Identification of aberrantly expressed miRNAs in rectal cancer

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Abstract. Disturbance of miRNA expression may play a key role in the initiation and progression of colorectal cancer (CRC). CRC should be viewed as a heterogeneous disease, but previous studies have only screened dysregulated miRNAs in CRC from a panel of 96, 145, 287 and 455 miRNAs, respectively. It is necessary to identify new aberrantly expressed miRNAs in rectal cancer. In this study tissue samples were derived from patients undergoing a surgical procedure to remove a portion of cancers. The expression profile of 904 miRNAs was analyzed using a miRCURY™ LNA Array from 6-paired rectal cancers and normal tissues. The expression levels of 4 miRNAs were compared by real-time PCR between colon and rectal cancer, and also the expression levels of metastatic miRNAs in different stages of rectal cancer were analyzed. We found that 67 miRNA precursors are upregulated in rectal cancer ($P<0.05$) and 21 of those have never been reported in colorectal cancer (CRC); 39 miRNA precursors are downregulated ($P<0.05$) and 24 novel dysregulated miRNAs were identified in rectal cancer. miR-31, miR-126 and miR-143 are differentially expressed between colon cancer and rectal cancer. Here, we report an miRNA profile of rectal cancer, and we identified differential expression patterns of miRNAs between rectal and colon cancers. This novel information may suggest the potential roles of these miRNAs in the diagnosis of rectal cancer.

Introduction

Colorectal cancer (CRC) is a frequent cause of cancer-related death in China. Screening for CRC allows early stage diagnosis of the malignancy and potentially reduces mortality of the disease. A growing number of direct and indirect evidence suggests the correlation between altered miRNA expression and cancers. miRNAs are important factors in tumorigenesis and have been the subject of research in many types of cancers. miRNAs are small 19 to 22 nucleotide sequences of RNA that participate in the regulation of cell differentiation, cell cycle progression, and apoptosis. miRNAs are believed to function primarily as negative regulators of gene expression following binding to conserved sequences within the 3' untranslated region of target mRNAs. While the biological roles of miRNA are under intense investigation, they are believed to define and maintain cellular fate in a manner similar to transcription factors, alterations in miRNA expression may be an important contributor to the development of cancers. miRNAs are also abnormally downregulated or upregulated in colon cancer tissue. Screening aberrantly expressed miRNAs may provide a basis for understanding the function of miRNAs in cancer (1).

Dysregulated miRNA screening in CRC by gene chip analysis or real-time RT-PCR has been reported. Bandrés and colleagues (2) firstly identified a group of 13 miRNAs whose expressions are significantly altered in colorectal cancer, and the most significantly deregulated miRNA being miR-31, miR-96, miR-133b, miR-135b, miR-145 and miR-183. Ng and colleagues (3) also used real-time PCR technique to analyze 95 miRNAs in plasma and tissue samples of CRC patients, and found miR-17-3p and miR-92 were significantly elevated in patients with CRC. Arndt and colleagues (4) used 287 miRNA gene chips to analyze the miRNA expression profile in CRC and CRC cell lines. Thirty-seven miRNAs were identified to have different expression between CRC and normal tissue. Furthermore, several of these miRNAs were associated with CRC tumor progression including loss of miR-133a and gain of miR-224. Motoyama and colleagues (5) used a miRNA microarray containing 455 human miRNA probes to identify aberrant expression miRNAs between surgically resected colorectal cancer and non-cancerous tissues. They found 21 miRNAs overexpressed in colorectal cancer tissues, and among them, the expression of miR-31, miR-183, miR-17-5p, miR-18a, miR-20a and miR-92 were confirmed to be significantly higher in cancer tissues than in normal tissues. The expression of miR-143 and miR-145 in cancer tissues were significantly

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lower than in normal tissues. It has been estimated that there are about 1,000 miRNAs in human, but previous studies have only screened miRNAs expression in CRC patients from less than 500 miRNAs. It is necessary to screen CRC with larger collections of miRNAs by gene chip.

Accumulating evidence suggests that colorectal cancer should be viewed as a heterogeneous disease, with proximal and distal CRC showing multiple biological and clinical differences. There is a significant difference in regional expression of 10 tumor-associated markers (CDX2, CD44v6, CD44s, TOPK, nuclear β-catenin, pERK, APAF-1, E-cadherin, p21 and Bcl2) and 4 immune response markers (CD68, CD163, FoxP3 and TIA-1). Tumor diameter, pT stage and MSI status are used to distinguish right-sided colon cancer from rectal cancer, and pT stage and E-cadherin are used to discriminate left-sided colon cancer and rectal cancer (6). Kim and colleauges (7) also demonstrated that distal sporadic MSI-high CRC formed a distinct subgroup with distinguished clinicopathological and molecular features from proximal MSI-high CRC. These data along with existing evidence for the presence of distinct regional embryological origin and gene expression profile are highly supportive of the concept that proximal and distal CRC are distinct clinicopathological entities. Unfortunately, previously studies work with the total concept of 'CRC' and did not screen dysregulated miRNAs of specific subtype of CRC, and the aberrant miRNAs were identified from tissues of different stages of CRC. Therefore, some CRC progression-related miRNAs may have been neglected in the chip analysis.

To resolve the issues, we have used 904 miRNA gene chips which include nearly all human miRNAs and analysed new aberrantly expressed miRNAs between rectal cancer and non-tumour tissues. We have also assessed miRNA expression levels between colon cancer and rectal cancer.

Materials and methods

Tissue procurement. The tissue samples analyzed in this study were derived from patients undergoing a surgical procedure to remove a portion of rectal cancer at the Xiangya Hospital, Central South University. The collection of samples conformed to the policies of China and practices of the facility of the Institutional Review Board. Upon removal of the surgical specimen, research personnel immediately transported the tissue to the Surgical Pathology Lab. Pathology faculty performed a gross analysis of the specimen and selected cancerous appearing rectal tissue and normal appearing rectal tissue for research. Each sample was placed in a cryovial and flash-frozen in liquid nitrogen until analysis. Subsequent pathological analysis by the institutes providing the surgical specimens confirmed the histopathology of the samples taken for research. Histological slides were prepared from the section of the frozen tissue directly adjacent to tissue from which RNA was isolated. These slides were examined by one of us to determine if the benign tissues contained any rectal tumor cells. Benign tissue that contained residual tumor was not included in the study.

Total RNA isolation and quality analysis. Frozen tissues (~10 mg) were first pulverized in a stainless steel mortar and pestle. Total RNA was isolated from the cell lines or tissues in 1 ml of TRIzol (Invitrogen, Carslbad, CA) and used RNeasy mini kit (Qiagen) to purify RNA according to the manufacturer's instructions. RNA concentration was determined by analyzing 1 μl of solution using the ND-1000 micro-spectrophotometer (NanoDrop Technologies, Wilmington, DE). RNA integrity was evaluated using the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). RNA integrity number (RIN) was determined using the RIN algorithm of the Agilent 2100 Expert Software (8).

miRNA precursor expression profiling. RNA (30 ng) of 6-paired samples were labeled with Hy5™ or Hy3™ by miRCURY™ Array Power Labeling kit (Exiqon). The two samples from the Hy5™ and Hy3™ labeling reactions are combined and hybridized with miRCURY™ Array LNA miRNA chip (v.14.0) on a hybridization station (Exiqon). After hybridization, scanning is performed with the Axon GenePix 4000B microarray scanner. GenePix pro V6.0 is used to read the raw intensity of the image. The ratio of red signal to green signal was calculated after background subtraction and normalization using the global Lowess (Locally Weighted Scatterplot Smoothing) regression algorithm (MIDAS, TIGR Microarray Data Analysis System), which produced the best within-slide normalization to minimize the intensity-dependent differences between the dyes. Replicated spots on the same slide have been averaged by getting a median ratio of replicated spots. Between slides normalization was performed by scale normalization to reduce between-slide variability (9). The statistical significance of differentially expressed miRNA was analyzed by fold change and t-test.

miRNA quantification by real-time RT-PCR. SYBR-Green qRT-PCR assay was used for miRNA quantification. In brief, 40 ng of total RNA containing miRNA was polyadenylated by poly(A) polymerase and reverse transcribed to cDNA using miScript Reverse Transcription kit according to the manufacturer's instructions (Qiagen, Valencia, CA, USA). Real-time qPCR was performed using miScript SYBR-Green PCR kit with the manufacturer provided miScript Universal primer (Qiagen) and the miRNA-specific forward primers in ABI PRISM 7900 Real-time PCR system (Applied Biosystems). Each reaction was performed in a final volume of 10 μl containing 2 μl of cDNA, 0.5 mM of each primer and 1X SYBR-Green PCR Master mix (Qiagen). The amplification program was: denaturation at 95˚C for 10 min, followed by 45 cycles of 94˚C for 15 sec, 55˚C for 30 sec and 70˚C for 30 sec, in which fluorescence was acquired. At the end of the PCR cycles, melting curve analyses were performed as well as electrophoresis of the products on 3.5% agarose gels in order to validate the specific generation of the expected PCR product. Each sample was run in triplicates for analysis. The expression levels of miRNAs were normalized to RNU6B. Relative gene expression was calculated as $2^{-(CTmiRNA - CTRNU6B RNA)}$. Except reference PCR primers U6B (5'-CGC TTC ACG AAT TTG CGT GTC AT-3'), all miRNA PCR assay kits were purchased from Qiagen Co. miR-29b (MPH01245A-200), miR-31(MPH00155A-200),miR-1259(MPH01747A-200),miR-886-5p (MPH00447A-200), miR-886-3p (MPH01372A-200), miR-126 (MPH01161A-200),miR-145 (MPH00046A-200),

Table I. The upregulated expression of miRNAs in the rectal cancer.

Gene name	Fold
hsa-miR-135b	**28.8**
hsa-miR-886-5p	26.6
hsa-miR-374a	**21.3**
hsa-miR-1259	20.2
hsa-miR-31	**18.2**
hsa-miR-20a	**16.4**
hsa-miR-32	**12.8**
hsa-miRPlus-E1016	10.9
hsa-miR-196b*	**10.6**
hsa-miR-196b	**9.4**
hsa-miR-96	**9.1**
hsa-miR-190	8.1
hsa-miRPlus-A1087	7.6
hsa-miR-182	**7.0**
hsa-miR-17	**7.0**
hsa-miR-20a*	**6.9**
hsa-miR-126*	**6.4**
hsa-miR-21	**6.2**
hsa-miR-362-3p	5.9
hsa-miR-335	5.7
hsa-miR-552	3.3
hsa-let-7f	**3.1**
hsa-miR-301a	**3.1**
hsa-miR-491-3p	3.0
hsa-miR-140-5p	3.0
hsa-miR-181d	**3.0**
hsa-miR-16	**2.9**
hsa-miR-203	**2.9**
hsa-miR-126	**2.8**
hsa-miR-142-3p	**2.8**
hsa-miR-374b	**2.8**
hsa-miR-29b	**2.7**
hsa-miR-141*	**2.6**
hsa-miR-1274a	2.6
hsa-miR-10a	**2.5**
hsa-miR-33a	**2.5**
hsa-miR-93	**2.4**
hsa-miR-98	2.4
hsa-miR-29a*	**2.3**
hsa-miR-452	2.3
hsa-miR-886-3p	5.5
hsa-miR-27a	5.3
hsa-miR-18a	**5.1**
hsa-miR-1308	5.0
hsa-miR-223	**4.9**
hsa-miR-9*	**4.9**
hsa-mir-106a	**4.8**
hsa-miR-18b	**4.4**
hsa-miR-421	4.4
hsa-miR-19a	**3.9**
hsa-miR-424	**3.8**
hsa-miR-1201	3.7
hsa-miR-7	**3.6**
hsa-miR-183	**3.5**
hsa-miR-492	**2.3**
hsa-miR-15a	**2.2**
hsa-miR-106b*	**2.2**
hsa-miR-200a*	**2.1**
hsa-miR-339-5p	2.1
hsa-miR-24-2*	**2.1**
hsa-miR-148a	2.0
hsa-miR-15b	**2.0**
hsa-miR-17*	**2.0**
hsa-miR-92a	**2.0**
hsa-miR-25	**2.0**
hsa-miR-10a*	**2.0**
hsa-miR-30c-1*	**2.0**

P<0.05 and miRNAs in bold have been reported in previous publications of colorectal cancer. The threshold value used to screen upregulated miRNAs is fold change ≥2.00 and P<0.05 (t-test).

miR-125b (MPH00023A-200), miR-133b (MPH00033A-200), miR-143 (MPH01177A-200), miR-375 (MPH00191A-200), miR-378 (MPH01284A-200), miR-381 (MPH00200A-200) (Qiagen).

Hierarchical clustering analysis. Prior to hierarchical clustering, miRNA profiles were standardized to have mean zero and standard deviation one. Clustering was performed with average linkage and Pearson's correlation. In brief, the object of this algorithm is to compute a dendrogram that assembles all elements into a single tree. For any set of *n* miRNA genes, an upper-diagonal similarity matrix is computed by using the metric described above, which contains similarity scores for all pairs of genes. The matrix is scanned to identify the highest value (representing the most similar pair of genes). A node is created joining these two genes, and a gene expression profile is computed for the node by averaging observation for the joined elements (missing values are omitted and the two joined elements are weighted by the number of genes they contain).

Statistical analysis. Results of experiments are expressed as mean ± SD. Student's unpaired t-test was used to compare values of test and control samples. P<0.05 indicated significant difference.

Results

miRNA expression profile in rectal cancer tissues. We used miRCURY™ Array LNA miRNA chip (v.14.0) to evaluate miRNA expression profiles between rectal cancer tissues and adjacent non-tumours tissues. All 6 paired rectal cancers were pathologically classified in TNM category of stage III

Table II. The downregulated expression of miRNAs in the rectal cancer.

Gene name	Fold
hsa-miRPlus-E1067	0.13
hsa-miR-1973	0.14
hsa-miR-375	0.21
hsa-miR-147b	**0.24**
hsa-miR-378/hsa-miR-378c	**0.25**
hsa-miR-7-2*	**0.30**
hsa-miR-513a-5p	0.31
hsa-miRPlus-F1027	0.32
hsa-miR-640	0.32
hsa-miR-381	0.32
hsa-miR-133b	**0.33**
hsa-miR-186*	0.35
h**sa-miR-29c**	**0.35**
hsa-miRPlus-E1033	0.36
hsa-miRPlus-E1238	0.36
hsa-miR-494	0.36
hsa-miRPlus-E1173	0.37
hsa-miR-125b	**0.37**
hsa-miRPlus-E1285	0.38
hsa-miR-145	**0.40**
hsa-let-7b	0.42
hsa-miR-139-5p	**0.42**
hsa-miRPlus-F1026	0.42
hsa-miRPlus-E1101	0.43
hsa-miRPlus-F1155	0.43
hsa-miR-1184	0.46
hsa-let-7d*	0.46
hsa-miR-155	**0.46**
hsa-miRPlus-E1172	0.47
hsa-miR-320a	**0.47**
hsa-miR-150	0.48
hsa-miR-518e*/hsa-miR-519a* /hsa-miR-519b-5p/ hsa-miR-519c-5p/hsa-miR-522* /hsa-miR-523*	0.49
hsa-miRPlus-F1215	0.49
hsa-miR-320b	**0.50**
hsa-miR-320d	**0.50**
hsa-miRPlus-C1110	0.38
hsa-miRPlus-E1192	0.39
hsa-miR-125a-5p	**0.50**
hsa-miR-320c	**0.50**

P<0.05 and miRNAs in bold have been reported in previous publications of colorectal cancer. The threshold value used to screen downregulated miRNAs is fold change ≥2.00 and P<0.05 (t-test).

and stage IV or Dukes' C and D. When setting average change ≥2-fold and P<0.05 as a cut-off level, 131 miRNA precursors were found aberrantly expressed in rectal cancer (Tables I and II), in which 67 miRNAs are upregulated and 39 miRNAs are downregulated. Comparing with previous literature of miRNAs and CRC, we found that 46 genes have been reported in CRC among 67 upregulated genes, including miR-200a, miR-92a, miR-96 and miR-492. New upregulated genes include recently named genes such as: miR-1201, miR-1247, miR-1259, miR-1308, miR-1298 and a number of hsa-miRPlus genes (for example: miRPlus-A1087, miRPlus-C1070, miRPlus-E1012, miRPlus-E1016, miRPlus-E1029, miRPlus-E1038 and miRPlus-F1104). Among 39 downregulated genes, 15 genes have been reported, such as: miR-125a-5p, miR-125b, miR-133b, miR-145, miR-378 miR-7-2*, and so on. The newly identified downregulated genes including recently named miR-1184, miR-523*, miR-640 and miR-513a-5p. Notably 13 miRPlus genes downregulated in rectal cancer tissues (miRPlus-F1027, miRPlus-E1269, miRPlus-E1033, miRPlus-E1238, miRPlus-E1173, miRPlus-E1285, miRPlus-C1110, miRPlus-E1192, miRPlus-F1026, miRPlus-E1101, miRPlus-F1155, miRPlus-E1172, miRPlus-F1215) (Tables I and II). Overall our study has identified 42 newly upregulated and 24 downregulated miRNAs in rectal cancer.

Comparison of miRNA expression level between RT-qPCR and chip analysis. The RT-qPCR (real-time quantitative PCR) system detects PCR products as they accumulate rather than measures final product concentrations after a fixed number of cycles. The iCycler sequence detection system (Bio-Rad Laboratories, Hercules, CA) provides a way to monitor in real-time the accumulation of DNA synthesized during the PCR process. It excites fluorescence of the selected dye and images the emitted light during each thermal cycle of the PCR run. To compare miRNA expression level between RT-qPCR and chip analysis, miR-31, miR-126, miR-29, miR-886-5p, miR-1259, miR-886-3p, miR-145, miR-381, miR-375, miR-378, miR-133b and miR-125b were selected and they are among the top 20 differentially expressed miRNAs by chip analysis (Tables I and II). We have analyzed the expression level of these miRNAs by RT-qPCR in 27-paired samples of rectal cancer, and found that miRNA expression in the paired benign and tumor tissue is consistently increased or decreased in all cases. We found that all 6 upregulated miRNAs in chip analysis have increased expression level in rectal cancer tissues, and 6 downregulated miRNAs also have decreased expression in normal tissues (Fig. 1). Expression levels of 6 upregulated miRNAs are lower than those in chip analysis, range from 11.88 to 39.09%, respectively. On the other hand, expression levels of 6 miRNAs are higher than those in chip analysis, range from 1.35 to 29.35%. The correlation of relative miRNAs expression levels was analyzed by cDNA array (array-fold) vs. RT-qPCR (qPCR-fold). Pearson correlation of downregulated miRNAs was 0.923 (P<0.01) and Pearson correlation of upregulated miRNAs was 0.707 (P<0.05). Pearson correlation of both down- and upregulated miRNAs was 0.958 (P<0.01) (Fig. 2C-E). This suggests that new aberrantly expressed miRNAs identified by gene chips from rectal cancer and normal tissues can be confirmed by real-time PCR.

Clustering analysis of the significantly changed genes. For clustering analysis, we set P-value <0.05 as a cut-off level.

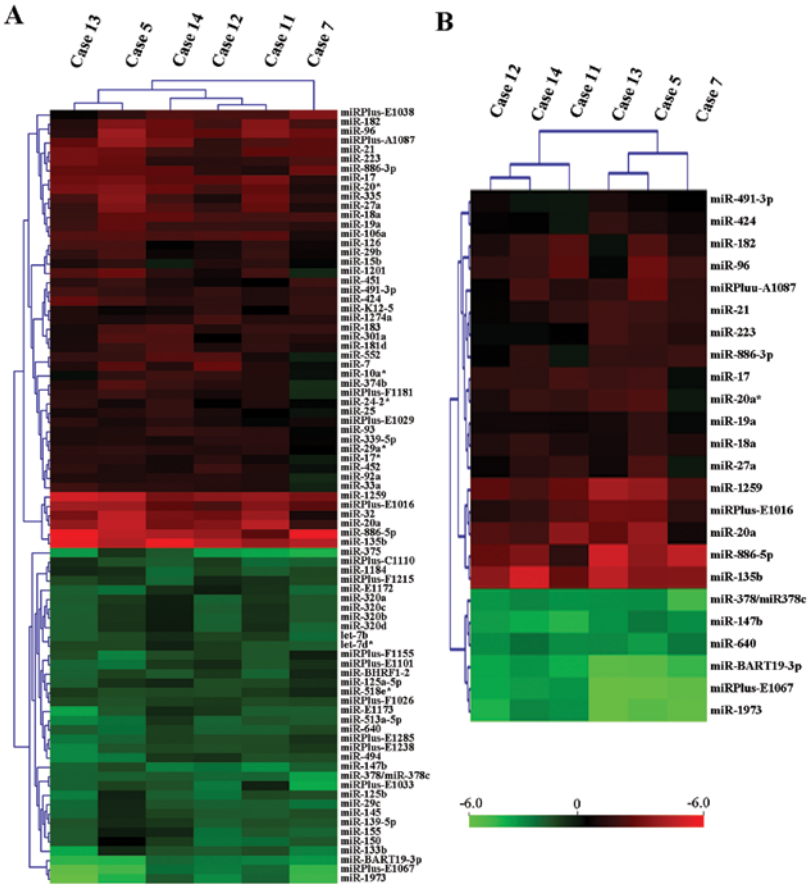

Figure 1. Hierachical cluster analysis of differentially expressed miRNAs. (A) Comparing with normal tissues, alteration of miRNA expression level is ≥2-fold (P<0.05). (B) Comparing with normal tissues, alteration of miRNA expression level is ≥2-fold (P<0.01). The heat-map shows accurate classification of rectal cancer in one subcluster and the normal samples in the other. Row, differentially expressed miRNAs; columns, tissue samples. Case 5, stage IV; case 7, stage III; case 11, stage IV; case 12, stage III; case 13, stage IV; case 14, stage IV.

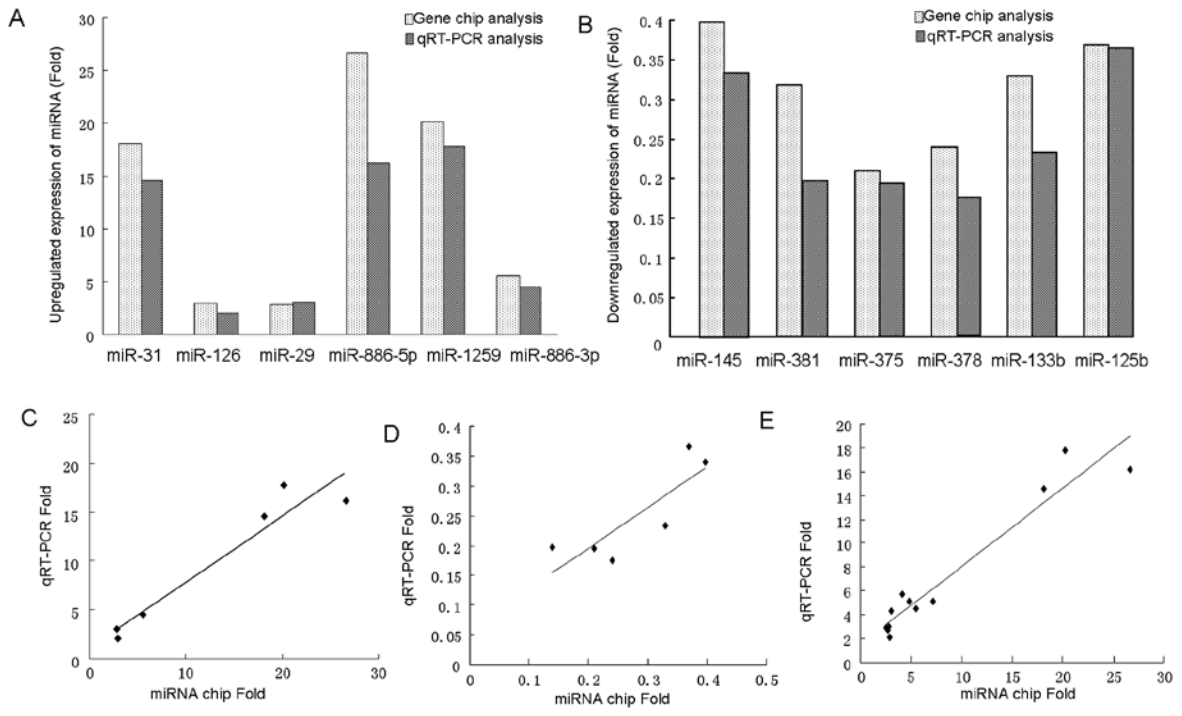

Figure 2. Comparison of miRNA expression level between chip and qRT-PCR. (A) Comparing upregulated miRNA expression levels between chip and qRT-PCR in paired rectal cancer. (B) Comparing downregulated miRNA expression levels between chip and qRT-PCR in paired rectal cancer. (C) Correlation of chip vs. qPCR data for upregulated miRNAs. (D) Correlation of chip vs. qPCR data for downregulated miRNAs. (E) Regression plot of increased or decreased fold change of miRNAs.

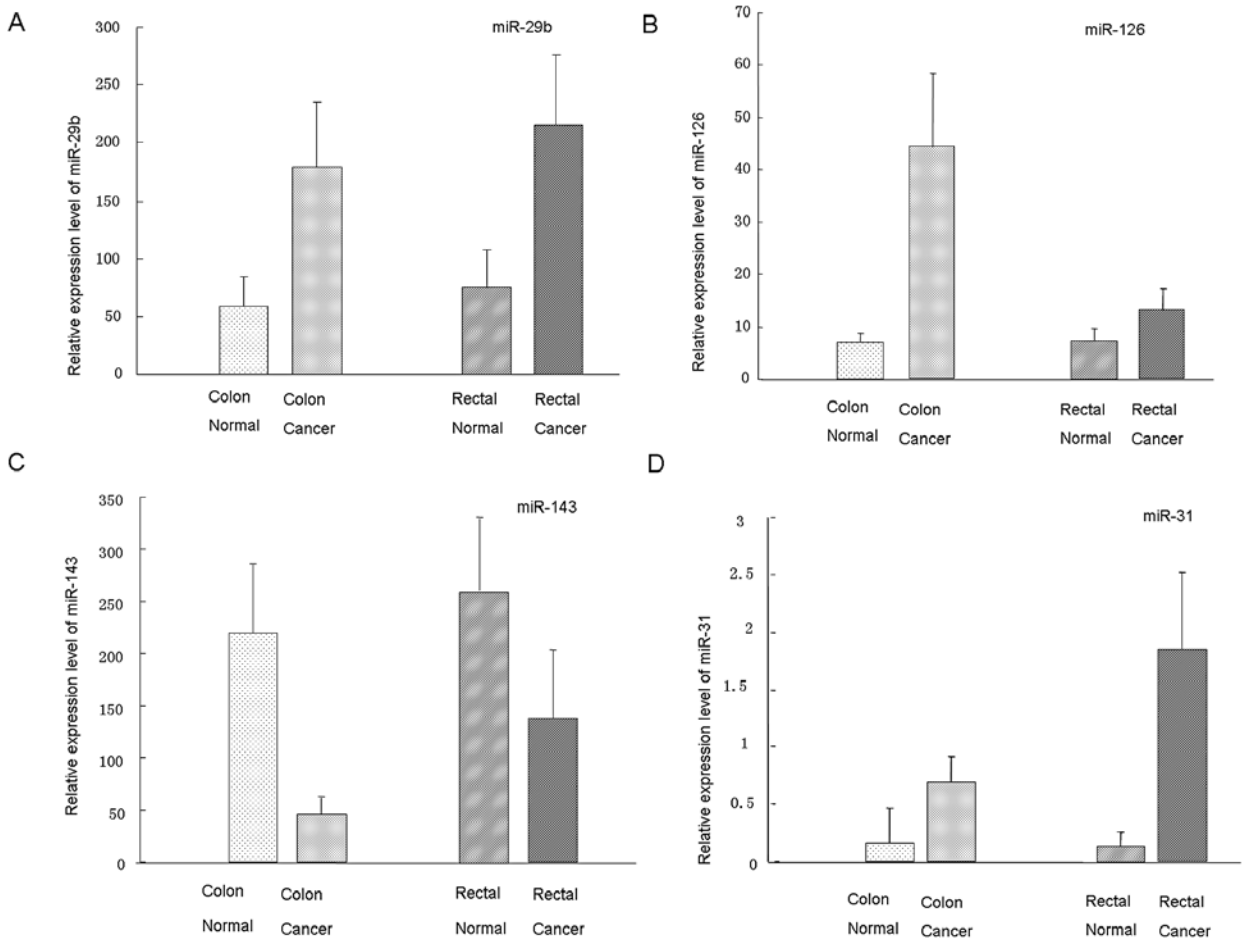

Figure 3. Different expression patterns between colon and rectal cancer. The numbers of stage I, II, III and IV are 21, 28, 22 and 18 cases respectively. (A) Real-time PCR expression of miR-29bR in paired colon and rectal cancer. (B) Real-time PCR expression of miR-126 in paired colon and rectal cancer. (C) Real-time PCR expression of miR-143 in paired colon and rectal cancer. (D) Real-time PCR expression of miR-31 in paired colon and rectal cancer.

Expression levels of upregulated genes and downregulated genes are analyzed by unsupervised hierarchical clustering. Our data showed that most of miRNAs showed very similar expression patterns and only miR-552, miR-374b, miR-17*, miR-33 and miR-92b expressed differently in 6 samples (Fig. 2A). We strictly selected those miRNA changes in each slide and $P<0.01$. Expression level of 18 upregulated genes and 5 dowregulated genes are analyzed by unsupervised hierarchical clustering. The heatmap demonstrated that most of miRNAs expressed similarly in 6 paired tissues of rectal cancer, and samples from stage IV group to the same subcluster, such as: case 5 and case 13 (Fig. 2B).

Different expression patterns of miRNAs between colon cancer and rectal cancer. Colorectal cancer should be viewed as a heterogeneous disease. To test if there is different expression pattern of miRNAs between colon cancer and rectal cancer, we selected 4 aberrant miRNAs of rectal cancer and analysed the expression level from 24-paired colon cancer and 89-paried rectal cancer. There were no statistically significant differences in gender, age and stages between thr groups. Comparing with paired normal tissues, the expression level of miR-29b in colon cancer is increased 3.03-fold. Similarly, it is increased 2.93-fold in rectal cancer (Fig. 3A). Expression of miR-126 is increased both in colon cancer and rectal cancer and $P<0.01$, but the increase is 6.34-fold in colon cancer and only 2.09-fold in rectal cancer, and there are significant differences between the subtype cancers ($P<0.01$) (Fig. 3B). miR-143 is downregulated in multiple cancers and it decreases 4.82-fold in colon cancer ($P<0.01$), but it does not change in rectal cancer ($P>0.05$) (Fig. 3C). miR-31 is increased 4.06-fold in colon cancer and it increase 13.02 in rectal cancer, and a significant difference exists between the subtype cancers ($P<0.01$) (Fig. 3D). Altogether, the data demonstrated that miRNAs have different expression patterns in the two subtype cancers and may have different pathological progress.

Discussion

Colorectal cancer should be viewed as a heterogeneous disease, with proximal and distal colorectal cancer showing multiple biological and clinical differences. Herein, we report the first comprehensive miRNA expression profiling in rectal cancer. In this study, we identified 106 miRNAs that are aberrantly expressed in rectal cancer and 45 miRNAs are newly found aberrant expression in rectal cancer. Comparing with mixed colorectal cancer, we identified more dysregulated expression miRNAs than any other reports, and it suggests that it is easier

to detect new aberrant miRNAs using subtype and similar stage cancers. Using the method, our data showed higher alteration of miRNAs expression levels (comparing with strictly paired cancer samples). Usually we set miRNA expression change at 2-fold as a cut-off, and this may neglect aberrant miRNAs in the chip analysis. We group subtype cancers to analyze the different expressions and resolve part of the issue.

Some of the differentially expressed miRNAs in rectal cancer were aberrantly expressed in other cancers. These include miR-155, which was increased in the present study and in diffuse large B-cell lymphoma and lymphoma (10,11). miR-21 was increased here and in glioblastomas (12), pancreatic cancer (13) and prostate cancer (14). miRNAs differentially expressed in other cancers were not deregulated to the same degree in rectal cancer. Expression of miR-17 and miR-19a was increased in the study and lymphoma (15), but was not significantly altered in lung cancer (16). Especially we compared aberrant miRNAs in rectal cancer and those in colorectal cancer, and half of upregulated miRNAs (46) also increase in colorectal cancer, such as: miR-374a, miR-20a, miR-32, miR-196b, miR-96 and miR-182. Near half of downregulated miRNAs (15) in rectal cancer also decrease in colorectal cancer. It suggests that miRNA expression pattern of rectal cancer are more like colon cancer than any other cancers. We report deregulation of a number of miRNAs in rectal cancer such as miR-886-5p, miR-1259 and miR-886-3p that have not been reported in any other cancers to our knowledge. Those new aberrant miRNA are highly expressed in rectal cancer and may regulate scores of mRNAs, and the impact on gene expression in rectal cancer may be profound. Thus, our results increase understanding of miRNAs in the carcinogenesis of colorectal cancer.

Colorectal cancer is used as one catalogue and is not suitable to explore the mechanism of carcinogenesis of rectal cancer. miRNA expression pattern of rectal cancer is like that of colorectal cancer, but some miRNAs showed different expression between them. Most of miRNAs in rectal cancer may be dysregulated as those in colorectal cancer, but some miRNA expression levels may show huge difference or even on the contrary, it is possible to identify relatively aberrant miRNAs in rectal cancer. miRNAs expressing different patterns of subtype cancers will increase our understanding of pathogenesis of colorectal cancer.

A couple of miRNAs were identified as invasion and metastasis-related genes (17). miRNA-31 functions as an oncogenic miRNA in mouse and human lung cancer cells. Engineered knockdown of miR-31 repressed lung cancer cell growth and tumorigenicity in a dose-dependent manner. miR-31 acts as an oncogenic miRNA in lung cancer by targeting tumor suppressor LATS2 and PPP2R2A for repression (18). On the contrary, miR-31 overexpression is associated with better prognosis in tumors from patients with advanced stage serous ovarian cancer (19). It seems the same miRNA is playing different roles in the various types of tumors. Our data showed miRNA-31 expression level may be related with different pathological stage of rectal cancer and the data resemble a recently report in colorectal cancer (20). Recent studies indicate that miR-145 is a tumor suppressor capable of inhibiting tumor cell growth both *in vitro* and *in vivo*. miR-145 is significantly downregulated in prostate cancer (21). It inhibits not only tumor growth but also cell invasion and metastasis in gastric cancer cells (22). We found downregulated miR-145 is related with stages of rectal cancer (data not shown), but Wang and colleagues (20) showed miR-145 expression was related to colorectal cancer site, and there was no relationship of miR-145 expression with other clinicopathological features. The difference may lies in different subtype of tumors and in fact they also found miR-145 expression was related to cancer location.

The molecular mechanisms responsible for the deregulated expression of miRNAs in CRC are poorly understood (23). Findings from this study further revealed that miR-18a, miR-135b, miR-21, miR-143 and miR-145 are overexpressed in rectal cancer. miR-18a functions as a tumor suppressor miRNA by targeting the K-RAS oncogene (24). miR-143 was also shown to inhibit translation of K-RAS (25). miR-135a directly targets the 3'-untranslated region of the APC gene, suppress its expression and activate Wnt signaling (26). On the contrary, APC regulates expression of the suppressor gene miR-122a and significantly downregulates miR-122a expression in gastrointestinal cell lines and tissues (27). Inactivation of APC is considered a gatekeeper event for the initiation of CRC. It suggests a miRNA-mediated mechanism for control of the APC gene and the activation of the Wnt signaling pathway in rectal cancer. Collectively, there is a growing appreciation for the role of miRNAs in CRC or rectal cancer. Our data may provide diagnostic biomarkers for rectal cancer and offer new clues to study the molecular mechanism of carcinogenesis of rectal cancer.

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