR29a and miR92a with $P<0.05$. In the setting of paired-samples t test, the mean (±SD) expression level of miR21 was apparently higher in tumor tissue than in corresponding normal tissue (4.122±1.973 vs. 1.825±0.661, $P=0.000$). So were miR155 (0.137±0.095 vs. 0.093±0.091, $P=0.043$), miR29a (2.220±0.834 vs. 1.863±0.730, $P=0.039$) and miR92a (1.437±0.581 vs. 0.761±0.241, $P=0.000$) (Figure 1).

Correlations between Clinicopathological Parameters and MicroRNAs Expression in Tumor Tissue

To evaluate the correlation between microRNAs expression and clinicopathological characteristics, patients were divided into different groups shown in the first column of Table 2. A statistically significant difference was observed between the group N0 and group N1-2 in miR21 miR155 and miR29a. The same phenomenon could be seen in Dukes stage (between the group A-B and group C-D). The results suggested that miR21, miR155 and miR29a might have potential association with tumor N stage (lymph node metastasis) and Dukes stage (positive metastasis).

For these 3 candidate microRNAs: miR21 miR155 and miR29a, bivariate and partial correlation analysis was used to further determine which microRNA had greater influence on N and Dukes stage. As Table 3 presented, a bivariate correlation analysis was used to hypothesis-test association and causality between tumor stage (N and Dukes stage) and microRNAs (miR21 miR155 and miR29a). From the results, these 3 microRNAs were all found to have relationship with N and Dukes stage in bivariate correlation. But when partial correlation analysis was used to remove the effects of any two microRNAs, spurious relationship was uncovered: only miR155 stood out and presented a strong correlation with

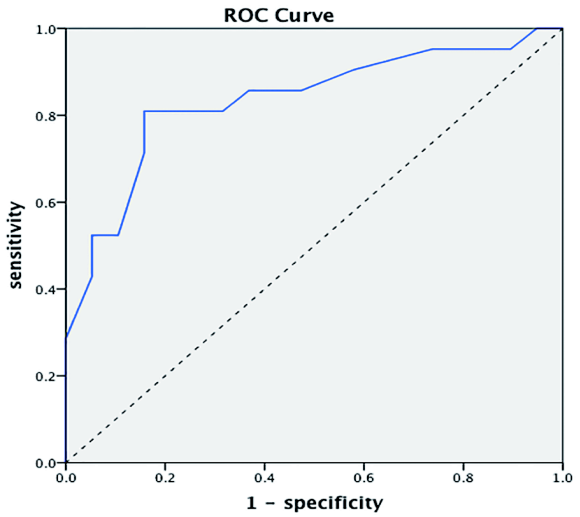

Figure 4. Dukes C-D Stage was Distinguished from A-B stage by MiR155. In Receiver Operating Characteristics (ROC) curve analysis, when C-D stage was differentiated from A-B stage by miR155 with an AUC of 0.835 (95% CI: 0.706-0.963), the best cutoff value was 0.125 with 81.0% sensitivity and 84.2% specificity

Table 3. Bivariate and Partial Correlation Between MicroRNAs and Tumor Stage

Variables	MicroRNAs and N stage				MicroRNAs and Dukes stage	
	No controlling		Controlling any two microRNAs		No controlling	
	Coefficient	2-tailed Sig.	Coefficient	2-tailed Sig.	Coefficient	2-tailed Sig.
MiR21	0.442	0.004*	0.161	0.333	0.225	0.164
MiR155	0.728	0.000*	0.474	0.003*	0.353	0.025*
MiR29a	0.388	0.013*	0.163	0.329	0.300	0.060

*P<0.05

N stage (coefficient=0.474, P=0.003) and Dukes stage (coefficient=0.353, P=0.025). That suggested miR155 could be used as a potential predictor for tumor N and Dukes stage.

The Predictive Value of MiR155 for N and Dukes Staging in Rectal Cancer

To further assess the ability of miR155 to distinguish tumor N and Dukes stages, receiver operating characteristics (ROC) curve analysis was used. As shown in Figure 2-4, N0 stage could be differentiated from N1-2 stage by miR155 with an AUC of 0.855 (95% CI: 0.730-0.980), so could N2 stage be differentiated from N0-1 stage with an AUC of 0.975 (95% CI: 0.930-1.000). Also, C-D stage could be apart from A-B stage with an AUC of 0.835 (95% CI: 0.706-0.963). For miR155, at the cutoff value of 0.125, the sensitivity, specificity and accuracy were 85.0%, 85.0% and 85.0%, the +LR (positive likelihood ratio) and –LR (the positive likelihood ratio) were 5.677 and 0.176 correspondingly in discriminating N0 from N1-2. At the cutoff value of 0.165, the sensitivity, specificity and accuracy were 90%, 96.7% and 95.0%, the +LR and -LR were 27.270 and 0.103 in discriminating N2 from N0-1. In discriminating C-D stage from A-B stage at the cutoff value of 0.125, the sensitivity, specificity and accuracy were 81.0%, 84.2% and 82.5%, the +LR and –LR were 5.127 and 0.266.

Discussion

In this study, we found that the expression levels of miR21, miR155, miR29a and miR92a in RC tissues were significantly higher than those in non-RC normal tissues. Expression levels of miR21, miR155 and miR29a in RC tissues seemed to have underlying relation with tumor stage. Although the increase of expression is much larger in miR21 (the largest) and miR29a than miR155 (Figure 1), further statistical analysis revealed a surprising result. After controlling any two factors, only miR155 still maintained the positive correlation with N and Dukes stages (Table 3).

For molecular mechanism, miR21, miR155, miR29a and miR92a are closely related to tumor development. MiR21 was demystified that it could down-regulate the protein expression of programmed cell death 4 (PDCD4), which plays a role as suppressors of transformation, tumor genesis, progression, invasion and metalloproteinase activation, and as an inducer of apoptosis (Asangani et al., 2008). Knockdown of PDCD4 decreased the expressions of epithelial-specific proteins, and increased the expressions of mesenchymal-specific proteins in vitro and in vivo, and the rate of wound closure and migration capacity in wound-healing assays and Boyden chamber migration assays, suggesting that knockdown of Pdcd4 results in epithelial to mesenchymal transition (EMT) and promotes cell migration (Wang et al., 2013). It was reported that miR155 could down-regulate TP53INP1, which is a pro-apoptotic stress-induced p53 target gene (Tomasini et al., 2002). It can interact with p53 and the homeodomain-interacting proteinkinase-2 within the promyelocytic leukemia nuclear bodies, modulating p53 transcriptional activity (Tomasini et al., 2003). Loss of p53 during tumor progression is associated with increased intestinal permeability, causing formation of an NF-κB-dependent inflammatory microenvironment and the induction of EMT (Schwitalla et al., 2013). It was revealed that miR29a was up regulated in mesenchyme, metastatic RasXT cells relative to epithelial EpRas cells, and could suppress the expression of tristetraprolin, a protein involved in the degradation of messenger RNAs with AU-rich 3'-untranslated regions, and led to EMT and metastasis in cooperation with oncogenic Ras signaling (Gebeshuber et al., 2009). Consequently, all of these three microRNAs could lead to EMT, a key process in the initiation of metastasis, but only miR155 was proved to be able to increase intestinal permeability that may be an enabling microenvironment for cancer cells migration. This may explain the reason why only miR155 has been found to have greater weight statistically in affecting N stage in partial correlation analysis, although there are larger increases of miR21 and miR29a in tumor tissues.

In previous studies, it was found high expression of miR21 in CRC tissue, which was associated with lymph node metastasis, distant metastasis and tumor staging (Slaby et al., 2008). A following joint research involving Chinese and American CRC patients uncovered the relation between low survival rate and high expression of miR21. The overexpression in C stage correlated with low chemotherapy sensitiveness and early recurrence

(Schetter et al., 2008). Other studies about miR155 demonstrated that over-expression of miR-155 was markedly related to both OS (Overall Survival) and RFS/CSS (Recurrence-free Survival or Cancer-specific Survival) in patients with digestion system cancer (Xu et al., 2013). MiR29a was detected overexpressing in CRC patients with liver metastasis (Wang et al., 2012). A recent meta-analysis also suggested that microRNAs might be potential novel biomarkers for detecting CRC with convincing sensitivity and specificity (Zhou et al., 2013). But these previous studies didn't present the mathematical positive correlation between microRNA expression and tumor N and Dukes stage. As far as we know, our study is the first report, which determined that miR155 had a positive correlation with N and Dukes stage of RC, and might have good sensitivity and specificity in N and Dukes staging. Moreover, the microRNAs in blood had acceptable diagnostic accuracy (Zhou et al., 2013), which provides important evidence for the further development of noninvasive method for diagnosing and staging CRC, even determining susceptibility to CRC through detecting single nucleotide polymorphisms (SNPs) on host microRNAs (Du et al., 2014).

Although CEA is a classic biochemical tumor marker, which is often used for early detection and progression monitoring in many malignant tumors, it showed no statistical relation with N and Dukes stages of RC in our study. Besides CEA, imaging examination is another routine test for early diagnosis and tumor stage prognosis in RC. But there are naturally limitations about evaluation of lymph node involvement. Firstly, imaging examination is not an 100% objective assessment for lymph node involvement. It can be influenced by radiologist's clinical experience and ability. Secondly, enlarged nodes may be benign and reactive, whereas small nodes may be infiltrated. For rectal cancer in particular, over half of the metastatic nodes are less than 5 mm, which result in difficulty to evaluate lymph node involvement (Dworak, 1989; Mönig et al., 1999). There is a wide variation in accuracy for metastatic nodal detection with CT (22–73%) and MRI (39–75%) (Karantanas et al., 2007). However, in our study, miR155 seemed to have acceptable accuracy (85%) for predicting tumor N stage, which might potentially improve the diagnostic capability of imaging staging.

Although our results are promising, there are still some limitations in this study. Firstly, the sample size of RC is relatively small, a larger size of samples is necessary; Secondly, our current research did not evaluate the health benefit and economic impact of detecting miR155 in clinical practices, and its relationship with recurrence and mortality rate. Our follow-up study will look into these issues.

In conclusion, our data demonstrate that miR155 appears to be a potential novel predictor for RC tumor staging. It could serve as the basis for further investigation, preferably in larger sample size and prospective studies.

Acknowledgements

We thank all colleagues of the Colorectal Group for clinical suggestions and Dr. Zhaohui Yang from University of Pennsylvania for reading and commenting the manuscript. Sichuan Science and Technology Agency funded this program (2011FZ0131) for scientific research.

References

Asangani I, Rasheed S, Nikolova D, et al (2008). MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. *Oncogene*, **27**, 2128-36.

Chiang Y, Song Y, Wang Z, et al (2012). MicroRNA-192, -194 and -215 are frequently downregulated in colorectal cancer. *Exp Ther Med*, **3**, 560-6.

Du W, Ma X L, Zhao C, et al (2014). Associations of single nucleotide polymorphisms in miR-146a, miR-196a, miR-149 and miR-499 with colorectal cancer susceptibility. *Asian Pac J Cancer Prev*, **15**, 1047-55.

Dworak O (1989). Number and size of lymph nodes and node metastases in rectal carcinomas. *Surg Endosc*, **3**, 96-9.

Esquela-Kerscher A, Slack FJ (2006). Oncomirs—microRNAs with a role in cancer. *Nat Rev Cancer*, **6**, 259-69.

Figueredo A, Coombes ME, Mukherjee S (2008). Adjuvant therapy for completely resected stage II colon cancer. *Cochrane Database Syst Rev*, **3**.

Gebeshuber CA, Zatloukal K, Martinez J (2009). MiR29a suppresses tristetraprolin, which is a regulator of epithelial polarity and metastasis. *EMBO Rep*, **10**, 400-5.

Gironella M, Seux M, Xie MJ, et al (2007). Tumor protein 53-induced nuclear protein 1 expression is repressed by miR-155, and its restoration inhibits pancreatic tumor development. *Proc Natl Acad Sci USA*, **104**, 16170-5.

Hashimoto Y, Akiyama Y, Yuasa Y (2013). Multiple-to-multiple relationships between microRNAs and target genes in gastric cancer. *PloS one*, **8**, e62589.

He L, Hannon GJ (2004). MicroRNAs: small RNAs with a big role in gene regulation. *Nat Rev Genet*, **5**, 522-31.

Iwaya T, Yokobori T, Nishida N, et al (2012). Downregulation of miR-144 is associated with colorectal cancer progression via activation of mTOR signaling pathway. *Carcinogenesis*, **33**, 2391-7.

Karantanas A, Yarmenitis S, Papanikolaou N, et al (2007). Preoperative imaging staging of rectal cancer. *Dig Dis*, **25**, 20-32.

Lai EC (2002). MicroRNAs are complementary to 3' UTR sequence motifs that mediate negative post-transcriptional regulation. *Nat Genet*, **30**, 363-4.

Mönig SP, Baldus SE, Zirbes TK, et al (1999). Lymph node size and metastatic infiltration in colon cancer. *Ann Surg Oncol*, **6**, 579-81.

Nishiumi S, Kobayashi T, Ikeda A, et al (2012). A novel serum metabolomics-based diagnostic approach for colorectal cancer. *PloS one*, **7**, e40459.

Schetter AJ, Leung SY, Sohn JJ, et al (2008). MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. *JAMA*, **299**, 425-36.

Schwitalla S, Ziegler PK, Horst D, et al (2013). Loss of p53 in enterocytes generates an inflammatory microenvironment enabling invasion and lymph node metastasis of carcinogen-induced colorectal tumors. *Cancer Cell*, **23**, 93-106

Shigoka M, Tsuchida A, Matsudo T, et al (2010). Deregulation of miR92a expression is implicated in hepatocellular carcinoma development. *Pathol Int*, **60**, 351-7.

Slaby O, Svoboda M, Fabian P, et al (2008). Altered expression of miR-21, miR-31, miR-143 and miR-145 is related to clinicopathologic features of colorectal cancer. *Oncology*, **72**, 397-402.

Song WF, Wang L, Huang WY, et al (2013). MiR-21 Upregulation induced by promoter zone histone acetylation is associated with chemoresistance to gemcitabine and enhanced malignancy of pancreatic cancer cells. *Asian Pac J Cancer Prev*, **14**, 7529-36

Tomasini R, Azizi Samir A, Pebusque MJ, et al (2002). P53-dependent expression of the stress-induced protein (SIP). *Eur J Cell Biol*, **81**, 294-301.

Tomasini R, Samir AA, Carrier A, et al (2003). TP53INP1s and homeodomain-interacting protein kinase-2 (HIPK2) are partners in regulating p53 activity. *J Biol Chem*, **278**, 37722-9.

Vandesompele J, De Preter K, Pattyn F, et al (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol*, **3**, research0034.

Wang LG, Gu J (2012). Serum microRNA-29a is a promising novel marker for early detection of colorectal liver metastasis. *Cancer Epidemiol*, **36**, e61-7.

Wang Q, Zhu J, Zhang Y, et al (2013). Down-regulation of programmed cell death 4 leads to epithelial to mesenchymal transition and promotes metastasis in mice. *Eur J Cancer*, **49**, 1761-70

Xu TP, Zhu CH, Zhang J, et al (2013). MicroRNA-155 expression has prognostic value in patients with non-small cell lung cancer and digestive system carcinomas. *Asian Pac J Cancer Prev*, **14**, 7085-90.

Yan LX, Huang XF, Shao Q, et al (2008). MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. *RNA*, **14**, 2348-60.

Zhou X J, Dong Z G, Yang Y M, et al (2013). Limited diagnostic value of microRNAs for detecting colorectal cancer: a meta-analysis. *Asian Pac J Cancer Prev*, **14**, 4699-704.