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Original Article

Increased expression of miRNA-182 in colorectal carcinoma: an independent and tissue-specific prognostic factor

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Abstract: Increasing evidence has revealed that miRNAs play a pivotal role in multiple processes of carcinogenesis, and are being explored as diagnostic, prognostic and predictive biomarker. In this study, we investigated the status of miR-182 expression in colorectal carcinoma (CRC) by in situ hybridization and its underlying clinicopathologic significance for patients with CRC. We found that 79/138 (57.25%) CRCs had high-level expression of miR-182, while 17/67 (25.37%) normal mucosa tissues had high-level expression of miR-182. The expression level of miR-182 was remarkably up-regulated in CRC tissues compared with non-neoplastic normal tissues ($P < 0.001$). The overexpression of miR-182 in cancer parenchyma cells in CRC were strongly correlated with T-stage ($P = 0.020$), lymph node metastasis ($P = 0.003$), distant metastasis ($P = 0.002$), and Dukes' stage ($P = 0.005$) in patients with CRC. Patients with high-level expression of miR-182 had short overall survival time than those with low-level expression of miR-182 ($P < 0.001$). Univariate and multivariate survival analyses further showed that miR-182 expression was a potential unfavorable prognostic factor for CRC, suggesting a potential application of miR-182 in prognosis prediction and therapeutic application in CRC.

Keywords: miR-182, in situ hybridization, prognosis, colorectal carcinoma

Introduction

Colorectal carcinoma (CRC) is one of the most common causes of cancer death worldwide [1]. Although several kinds of treatment modalities have been developed recently for the patients with CRC, the clinical outcome of prognosis continues to be poor in patients with advanced CRC [2]. Metastasis of CRC cells to vital organs is responsible for the majority of cancer deaths. Therefore, it is of great interest to search for valuable factors for early diagnosis for patients with a high risk of metastasis, prognosis prediction and novel therapeutic strategies.

microRNAs (miRNAs) are regulatory, noncoding RNAs about 23 nucleotides in length and widely express in living organisms. They bind with the 3'UTR of mRNA, causing either mRNA molecule degradation or translational inhibition [3]. miRNAs are involved in many biologic and pathologic processes [4, 5]. Moreover, several miRNAs with potential biological and clinical relevance have been identified and are being explored as diagnostic, prognostic and predictive biomarker [6-8].

miR-182 is located at chromosome 7q32.2, and belongs to the miR-183 family which is comprised of miR-96, miR-182 and miR-183. Researches have illustrated that miR-182 is abnormally expressed in various tumors [9, 10] and directly involves in human cancer processes, such tumorigenesis, migration and metastasis [11-14]. Several studies using quantitative real-time RT-PCR (qRT-PCR) have identified that the expression of miR-182 is up-regulated in CRC tissue blocks [10, 15, 16]. Liu et al. reported that the up-regulated expression of miR-182 correlated with the poor prognosis of patients with CRC by using qRT-PCR [10]. However, the expressed location and the possible prognostic role of miR-182 in CRC have not been completely understood yet. In the present study, we

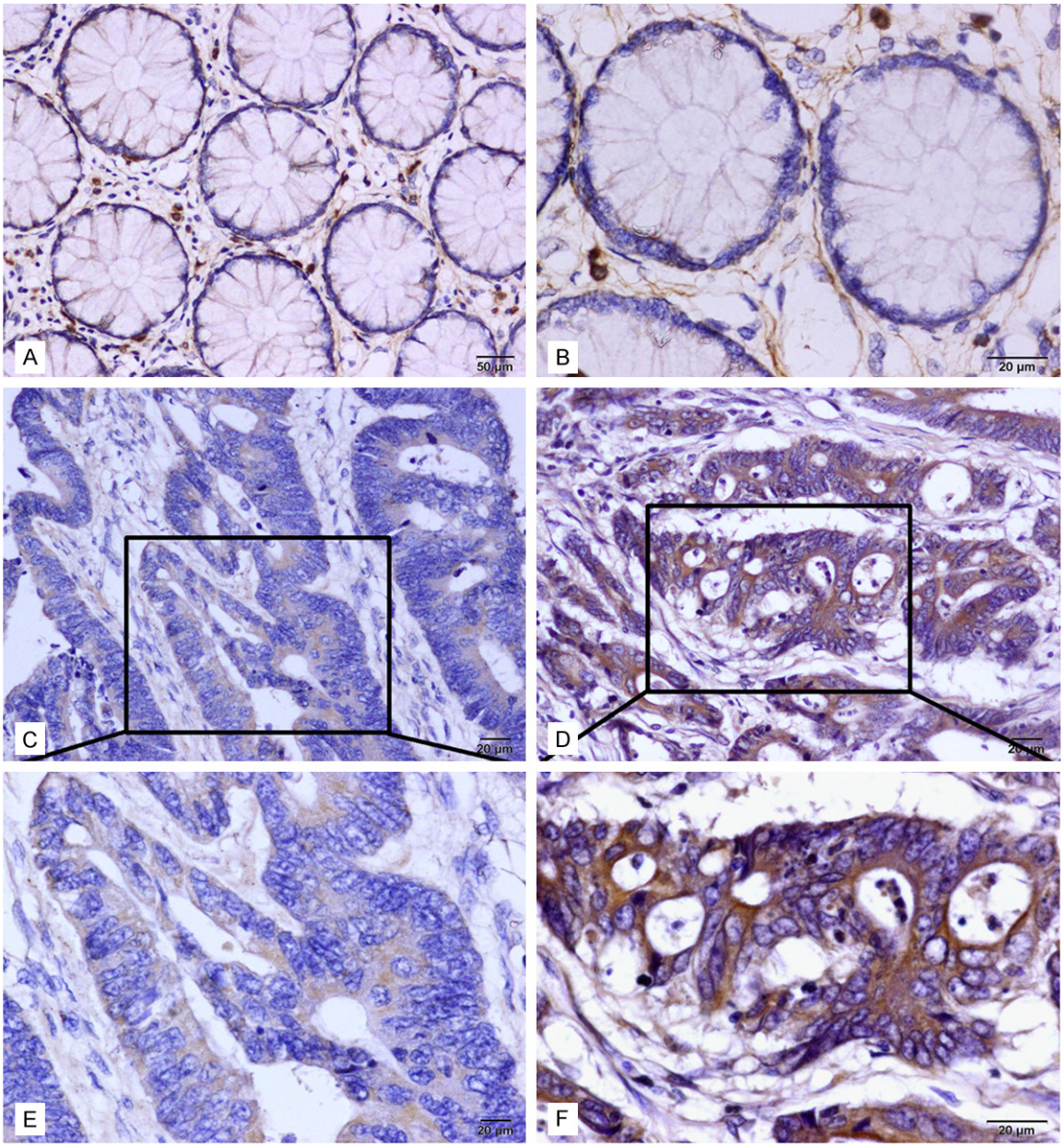

Figure 1. Expression analysis of miR-182 in normal colorectal mucosa and CRC tissues by in situ hybridization. A, B: Negative expression of miR-182 in normal colorectal mucosa (original magnification 200× and 400×, respectively); C-F: miR-182 expression in human CRC tissue samples. C, E: Low expression of miR-182 in CRC tissues (original magnification 200× and 400×, respectively); D, F: High expression of miR-182 in CRCs (original magnification 200× and 400×, respectively).

explore the unambiguous expression analysis of miR-182 in CRC using in situ hybridization. We found that the expression level of miR-182 was higher in CRCs than that in normal colon mucosa. Furthermore, the upregulation of miR-182 in cancer cells is associated with the aggressive phenotypes of CRC and poor prognosis in patients with CRC. In our studies, miR-182 was suggested as a new prognostic marker for CRC patients.

Materials and methods

Tissue specimens

Formalin-fixed, paraffin-embedded, CRC tissue samples were obtained from patients with a

Table 1. Correlations between the clinicopathologic features and expression of miR-182

Characteristics	n	miR-182		*P*	χ^2
		Low (%)	High (%)		
Gender					
Male	83	30 (36.14)	53 (63.86)		
Female	55	29 (52.73)	26 (47.27)	0.054	3.717
Age (y)					
< 50	63	32 (50.79)	31 (49.21)		
≥ 50	75	27 (36.00)	48 (64.00)	0.080	3.062
Tumor site					
Proximal colon	42	19 (45.24)	23 (54.76)		
Distant colon	33	14 (42.42)	19 (57.58)		
Rectum	63	26 (41.27)	37 (58.73)	0.921	0.164
Tumor size (cm in diameter)					
< 5	46	17 (36.96)	29 (63.04)		
≥ 5	92	42 (45.65)	50 (54.35)	0.330	0.947
Tumor differentiation					
Good	12	8 (66.67)	4 (33.33)		
Moderate	103	39 (37.86)	64 (62.14)		
Poor	23	12 (52.17)	11 (47.83)	0.092	4.644
T-satge					
1-2	6	5 (83.33)	1 (16.67)		
3	113	50 (44.25)	63 (55.75)		
4	19	4 (21.05)	15 (78.95.)	0.020	7.796
N-stage					
1-2	55	15 (27.27)	40 (72.73)		
0	83	44 (53.01)	39 (46.99)	0.003	0.954
Distant metastasis					
1	37	8 (21.62)	29 (78.38)		
0	101	51 (50.50)	50 (49.50)	0.002	9.224
Dukes' stage					
A+B	70	38 (54.29)	32 (45.71)		
C+D	68	21 (30.88)	47 (69.12)	0.005	7.719

diagnosis of primary CRC and then underwent elective surgery in Nanfang Hospital, Southern Medical University (Guangzhou, China). The use of tissues for this study has been approved by the ethics committee of Nanfang Hospital, Southern Medical University. A total of 138 cases of archived CRC tissue samples were collected and used in clinicopathological and prognostic investigation. No patient received any pre-operative chemotherapy or radiotherapy. A comprehensive set of clinicopathological data were possessed, including age, gender, size of primary tumor, tumor differentiation, T stage, lymph node metastasis and distant metastasis. Complete follow-up, ranging from 1-96 months, was available for the cohort of 138 patients, and the median survival was 54 months.

In situ hybridization and evaluation of staining of miR-182

In situ hybridization (ISH) was performed, as previously described [17]. The sections were prehybridized in a hybridization solution with 40 nM of a locked nucleic acid-modified, 5' digoxigenin (DIG)-labeled oligonucleotide probe of hsa-miR-182 or a scrambled control probe (Exiqon) at a temperature of 50°C overnight. The ISH stained tissue sections were reviewed and scored separately by two pathologists blind to the study. Staining for miR-182 was assessed using a method described previously [18, 19]. On a scale of 0 to 3, the staining intensity was scored as follows: negative (no staining, 0), weak (light yellow, 1), medium (yellowish brown, 2), or strong (brown, 3). The extent of the staining is defined as the percentage of positive staining areas of tumour cells or normal mucosa epithelial cells in relation to the whole tumour area or entire section for the normal samples. The extent of staining was scored on a scale of 0 to 4 as follows: 0, 0%; 1, 1-25%; 2, 26-50%; 3, 51-75%; and 4, 76-100%. The sum of the staining-intensity and staining-extent scores was used as the final staining score for miR-182 (0-7). For statistical analysis, a final staining score of ≥ 3 was regarded as high expression.

Statistical analysis

All statistical analyses were performed using the SPSS 16.0 statistical software package. Differences between variables were assessed

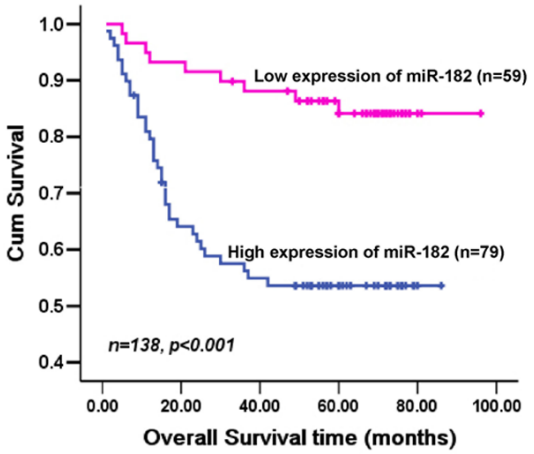

Figure 2. Kaplan-Meier survival analysis for overall survival duration in the 138 CRC patients according to miR-182 expression. The log-rank test was used to calculate *P* value.

by the χ^2 test. Survival curves for the patients with different levels of miR-182 expression were plotted using the Kaplan-Meier method and compared using the log-rank test. Multivariate survival analysis was performed on all parameters that were found to be significant in univariate analysis using the Cox repression model. *P* value of < 0.05 was considered significant.

Results

miR-182 was up-regulated in human primary CRC tissues

We measured miR-182 expression in a large cohort of 138 archived paraffin-embedded CRC and normal colon tissues using in situ hybridization (ISH). We observed miR-182 expression in the cytoplasm of benign and malignant epithelial cells (**Figure 1**). We also observed that 79/138 (57.25%) CRCs had high-level expression of miR-182, while 17/67 (25.37%) normal mucosa tissues had high-level expression of miR-182. The expression level of miR-182 was remarkably up-regulated in CRC tissues compared with non-neoplastic normal tissues (*P* < 0.001).

Overexpression of miR-182 is associated with aggressive phenotypes

The correlation analysis between clinicopathological characteristics and miR-182 level showed high-level expression of miR-182 was significantly associated with T-stage (*P* = 0.020), N-stage (*P* = 0.003), distant metastasis (*P* = 0.002), and Dukes' stage (*P* = 0.005) in patients with CRC, however, not associated with age, sex, tumor site, tumor size, and tumor differentiation degree (*P* > 0.05, **Table 1**).

Overexpression of miR-182 is associated with poor prognosis of patients with CRC

To further evaluate the prognostic value of miR-182 for CRC patients, we also analyzed the association between miR-182 expression and survival duration using Kaplan-Meier analysis with the log-rank test. The results revealed that high-level expression of miR-182 was correlated with short survival time of patients with CRC (Log Rank = 15.228, *P* < 0.001, **Figure 2**). High-level of miR-182 was associated with short survival time (53.02 ± 4.10 *vs.* 85.02 ± 3.47).

Univariate and multivariate analyses of prognostic variables in CRC patients

To determine whether expression of miR-182 is an independent prognostic factor for CRC, univariate and multivariate analyses were performed to determine the prognostic value of clinicopathological variables including sex, age, tumour site, tumour size, differentiation grade, T-stage, N-stage, distant metastasis, and Dukes' stage in patients with CRC. The results showed that high-level expression of miR-182 is an independent prognostic factor for poor survival of patients with CRC (*P* < 0.05, **Table 2**).

Discussion

Over the last decade, it was become evident that miRNAs are involved in the pathogenesis of several human diseases, including cancer. Dysregulated expression of miR-182 has been reported in a number of cancers [9, 10] and directly involves in human cancer processes, such tumorigenesis, migration and metastasis [11-14, 20]. Theses results suggested that miR-182 may play important roles in cancer progression and exert different effects in various types of cancer. However, little is know about the relationship between the expressions of miR-182 in CRC with the prognosis of CRC patients. This study focused on the potential relationship between the expression level of miR-182 and various clinicopathological char-

Table 2. Summary of Overall survival analyses by univariate and multivariate COX regression analysis

Variables	Univariate analysis			Multivariate analysis		
	P value	HR	95% CI	*P* value	HR	95% CI
Gender	0.708	0.891	0.488-1.628			
Age	0.699	0.891	0.496-1.600			
Tumor site	0.957	1.009	0.716-1.424			
Tumor size	0.513	1.240	0.651-2.634			
Tumor differentiation	0.067	1.841	0.957-3.540			
T-stage	0.010	2.128	1.200-3.773	0.240	1.471	0.772-2.904
N-stage	0.000	3.390	1.851-6.207	0.528	1.256	0.619-2.547
M-stage	0.000	10.286	5.449-19.417	0.001	3.880	1.700-8.852
Dukes' stage	0.000	10.158	4.286-24.072	0.042	3.393	1.044-11.033
miR-182 expression	0.012	3.853	1.853-8.013	0.013	2.624	1.222-5.633

acteristics of CRC patients, as well as overall survival. miRNAs are well preserved in formalin-fixed tissue, making them attractive candidates for use in routinely processed tissue materials [21]. Using qRT-PCR, previous studies have revealed that the miR-182 expression is associated with poor overall survival of malignant glioma [22] or CRC patients [10]. The studies on miRNA clinical-prognosis were done by qRT-PCR using RNA extracted from human cancer tissue blocks. Cancer tissue block contains a mixture of neoplastic tumor cells and tumor related stromal cells. Using RNA extracts from whole tumors may have probably led to erroneous results for biomarker [18]. Recent data have suggested that some miRNAs had high expression levels in stromal cells [23, 24]. A major advantage of *in situ* hybridization is the ability to precisely identify positive signals at the celluar level.

In our study, we observed that miR-182 expression in the cytoplasm of benign and malignant epithelial cells and that miR-182 overexpression was significantly associated with T-stage, lymph node metastases, distant metastasis and Dukes' stage of CRC patients. The expression level of miR-182 was inversely correlated with overall survival. CRC patients with higher miR-182 had a shorter survival. A multivariate Cox proportional hazard regression analysis revealed that miR-182 overexpression was a worse prognostic impact on the overall survival of CRC patients independent of distant metastasis. The results indicate that, as an independent risk factor, miR-182 could serve as a prognostic marker for the survival of CRC patients. To date, several studies have revealed the prognostic significance of miR-182 in various carcinomas, such as malignant glioma [22] or CRC [10], which indicated that the up-regulation of miR-182 could be regarded as a predicted factor of cancer patients. To the best of our knowledge, we present the first large-scale study that combines in situ hybridization to evaluate the prognostic impact of miR-182 expression in CRC.

In summary, the results of our study indicate that the expression of miR-182 is strongly correlated with cancer progression and overall survival times of patients with CRC, providing evidence that up-regulation of miR-182 might play an important role in the progression of CRC. It suggests that miR-182 could be considered as a novel therapeutic target for patients with CRC.

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Disclosure of conflict of interest

None.

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