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A microRNA expression signature as a predictor of survival for colon adenocarcinoma

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Colon cancer is a major cause of cancer mortality worldwide and most colon cancers are adenocarcinoma. MicroRNA (miRNA) expression signature has been shown to be able to predict progression and prognosis of various cancers. The aim of our study was to explore a novel signature of microRNA expression for predicting survival of colon adenocarcinoma patients. By analyzing the miRNA expression profiles and clinical information of 329 colon adenocarcinoma patients derived from The Cancer Genome Atlas database. 129 miRNAs were identified to be expressed differentially between the cancer and adjacent tissues. Among them, 27 miRNAs were found to be associated with the corresponding clinical characteristics of the patients. Furthermore, 7 miRNAs (let-7a-2, mir-32, mir-181a-1, mir-197, mir-328, mir-505 and mir-652) were found to be significantly correlated with the patient survival. The risk established by the 7-miRNA signature we built was proved be an independent prognostic factor (Hazard ratio [HR] = 2.048; 95% CI = 1.144-3.664; p, 0.016). In summary, our study identified miRNAs correlated with progression and prognosis of colon adenocarcinoma and built a 7-microRNA expression signature for prediction of the survival of the patients with colon adenocarcinoma.

Key words: colon cancer, biomarker, microRNA, survival

Colon cancer is a common gastrointestinal cancer with a significant threat to the patient health and the third leading cause of cancer mortality in the United States [1]. Prognosis for patients with colon cancer has significantly improved in past decades with technological advances in early detection and intervention [2]. Although, prognosis remains poor for those with advanced stage colon cancer even they received standard care treatment including surgery, chemotherapy and radiotherapy [3-5]. Colon cancer has various histological subtypes and 90% of them are adenocarcinoma [2, 6]. Identifying novel biomarkers at a molecular level to predict prognosis and improve treatment outcome for afflicted patients would have a very important practical significance.

MicroRNAs (miRNAs) are a group of 19 to 25 nucleotides in length small non-coding RNAs that regulate gene expression at the post-transcriptional level through binding to the sequences of their target mRNAs' 3'untranslated region [7]. They participate in regulating many cellular processes such as proliferation, differentiation, and apoptosis [8, 9]. Acumulating studies have implied that miRNAs are mutated or expressed aberrantly in human cancers, exerting critical functions in the pathogenesis of cancers [10, 11]. Expression signatures of miRNAs have been proposed to be effective biomarkers for detection, intervention and prognosis of cancers [12, 13]. The prognostic role of miRNAs´ expressions in colon cancer has been reported [14-17]. For example, higher expression of miR-29a in stage II colon cancer was associated with a longer disease-free survival of the patients [17, 18], and higher miR-21 expression in colon adenocarcinoma was associated with low survival rate and resistance to chemotherapy [15]. In this study, we analyzed miRNA expression data and corresponding clinical information of 329 colon adenocarcinoma patients from the latest gathered data in The Cancer Genome Atlas (TCGA) database, and found that some miRNAs were expressed differentially in colon adenocarcinoma. We then generated a novel miRNA expression signature model that could be used to predict survival of the patients.

Patients and methods

Patient information and miRNA microarray data. The miRNA expression data and the corresponding clinical in-

formation of the patients with colon cancer were downloaded from the TCGA data portal (http://cancergenome.nih.gov/) in October 2015. The downloaded file contained 444 cases of miRNA expression and 459 cases of clinical information. We screened the data from these cases, and selected the patients for the present study according to the following criteria: (1) the tumor histological type was colon adenocarcinoma; (2) the clinical information was complete and evaluable; (3) the patients didn't die for other non-cancer reasons during the following-up investigation. Overall, 329 patients were enrolled in the present study, and their demographic characteristics and clinical information were summarized in Table 1. Among the 329 patients (Cohort T), 8 patients provided the corresponding adjacent non-tumor tissues (Cohort M). The TCGA project collected and processed patient data following the data access policies approved by their ethics committee, so no further ethical approval was required for this study.

The expression data of the miRNAs were obtained by miRNA sequencing performed with the Illumina Genome Analyzer and HiSeq platforms (Illumina Inc, San Diego, CA, USA). Raw expression data were standardized into level 3 data with the expression quantity demonstrated as reads per million (RPM). We downloaded the level 3 data and analyzed them with BRB-Array tools (version 4.4.0) developed by Dr. Richard Simon and the BRBA-array Tools Development Team [19]. We excluded non-qualified miRNAs from the data according to the following criteria: (1) the expression was less than 1 RPM in at least 90% of the samples; (2) the expression had less than 1.5 folds changes from the median value in at least 80% of the samples. Then, RNAseq expression data log2 transformed were conducted to standardize the expression values of each filtered miRNA [20].

Target gene prediction and pathway analysis. DIANA-mirPath [21] tools were employed to do pathway analysis of miRNAs signature. Target genes were predicted by the microT-CDS algorithm and some experimentally validated miRNA interactions were derived from the DIANA-TarBase database. KEGG pathway enrichment and GO annotation of these target genes were conducted by the mirPath v.3 with default settings.

Statistical analysis. The continuous variables were presented as mean ± standard deviation (SD) and the categorical variables were expressed as counts and percentages. Chi-square test was used to analyze the different distribution of clinical variables (gender, AJCC stage, depth of invasion, lymph node invasion and distant metastasis) between Cohort M and Cohort T, while Student t test was used to examine the difference of age. To determine the difference of miRNA expression between cancerous and matched noncancerous tissues, paired-sample t test was conducted (significant p-value was set as 0.001). The unpaired t-test was employed to analyze the expression levels of miRNAs between different clinicopathological groups (significant p-value was set as 0.01).

To explore the relationship between miRNA expression and patients' survival time, the univariate Cox proportional hazards regression model was conducted (significant p-value was set as 0.001). After the significant miRNAs were selected, the Kaplan-Meier method and the log-rank test were performed to study the distribution of survival time in different expression states. Expression value of the miRNAs and survival status of the patients were used to compute the risk score, and an expression signature was generated by the principal component model. For further exploring prognostic values of the microRNA expression signature, the univariate Cox regression analysis was conducted to identify the effects of risk score (high risk vs low risk), AJCC stage (stage III+IV vs stage I+II), depth of invasion (T3+T4 vs T1+T2), lymph node invasion (N1+N2 vs N0) and distant metastasis (M1 vs M0) on patient survival. Then, multivariate Cox regression analysis was applied to combine the significant factors (p-value <0.05 in the univariate Cox regression analysis). The results generated by Cox regression analysis were expressed as hazard ratio (HR) and 95% confidential interval (95% CI). Unless specifically indicated, all tests were two-sides and p-value < 0.05 was regard as statistically significant. All the statistical analysis was performed by the SPSS 19 (IBM, USA) and BRB-Array Tools 4.0.

Table 1. Clinical characteristics of patients with colon adenocarcinoma

Category	Cohort M (n = 8)	Cohort T (n = 329)	P value [a]
Age	75.4±15.2	66.9±13.1	**0.013**
Gender			0.154
Male	6	152	
Female	2	177	
AJCC stage			0.768
I	1	53	
II	4	132	
III	1	91	
IV	2	45	
NA	0	8	
Tumor size			0.946
T1	0	9	
T2	1	56	
T3	6	229	
T4	1	35	
Lymph node			0.669
N0	6	197	
N1	1	80	
N2	1	52	
Metastasis status			0.688
M0	6	253	
M1	2	45	
MX	0	26	
NA	0	5	
Vital status			0.661
Live	6	264	
Dead	2	65	

AJCC: American Joint Committee on Cancer; MX: metastasis status unknown; NA: Not Available. [a]Statistical significant results (in bold)

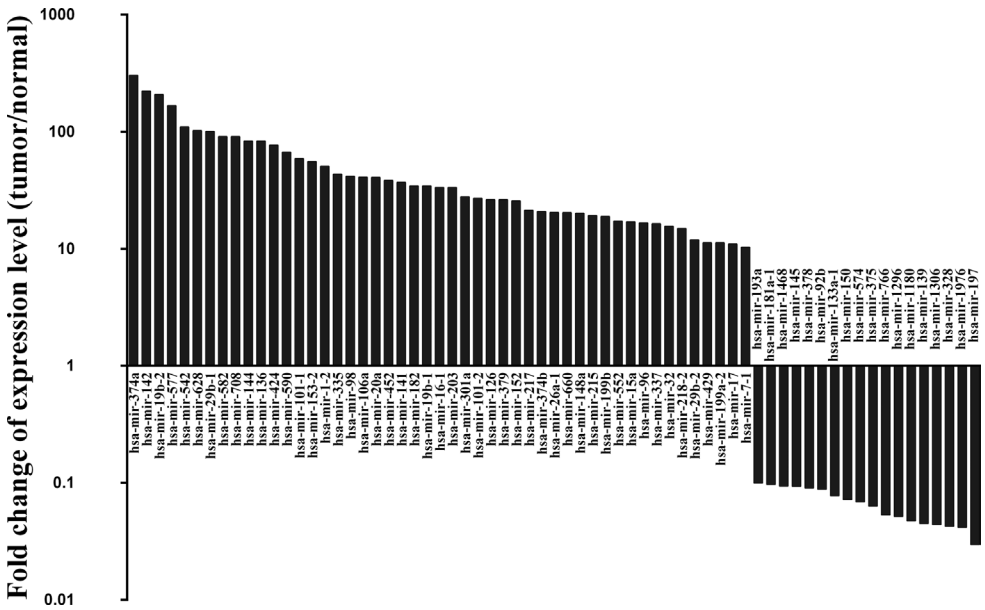

Figure 1. Differentially expressed microRNAs between colon adenocarcinoma and adjacent tissues with fold-change ≥10.

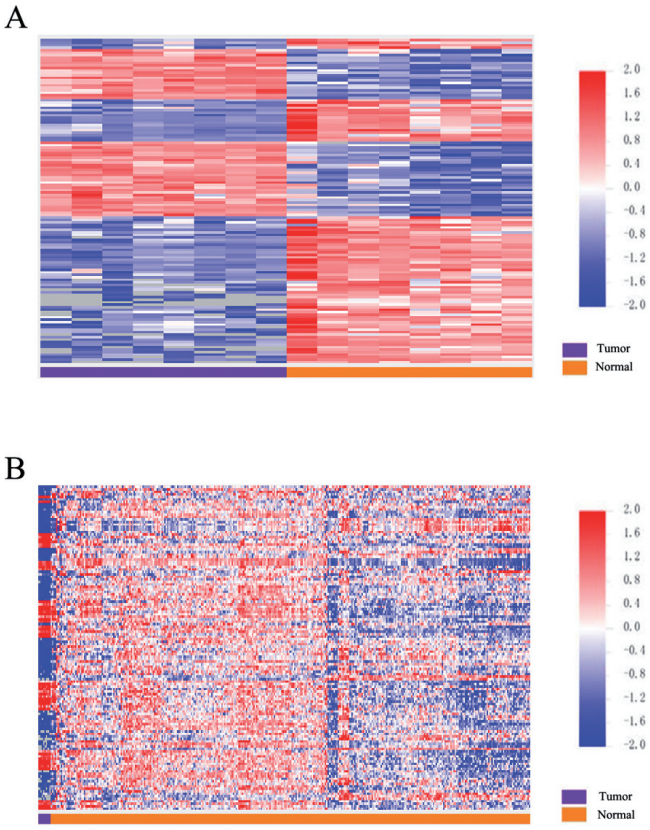

Figure 2. Unsupervised hierarchical cluster analysis of 129 differentially expressed miRNAs. A. Unsupervised hierarchical cluster analysis of 129 differentially expressed miRNAs in paired samples. B. Unsupervised hierarchical cluster analysis of 129 differentially expressed miRNAs in unpaired samples.

Results

Patient characteristics. There were a total of 329 patients enrolled in this study and among them (Cohort T), 8 patients provided corresponding the adjacent tissues (Cohort M). All of these patients were clinically diagnosed with colon adenocarcinoma. Their ages were 66.9 ± 13.1 (mean ± SD) years old, and the follow-up time was 30.8 ± 26.2 (mean ± SD) months. Overall, 14 (4.3%) patients' follow-up time were less than 31 days and 46 (14.0%) patients died after the mean follow-up time. The detailed demographic and clinical information with their distributions between the two cohorts are summarized in Table 1.

Differential expression analysis of miRNA. There were a total of 129 miRNAs expressed differentially between the cancer and the matched adjacent tissues (p-value <0.001). Among these 129 miRNAs, 80 miRNAs (61.5%) were up-regulated in the cancer tissues, while the remaining 49 miRNAs (38.5%) were downregulated (Table S1). In consideration of the fold change, 67 of them expressed a greater than ten-fold in the transformed values (Figure 1). In addition, the unsupervised hierarchical clustering could discriminate the cancer and the normal class clearly with these differentially expressed miRNAs (Figure 2). Besides, class comparison analysis was applied to found out miRNAs associated with cancer progression. A total of 27 differentially expressed miRNAs were identified to be associated with the corresponding clinical characteristics of the patients. Among them, 4 miRNAs was associated with AJCC tumor stage, 5 with pathologic T, 9 with pathologic N, and 9 with pathologic M (Table S2).

MiRNA expression associated with patient survival. Seven miRNAs (let-7a-2, mir-32, mir-181a-1, mir-197, mir-

328, mir-505 and mir-652) were identified to be related to the survival of the patients with colon adenocarcinoma by the univariate Cox regression analysis (Table 2). Kaplan–Meier survival curves indicated that all of them were risky miRNAs (Figure 3). Then, a seven-miRNA expression signature was obtained by the principal component analysis with Cox pro-

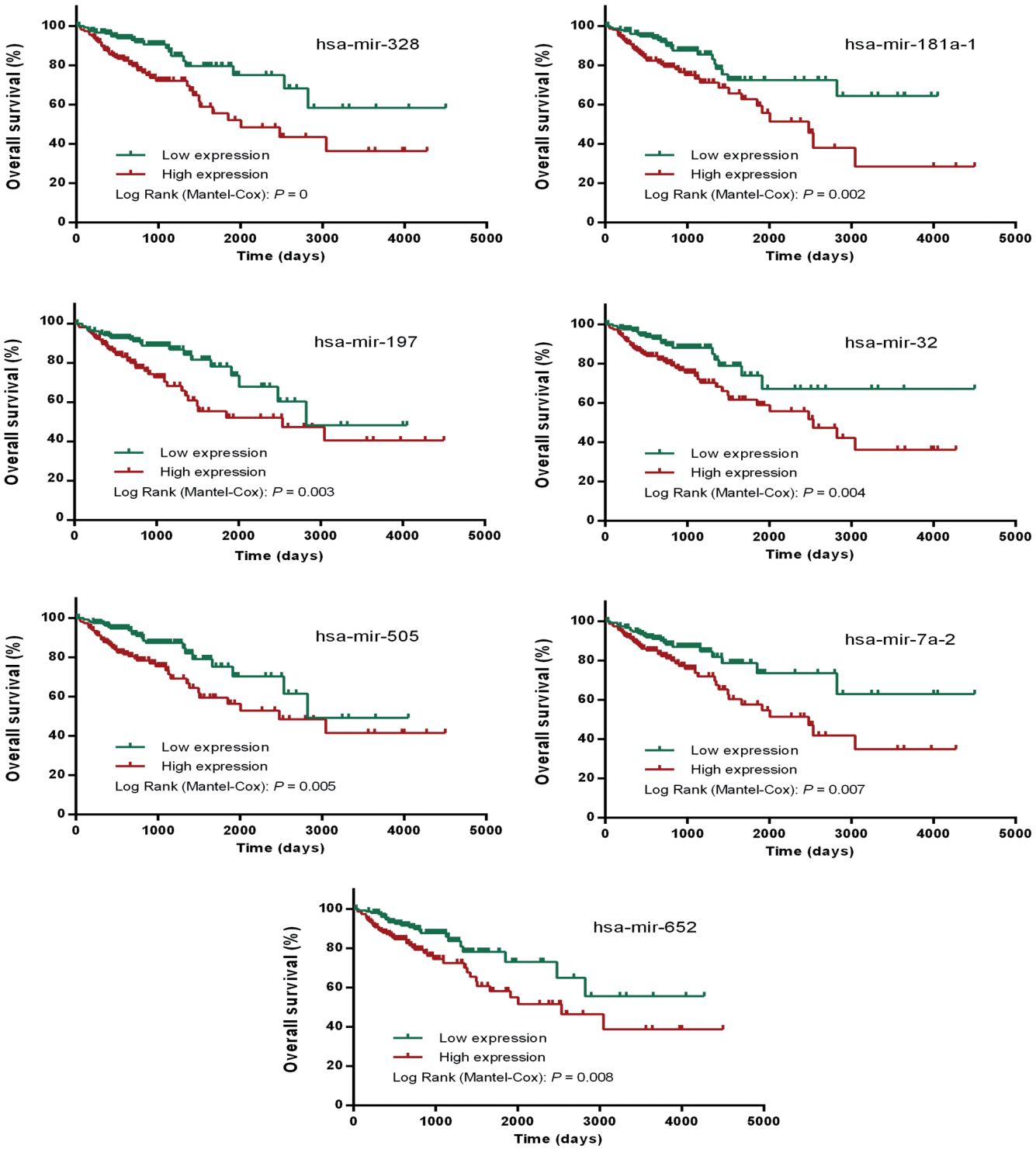

Figure 3. Kaplan-Meier survival curves for 7 survival related miRNAs. All miRNAs (hsa-mir-328, hsa-mir-181a-1, hsa-mir-197, hsa-mir-32, hsa-mir-505, hsa-let-7a-2 and hsa-mir-652) were negatively associated with overall survival. (Horizontal axis: overall survival time; vertical axis: survival function).

Table 2. Univariate analysis of miRNAs associated with overall survival in colon adenocarcinoma patients.

MiRNAs	HR	95% CI	P value [a]
let-7a-2	1.419	1.097-1.835	**0.008**
mir-32	1.465	1.120-1.916	**0.005**
mir-181a-1	1.483	1.146-1.919	**0.003**
mir-328	1.574	1.209-2.049	**0.001**
mir-197	2.11	1.266-3.518	**0.004**
mir-505	1.439	1.112-1.862	**0.006**
mir-652	1.401	1.085-1.809	**0.01**

HR hazard ratio; CI confidential interval.
[a] Statistical significant result (in bold)

portional hazards regression [22]. Risk score for each patient was computed by a math formula ∑iwi xi – 7.679485 where wi and xi are the weight and logged miRNA expression for the i-th miRNA (Risk score = (0.148126 x logged expression of hsa-mir-197) + (0.123695 x logged expression of hsa-mir-328) + (0.186403 x logged expression of hsa-mir-505) + (0.190766 x logged expression of hsa-mir-32) + (0.144632 x logged expression of hsa-let-7a-2) + (0.110015 x logged expression of hsa-mir-181a-1) + (0.241215 x logged expression of hsa-mir-652) – 7.679485). A patient would be deem as high (low) risk if his risk score was larger (smaller) than 0.011828.

Validation of the 7-miRNA signature as an independent prognostic factor. Clinical parameters (age, gender, AJCC tumor stage, pathologic T, pathologic N and pathologic M) and the 7-miRNAs signature in the patients with colon adenocarcinoma were conducted the univariate Cox regression analyses. The result indicated AJCC tumor stage (P = 0.001), pathologic T (P = 0.044), pathologic N (P = 0.0001), pathologic M (P = 0.0001) and risk score (P = 0.001) were significantly associated with survival of the patients (Table 3). Then, Kaplan-Meier survival curves demonstrated the distribution of survival time in different states of all significant variables (Figure 4). Furthermore, the 7-miRNAs signature (HR = 2.048; 95% CI = 1.144-3.664; p, 0.016) and pathologic M (HR = 1.686; 95% CI = 1.196-2.375; p, 0.003) passed the multivariate Cox regression analysis with all significant variables to be proven as an independent prognostic factors (Table 3).

Target gene prediction and pathway enrichment analysis. A summary of 1773 target genes of the 7 miRNAs in the signature were predicted by the microT-CDS algorithm. These target genes were then subjected to the GO annotation and KEGG analyses by DIANA-mirPath v.3. The GO annotation showed these target genes' functions were related to biosynthetic processes, cellular protein modification processes, nucleic acid binding transcription factor activity, and so on (Table 4). Besides, the results of KEGG analyses demonstrated that these target genes were mostly associated with cancer-related pathways such as the Wnt signaling pathway, the p53 signaling pathway and the colorectal cancer signaling pathway. More pathways are listed on Table 4.

Discussion

Colon adenocarcinoma is the most prevalent subtype of colon cancer with highly variable clinical features [2, 23, 24]. Traditional models to predict patient survival are primarily established on imaging and pathohistologic examination without disease information at the molecular level [25, 26]. Aberrant expression of miRNAs have been documented to be highly correlated with cancer pathogenesis and have potential to be used as a predictor for patient prognosis [23, 27, 28]. In this study, we analyzed the aberrant miRNA expression profiles in colon adenocarcinoma with the aim to find a miRNA expression signature which could serve as an independent predictor of survival for the patients with the cancer.

As a family of short, single-stranded and non-coding endogenous RNAs, miRNAs can regulate gene expression at the post-translational level. They are involved in various biological processes through binding to 3'untranslated regions of their target genes [29, 30]. Emerging evidence has suggested that miRNAs play an important role in the development and progression of cancers, and identification of miRNA biomarkers is becoming an important field in cancer research [12, 13]. In fact, a number of miRNAs were reported to be correlated with the prognosis of colon adenocarcinoma. Schetter et al. reported that higher miR-21 expression in colon adenocarcinoma tissue was associated with a poor prognosis and resistance to chemotherapeutic drug [15]. Brenner et al. demonstrated that higher expression of miR-29a was associated with a longer disease-free

Table 3. Univariate and multivariate analysis of parameters associated with overall survival.

		Univariate analysis		Multivariate analysis	
		HR (95% CI)	P value [a]	HR (95% CI)	P value [a]
7-miRNA Signature	High risk vs. Low risk	2.583 (1.498-4.454)	**0.001**	2.048 (1.144-3.664)	**0.016**
AJCC Pathological Stage	III + IV vs. I + II	1.521 (1.178-1.964)	**0.001**	1.144 (0.575-2.277)	0.702
AJCC Pathological T	T3+T4 vs. T1+T2	1.603 (1.013-2.534)	**0.044**	1.767 (0.853-3.661)	0.126
AJCC Pathological N	N1+N2 vs. N0	1.597 (1.246-2.048)	**<0.001**	1.120 (0.598-2.099)	0.723
AJCC Pathological M	M1 vs. M0	2.132 (1.603-2.841)	**<0.001**	1.686 (1.196-2.375)	**0.003**

AJCC American Joint Committee on Cancer; HR hazard ratio; CI confidential interval; vs. versus
[a] Statistical significant results (in bold)

survival of stage II colon cancer patients [17]. However, exact biological functions and potential prognostic characteristics of miRNAs in colon adenocarcinoma remain to be elucidated.

In the present study, we explored genome wide miRNA expression profiles and the corresponding clinical data of 329 colon adenocarcinoma patients selected from the TCGA database. We identified 129 miRNAs that were differentially expressed between the cancer and adjacent tissues. Robustness of the classifier with all these differentially expressed miRNAs were validated in unpaired conditions. Among

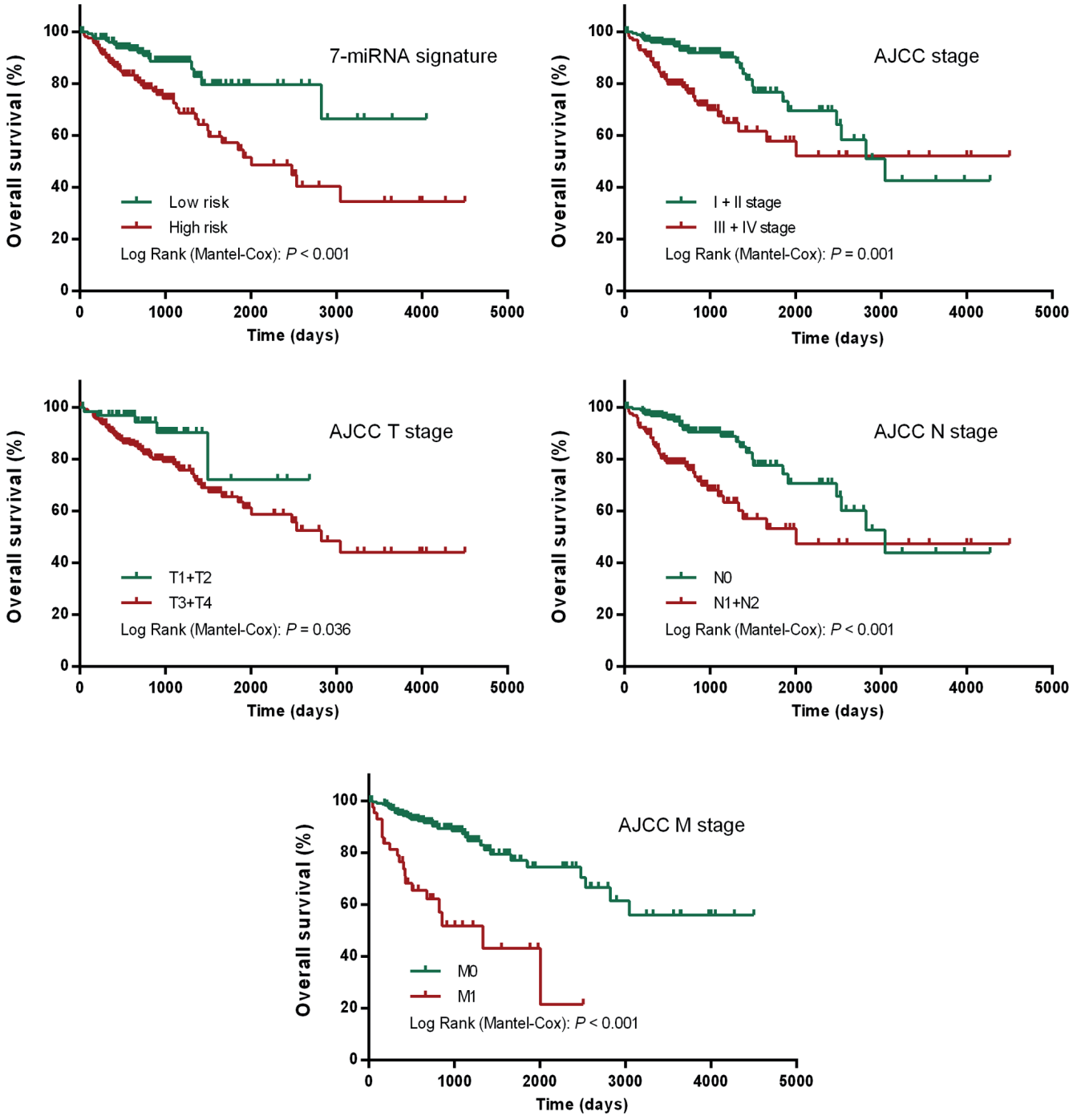

Figure 4. Kaplan–Meier survival curves for colon adenocarcinoma patients. 329 colon adenocarcinoma patients were compared in two groups according to: 7-miRNA signature (high risk vs. low risk); AJCC pathological (III + IV vs. I + II stage); AJCC T stage (T3+T4 vs. T1+T2); AJCC N stage (N1+N2 vs. N0) and AJCC M stage (M1 vs. M0). (Horizontal axis: overall survival time; Vertical axis: survival function).

Table 4. GO and KEGG analysis of the seven miRNAs' target genes.

GO Analysis	Number of miRNAs	Number of genes	P-value
Organelle (GO:0043226)	7	889	2.56E-52
Ion binding (GO:0043167)	7	623	1.64E-51
Cellular nitrogen compound metabolic process (GO:0034641)	7	497	3.92E-46
Biosynthetic process (GO:0009058)	7	407	1.27E-29
Cellular protein modification process (GO:0006464)	7	254	8.10E-23
Molecular_function (GO:0003674)	7	1403	1.82E-17
Protein binding transcription factor activity (GO:0000988)	7	73	2.06E-14
Gene expression (GO:0010467)	7	71	3.67E-13
Nucleoplasm (GO:0005654)	7	138	3.67E-13
Nucleic acid binding transcription factor activity (GO:0001071)	7	117	9.95E-13
Cellular_component (GO:0005575)	7	1394	3.19E-11
Enzyme binding (GO:0019899)	7	137	1.55E-10
Cellular component assembly (GO:0022607)	7	136	3.30E-10
Protein complex (GO:0043234)	7	340	5.99E-10
Catabolic process (GO:0009056)	7	183	9.91E-10
KEGG Pathway Analysis	**Number of miRNAs**	**Number of genes**	**P-value**
Wnt signaling pathway	7	26	2.29E-08
Glycosaminoglycan biosynthesis – heparan sulfate / heparin	3	5	5.58E-04
Adherens junction	5	11	3.29E-03
Focal adhesion	5	22	7.26E-03
ErbB signaling pathway	5	11	7.58E-03
PI3K-Akt signaling pathway	5	32	8.61E-03
Drug metabolism – cytochrome P450	2	3	1.43E-02
VEGF signaling pathway	6	9	1.83E-02
p53 signaling pathway	5	9	2.32E-02
TGF-beta signaling pathway	4	10	2.60E-02
RNA transport	7	15	3.04E-02
MAPK signaling pathway	6	26	3.17E-02
Colorectal cancer	4	9	3.98E-02
HIF-1 signaling pathway	4	12	4.37E-02

(A) GO analysis of the target gene; (B) KEGG analysis of the target gene.

these 129 miRNAs, 67 miRNAs had more than ten folds change in expression levels. With regard to the clinical pathological feature, 4 of the aberrantly expressed miRNAs were identified to be associated with AJCC tumor stage, 5 with pathologic T, 9 with pathologic N, and 9 with pathologic M. Subsequently, seven miRNAs (let-7a-2, mir-32, mir-181a-1, mir-197, mir-328, mir-505 and mir-652) were ascertained to be significantly associated with overall survival of the patients with colon adenocarcinoma. Kaplan-Meier survival curves indicated that all of them were risky miRNAs. After the stepwise univariate Cox regression analysis and the multivariate Cox regression analysis, a seven-miRNAs signature was proved to be an independent prognostic factor. Among these prognosis associated miRNAs, mir-197 and mi-328 were previously reported to be related with colon cancer. Zhou et al reported downregulation of mir-197 in colon cancer cells upon administration of chemotherapeutics in vitro [31]. Xu et al documented that miR-328 could be interacting with ABCG2 gene to overcome drug resistance in colorectal cancer cells [32]. Some of the miRNAs in the signature were also to be associated other cancers. Lower expression of let-7a-2 and mir-32 was identified to be correlated with poor survival of lung cancer [33, 34]. MiRNA-32 was reported to promote hepatocellular carcinoma cell proliferation, migration and invasion [35]. As a member of the mir-181 family, expression of mir-181a-1 proved to be an independent diagnostic indicator jointed with KAT2B gene expression in gastric cancer [36]. The KEGG enrichment analysis indicated the 7 miRNAs in the signature could regulate important cancer related pathways such as Wnt signaling pathway, the p53 signaling pathway, and the colorectal cancer signaling pathway. More biological functions of these miRNAs in colon adenocarcinoma remain to be clarified.

Some limitations should be acknowledged in interpreting the above results. First, there were 329 cancer tissues but only 8 paired cancer adjacent tissues conducted to identify the differentially expressed miRNAs between them. The sample numbers of the latter were relatively small which could re-

duce the statistical power. Besides, although we selected and analyzed the data strictly, external biological and clinical experiment still need to be performed to validate the prognostic model. Among those prognosis related miRNAs, 6 were downregulated (let-7a-2, mir-181a-1, mir-197, mir-328, mir-505 and mir-652) in tumors, and 1 was upregulated (mir-32). But, they were all identified to be risky miRNAs by Cox regression analysis and the Kaplan-Meier method. The following reasons may explain the controversial results. First, although oncogenes were usually highly expressed in tumors, no theory exists to validate the universality of their relationship. Second, some miRNAs may play different roles at different stages of tumor development like transforming growth factor-beta (TGF-β) that functions as a tumor suppressor in normal and early-stage cancers and as a tumor promoter in their late-stage counterparts [37, 38]. Indeed, Meng et al. reported that some miRNAs are expressed in a wave-like manner during carcinogenesis [39]. Finally, compared with the survival analysis, the number of samples was relatively small in paired class comparison for screening differentially expressed genes, and this could reduce the statistical power and generate some false positive results.

In summary, by analyzing an independent colon adenocarcinoma patient cohort derived from the TCGA database, our study identified a seven-miRNA expression signature which could serve as an independent prognostic factors to predict survival of the patients with colon adenocarcinoma.

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Table S1. Summary of differentially expressed microRNAs between cancer and adjacent tissues.

MicroRNA	Expression Level [a]		Fold Change	*P* value	FDR
	Tumor	Adjacent			
hsa-mir-423	65.21	636.12	0.10245902	< 1e-07	< 1e-07
hsa-mir-141	1267.28	34.11	37.037037	< 1e-07	< 1e-07
hsa-mir-182	13598.48	390.03	34.4827586	< 1e-07	< 1e-07
hsa-mir-378	528.42	5851.17	0.09033424	< 1e-07	< 1e-07
hsa-mir-19b-2	141.94	0.68	208.333333	< 1e-07	< 1e-07
hsa-mir-16-1	705.55	21.08	33.3333333	< 1e-07	< 1e-07
hsa-mir-582	370.76	4.25	90.9090909	< 1e-07	< 1e-07
hsa-mir-98	36.21	0.86	41.6666667	< 1e-07	< 1e-07
hsa-mir-328	14.71	346.45	0.04246285	< 1e-07	< 1e-07
hsa-mir-374a	675.62	2.21	303.030303	< 1e-07	< 1e-07
hsa-mir-203	20718.08	631.04	33.3333333	< 1e-07	< 1e-07
hsa-mir-126	2562.93	97.79	26.3157895	< 1e-07	< 1e-07
hsa-mir-142	3219.77	14.51	222.222222	< 1e-07	< 1e-07
hsa-mir-1180	3.76	79.41	0.04730369	< 1e-07	< 1e-07
hsa-mir-542	138.18	1.26	109.89011	< 1e-07	< 1e-07
hsa-mir-29b-1	445.17	4.61	100.4908	< 1e-07	< 1e-07
hsa-mir-101-1	5942.65	103.44	58.8235294	< 1e-07	< 1e-07
hsa-mir-574	27.06	391.86	0.06906077	< 1e-07	< 1e-07
hsa-mir-152	212.96	8.42	25.7294	< 1e-07	< 1e-07
hsa-let-7b	11906.26	97941.41	0.12150668	< 1e-07	< 1e-07
hsa-mir-1976	3.07	73.54	0.04177109	< 1e-07	< 1e-07
hsa-mir-193a	78.68	789.28	0.0997009	< 1e-07	< 1e-07
hsa-mir-335	148.8	3.39	43.4782609	< 1e-07	< 1e-07
hsa-mir-197	136.84	4595.45	0.02977963	< 1e-07	< 1e-07
hsa-mir-26a-1	10.79	0.53	20.4081633	< 1e-07	< 1e-07
hsa-mir-20a	345.99	8.56	40.65392	< 1e-07	< 1e-07
hsa-mir-106a	47.42	1.18	40.83096	< 1e-07	< 1e-07
hsa-mir-324	19.56	96.96	0.2016129	< 1e-07	< 1e-07
hsa-let-7d	500.2	4547.88	0.110011	< 1e-07	< 1e-07
hsa-mir-148a	75911.95	3768.94	20.10935	< 1e-07	< 1e-07
hsa-mir-379	656.61	25.1	26.3157895	< 1e-07	< 1e-07
hsa-mir-199b	4325.75	228.74	18.8679245	< 1e-07	< 1e-07
hsa-mir-766	3.26	61.19	0.0532198	< 1e-07	< 1e-07
hsa-mir-101-2	14.69	0.54	27.027027	< 1e-07	6.55E-07
hsa-mir-29b-2	483	40.45	11.9047619	< 1e-07	6.55E-07
hsa-mir-452	59.68	1.53	38.4615385	< 1e-07	6.55E-07
hsa-mir-429	218.79	19.45	11.2359551	1.00E-07	6.55E-07
hsa-let-7a-3	2907.3	12417.59	0.23419204	2.00E-07	1.19E-06
hsa-mir-19b-1	13.78	0.4	34.4827586	2.00E-07	1.19E-06
hsa-mir-375	15165.46	239661.69	0.06329114	2.00E-07	1.19E-06

hsa-let-7a-1	2852.36	11482.82	0.24813896	2.00E-07	1.19E-06
hsa-mir-652	13.06	59.33	0.22026432	3.00E-07	1.56E-06
hsa-mir-199a-1	1602.3	183.74	9.09090909	3.00E-07	1.56E-06
hsa-mir-199a-2	2988.74	265.91	11.2359551	3.00E-07	1.56E-06
hsa-mir-17	1039.39	94.52	10.989011	3.00E-07	1.56E-06
hsa-mir-200c	6221.95	33750.22	0.18450185	3.00E-07	1.56E-06
hsa-mir-144	55.61	0.65	83.3333333	3.00E-07	1.56E-06
hsa-mir-139	26.83	595.79	0.04502476	4.00E-07	2.03E-06
hsa-mir-589	29.17	170.66	0.17094017	5.00E-07	2.39E-06
hsa-mir-30e	11449.76	1846.59	6.252009	5.00E-07	2.39E-06
hsa-mir-1306	2.65	60.15	0.04409171	5.00E-07	2.39E-06
hsa-mir-136	59.75	0.73	83.3333333	6.00E-07	2.77E-06
hsa-mir-125a	293.85	2805.77	0.10471204	6.00E-07	2.77E-06
hsa-mir-660	25.85	1.3	20.37497	7.00E-07	3.06E-06
hsa-let-7a-2	7409.83	25070.24	0.29585799	7.00E-07	3.06E-06
hsa-mir-1266	4.74	29.97	0.15822785	7.00E-07	3.06E-06
hsa-mir-92b	28.45	323.2	0.08802817	8.00E-07	3.43E-06
hsa-mir-10a	53382.96	7757.98	6.66666667	9.00E-07	3.80E-06
hsa-mir-92a-2	9256.72	57133.31	0.16207455	1.30E-06	5.22E-06
hsa-mir-181a-1	605.37	6230.26	0.09718173	1.30E-06	5.22E-06
hsa-mir-374b	32.43	1.56	20.8333333	1.30E-06	5.22E-06
hsa-mir-140	399.89	1637.69	0.24390244	1.50E-06	5.93E-06
hsa-mir-340	10.07	1.17	8.33333333	1.60E-06	6.22E-06
hsa-mir-424	86.27	1.09	76.9230769	1.90E-06	7.28E-06
hsa-mir-92a-1	375.16	2815.5	0.13333333	2.00E-06	7.32E-06
hsa-mir-150	270.22	3745.44	0.07215007	2.00E-06	7.32E-06
hsa-mir-15a	90.52	5.36	16.9491525	2.00E-06	7.32E-06
hsa-mir-1307	1078.6	9451.56	0.11415525	2.10E-06	7.58E-06
hsa-mir-183	7036.58	899.5	7.69230769	2.70E-06	9.60E-06
hsa-mir-744	27.65	114.43	0.24154589	3.20E-06	1.12E-05
hsa-mir-99b	18129.17	94070.15	0.19267823	4.00E-06	1.38E-05
hsa-mir-628	42.84	0.42	102.040816	4.50E-06	1.53E-05
hsa-mir-671	3.4	22.18	0.15360983	4.60E-06	1.55E-05
hsa-mir-196b	3664.06	396.47	9.09090909	6.10E-06	2.03E-05
hsa-let-7f-1	9.19	1.22	7.69230769	6.50E-06	2.13E-05
hsa-mir-153-2	32.08	0.57	55.5555556	7.90E-06	2.55E-05
hsa-mir-151	1431.77	477.39	3.03030303	8.60E-06	2.75E-05
hsa-mir-32	20.14	1.29	15.625	1.02E-05	3.21E-05
hsa-mir-361	126.94	496.56	0.25575448	1.05E-05	3.27E-05
hsa-mir-15b	97.53	631.27	0.15455951	1.18E-05	3.63E-05
hsa-mir-24-2	1383.13	265.79	5.26315789	1.26E-05	3.83E-05
hsa-mir-1296	1.29	24.93	0.05167959	1.31E-05	3.93E-05
hsa-mir-484	38.06	181.49	0.20964361	1.37E-05	4.06E-05

hsa-let-7f-2	3102.88	373.88	8.33333333	1.46E-05	4.28E-05
hsa-mir-30b	148.68	20.74	7.14285714	1.62E-05	4.69E-05
hsa-mir-338	361.49	47.02	7.69230769	2.47E-05	7.07E-05
hsa-mir-708	54.11	0.61	90.9090909	4.16E-05	0.000118
hsa-mir-874	10.8	60.97	0.17730496	4.45E-05	0.000124
hsa-mir-145	2215.72	23665.56	0.09363296	4.50E-05	0.000125
hsa-mir-10b	35676.39	8227.69	4.34782609	5.44E-05	0.000149
hsa-mir-7-1	27.69	2.85	10.293647	5.55E-05	0.00015
hsa-mir-218-2	13.4	0.89	14.9253731	6.04E-05	0.000162
hsa-mir-577	85.07	0.51	166.666667	7.22E-05	0.000191
hsa-mir-132	52.46	187.64	0.27932961	7.88E-05	0.000207
hsa-mir-95	5.59	0.83	6.66666667	8.31E-05	0.000216
hsa-mir-185	35.71	10.8	3.33333333	8.70E-05	0.000223
hsa-mir-1468	1.27	13.51	0.09372071	8.91E-05	0.000224
hsa-mir-193b	19.21	158.59	0.12106538	8.91E-05	0.000224
hsa-mir-196a-1	161.06	26.8	5.88235294	9.43E-05	0.000235
hsa-mir-30d	3412.72	10199.49	0.33444816	9.92E-05	0.000245
hsa-mir-223	215.25	29.24	7.14285714	0.000115	0.000281
hsa-mir-188	2.52	0.51	5.296312	0.00016	0.000386
hsa-mir-552	38.97	2.24	17.2413793	0.000166	0.000398
hsa-mir-181d	9.46	1.8	5.26315789	0.00017	0.000403
hsa-mir-339	19.18	86.07	0.22271715	0.000173	0.000405
hsa-mir-301a	15.91	0.57	27.7777778	0.000174	0.000405
hsa-mir-1-2	36.7	0.74	50.782093	0.000183	0.000421
hsa-mir-186	249.24	63.59	3.84615385	0.000199	0.000453
hsa-mir-23a	3165.31	1208.86	2.63157895	0.000222	0.000502
hsa-let-7g	445.11	133.87	3.33333333	0.000242	0.000543
hsa-mir-330	7.76	28.83	0.26954178	0.000249	0.000554
hsa-mir-505	33.73	87.27	0.38610039	0.000279	0.000615
hsa-mir-26b	379.75	82.39	4.54545455	0.00029	0.000633
hsa-mir-34a	61.19	14.44	4.16666667	0.000298	0.000645
hsa-mir-133a-1	12.63	162.77	0.07757952	0.000306	0.000657
hsa-mir-337	23.77	1.45	16.3934426	0.000357	0.000759
hsa-mir-576	7.96	1.24	6.254326	0.000393	0.000829
hsa-mir-27a	883.97	223.58	4.293648	0.000494	0.00103
hsa-mir-22	45125.04	16370.5	2.77777778	0.000515	0.00107
hsa-mir-590	24.02	0.35	66.6666667	0.000571	0.00117
hsa-mir-30a	9018.02	2905.33	3.1253497	0.000605	0.00124
hsa-mir-200a	1738.33	829.87	2.08333333	0.000693	0.0014
hsa-mir-539	17.71	3.83	4.54545455	0.000808	0.00162
hsa-mir-28	5013.11	10840.79	0.46296296	0.000823	0.00164
hsa-mir-451	136.62	22.89	5.88235294	0.00086	0.0017
hsa-mir-96	11.85	0.71	16.6666667	0.00088	0.00173

hsa-mir-215	290.73	15.18	19.2307692	0.00091	0.00177
hsa-mir-217	23.49	1.09	21.2765957	0.000935	0.00179
hsa-mir-125b-1	191.76	1053.42	0.18214936	0.000937	0.00179

FDR, false discovery rate
[a] mean expression data

Table S2. MicroRNAs associated with the progression of colon adenocarcinoma

MicroRNA	Expression Level [a]		Fold Change	*P* value	FDR
AJCC stage	**High stage**	**Low stage**			
hsa-mir-30a	5742.86	4783.97	1.2	0.0055929	0.386
hsa-mir-625	320.23	407.28	0.79	0.0058302	0.386
hsa-mir-195	30.26	22.94	1.32	0.007238	0.386
hsa-mir-942	8.05	10.26	0.78	0.0073537	0.386
Pathologic T	**T3 + T4**	**T1 + T2**			
hsa-mir-379	763.83	577.85	1.32	0.0041091	0.444
hsa-mir-337	23.37	18.25	1.28	0.0042134	0.444
hsa-let-7e	540.85	401.66	1.35	0.0094846	0.444
hsa-mir-106a	28.74	41.71	0.69	0.0095788	0.444
hsa-mir-493	12.62	9.83	1.28	0.0098845	0.444
Pathologic N	**N1 + N2**	**N0**			
hsa-mir-942	8.26	10.66	0.77	0.0020626	0.269
hsa-mir-30a	5655.2	4778.2	1.18	0.0054794	0.269
hsa-mir-1-2	31.32	21	1.49	0.0066475	0.269
hsa-mir-125a	289.01	240.97	1.2	0.0068128	0.269
hsa-mir-625	326.76	409.47	0.8	0.0070591	0.269
hsa-mir-146b	416.02	501.99	0.83	0.0075198	0.269
hsa-mir-133a-1	13.59	9.1	1.49	0.0083855	0.269
hsa-mir-149	9.76	7.44	1.31	0.0090838	0.269
hsa-mir-629	104.19	121.09	0.86	0.0098188	0.269
Pathologic M	**M1**	**M0**			
hsa-mir-96	22.2	14.52	1.53	0.0014243	0.253
hsa-mir-365-1	15.26	9.59	1.59	0.0020284	0.253
hsa-mir-337	28	20.85	1.34	0.0032715	0.253
hsa-mir-29b-1	465.81	363.38	1.28	0.0058784	0.253
hsa-mir-1307	1179.16	1476.49	0.80	0.0067812	0.253
hsa-mir-365-2	14.21	9.62	1.48	0.0073563	0.253
hsa-mir-29b-2	463.23	365.76	1.27	0.0080762	0.253
hsa-mir-625	277.31	380.32	0.73	0.0081321	0.253
hsa-mir-23b	1277.04	1049.97	1.22	0.0092378	0.256

FDR, false discovery rate

[a] mean expression data