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Upregulated miR-410 is linked to poor prognosis in colorectal cancer

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ABSTRACT

Background: Although miR-410 acts as a cancer inducer in colorectal cancer, there is limited data on the clinical implications of miR-410 expression levels in patients. We hypothesized a link between miR-410 expression and its potential clinical values in patients with colorectal cancer.

Material and methods: 120 colorectal cancer tissue specimens and 120 adjacent non-tumour tissues were obtained. Quantification of miR-410 expression levels was determined by, quantitative RT-PCR. Expression was analysed by clinical features.

Results: miR-410 was up-regulated in malignant tissues compared with corresponding normal tissues ($P < 0.01$), with TNM stage and lymph node metastasis ($P = 0.03$, $P = 0.004$, respectively), and with worse overall survival ($P = 0.002$). Multivariate survival analysis identified it as an independent risk factor for outcome ($P = 0.021$, HR = 2.19; 95% CI = 1.12–4.25).

Conclusion: Compared to normal non-cancerous tissues, miR-410 was overexpressed in tumour tissues and is independently associated with the unfavourable outcome. Levels of MiR-410 might a useful laboratory tool in managing and predicting the prognosis of colorectal cancer.

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Introduction

Colorectal cancer is the most frequent type of cancer worldwide [1,2]. Whilst recent advances in diagnosis and treatment led to improved survival, due to insufficient predictive markers for clinical outcomes, it is still a leading causes of cancer mortality. Therefore, discovering novel and reliable predictive and prognostic biomarkers is critical for effective treatment and improving the clinical outcome of patients.

MicroRNAs (miRNAs) are single-stranded, short (comprised of 19–25 nucleotide in length), non-coding RNAs that function in the regulation of gene expression by degrading target messenger RNA (mRNA) or repressing translation of protein [3]. By targeting about 60% of protein-coding genes, miRNAs contribute to regulating a wide array of cellular functions such as cell cycle regulation, cell proliferation, cell differentiation, and apoptosis [4]. Furthermore, miRNAs are believed to involved in carcinogenesis and their deregulation is related to development, progression, invasion, and metastasis of different types of malignancy including colorectal cancer [4–7]. Moreover, specific miRNAs represent significant alterations of expression in cancerous colorectal cancer tissues [8]. Therefore, altered expression levels of miRNAs are considered to be a potential diagnostic, predictive and prognostic factor in colorectal cancer [9].

MiR-410 has the potential to function as a tumour inducer or a tumour suppressor in various types of cancers [10]. Its oncogenic functions have been reported in retinoblastoma, lung, liver and colorectal cancers where it regulates BAK1, FHL1, CETN3, and BRD7, and functions as a tumour suppressor in pancreatic, bone, gastric and breast cancers by regulating c-MET, AGTR1, and SNAIL [10]. In colorectal cancer, miR-410 is involved in different tumorigenic processes including proliferation, migration, invasion, and apoptosis by regulating FHL1, ITPKB, DKK-1, and Bak1 [11–14]. Furthermore, overexpression of miR-410 was previously reported in colorectal cancer patients and cell lines [11,12,14]. However, there is limited knowledge concerning the clinical implication of miR-410 expression in colorectal cancer. We therefore hypothesized links between the expression of mi-410 in malignant tissue and clinical features, and outcome survival in patients with colorectal cancer.

Materials and methods

Formalin-fixed paraffin-embedded (FFPE) malignant and nearby normal tissues were obtained from 120 patients with colorectal cancer. Patients who received any chemo/radiotherapy before surgery were excluded. Table 1 shows clinicopathological parameters of patients in the

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Table 1. The correlation between clinicopathological variables and miR-410 expression.

Variables	High miR-410 expression	Low miR-410 expression	Hazard ratio (95% CI)	P-value
Gender Female/ Male	38/30	22/30	1.31 (0.92–1.86)	0.14
Age (years)	60.4 [11.8]	61.6 [10.5]	-	0.56
Tumour location: Rectum/Colon	30/30	21/39	1.35 (0.95–1.93)	0.09
Local invasion T1-T2/T3-T4	41/19	35/25	1.25 (0.84–1.86)	0.25
Differentiation Well-Moderate /Poor	28/32	35/25	0.79 (0.55–1.13)	0.20
Lymph node metastasis Present/Absent	28/32	13/47	1.69 (1.20–2.37)	0.004
TNM stage I–II/III–IV	28/32	40/20	0.67 (0.47–0.95)	0.03

Age expressed as mean [SD].

study. The overall survival was measured from the date of initial surgery to date of death or the last contact. Tissues were staged by the tumour node metastasis (TNM) staging system [15]. This study was performed in accordance with the Declaration of Helsinki and informed consent was obtained from all participants.

Four to five sections (10 µm) per sample from the interior of the FFPE blocks were used for total RNA preparation. The sections were placed in 1.5 mL microtube and 1 ml xylene was added to each tube and mixed thoroughly. Afterwards, the tubes were incubated at room temperature for 2 min after vortexing centrifuged at maximum speed for 1 min. The supernatant was removed and pellets were rinsed twice with 1 ml absolute ethanol to remove residual xylene. Finally, the samples were air-dried until evaporation of all residual ethanol. Total RNA was extracted through an RNeasy FFPE kit (Qiagen, Hilden, Germany) based on the manufacturer's instruction. RNA concentration and purity were assessed using a NanoDrop Spectrophotometer.

MiR-410 expression levels were quantified by the stem-loop RT-qPCR assay. The stem-loop primers for the reverse transcription (RT) and amplification primers were designed according to the previously developed method [16]. After reverse transcription of 100 ng of isolated RNA using the PrimeScript RT reagent kit (Takara, Japan) and a mixture of stem-loop RT primers, cDNA was used for RT-qPCR on StepOnePlus Real-Time PCR System (Applied Biosystems, USA). The sequences of the stem-loop primers were as follows: 5′ CGTCGTACTCAACGTGGTTA GGGTCCGAGGTATAGGTTCCCACGTGGAGGACGACGACA GGC-3′ (miR-410) and 5′-CGTCGTACTCAACGTGGTTA GGGTCCGAGGTATAGGTTCCCACGTGGAGGACGACGAAT-ATG-3′ (U6). SYBR Green-based RT-qPCR was done using the following primers: 5′-GGGCGCGAATATAACACAGAT -3′ (specific forward primer for miR-410), 5′- GGATG ACGCAAATTCGTGAAGC-3′ (specific forward primer for U6) and reverse universal primer 5′- CGTGGTTA GGGTCCGAGGTA-3′. The 25-µl RT-qPCR reactions mixture contained 12.5 µl of RealQ Plus 2x Master Mix Green (Ampliqon), 2 µl of cDNA, 0.5 mL of each forward and reverse primer and 9.5 mL ddH2O. The RT-qPCR condition was as follows: 95°C for 15 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. To estimate the specificity of the primers, the melt curve was conducted at the end of each RT-qPCR run. All RT-qPCR assays were run in duplicate. miR-410 expression levels were normalized to the U6 snRNA as a reference gene. The relative miR-410 expression levels were determined using the $2^{-\Delta Ct}$ method where ΔCt = CtmiR-410-CtU6.

Data were statistically analysed using SPSS 20.0 (IBM Corporation, Chicago, NY, USA) and GraphPad Prism 7 (GraphPad Software, Inc, La Jolla, CA, USA) software. Comparison between matched tumour and normal tissues according to the miR-410 expression levels was made using paired samples t-test. The prevalence of clinical-pathological variables among different miR-410 expressing groups was compared by Pearson's Chi-square test. For comparison of the overall survival between different miR-410 expressing groups of patients Kaplan–Meier curves with log-rank test was plotted. Univariate and multivariate analyses were conducted with Cox proportional hazard model to evaluate the prognostic utility of miR-410 expression for the outcome survival of patients [17]. A P-value < 0.05 was considered as statistically significant.

Results

We applied quantitative RT-qPCR assay to quantify miR-410 expression in the cancerous and paired non-cancerous tissues in 120 colorectal cancer patients. As depicted in Figure 1, our results revealed marked miR-410 up-regulation in malignant tissues at median [IQR] 1.37 [0.46–2.59] compared to the paired matched

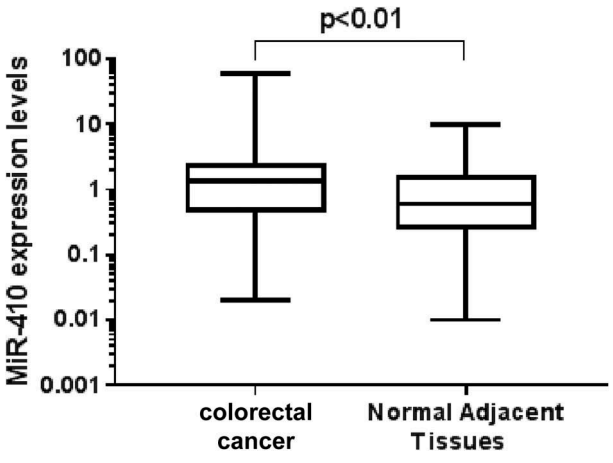

Figure 1. Comparison of the miR-410 expression determined by RT-qPCR in 120 matched cancerous vs 120 non-cancerous tissues.

normal tissues at 0.61 [0.25–1.64] ($P < 0.01$). To evaluate the clinicopathological relevance of aberrant miR-410 expression in colorectal cancer patients, the median expression level of miR-410 was used to categorize the patients into low-expressed and high-expressed miR-410 groups. Up-regulation of miR-410 was linked with lymph node metastasis and TNM stage but not with sex, age, tumour location, local invasion or tumour grade (Table 1).

High-expressed miR-410 patients exhibited a poorer outcome survival ($P = 0.002$), compared with low-expressed miR-410 patients (Figure 2). The predictive role of miR-410 expression in the clinical outcome was confirmed by Cox regression analyses (HR = 2.71, 95% CI, 1.41–5.23, $P = 0.003$). Moreover, multivariate Cox analysis showed that up-regulated miR-410 was a prognostic predictor for worse outcome survival that is independent of other risk factors (Table 2), although of less predictive value than both degree of differentiation and TNM stage.

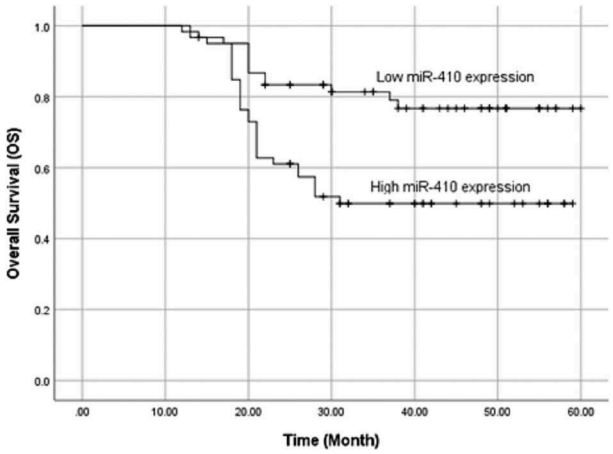

Figure 2. Kaplan–Meier curves with log-rank test of overall survival rates for 120 matched cancerous vs 120 non-cancerous tissues, stratified by miR-410 expression. The higher miR-410 expression group tended to have an inferior survival compared to low-expressed miR-410 group ($P = 0.002$).

Table 2. Univariate and multivariate analyses of risk factors for overall survival.

	Univariate analysis		Multivariate analysis	
Parameters	Risk ratio (95% CI)	*P*-value	Risk ratio (95% CI)	*P*-value
Sex (men vs women)	1.33 (0.72–2.44)	0.35	-	-
Age (>60 vs <60)	1.72 (0.94–3.16)	0.08	-	-
Differentiation (poor vs well/ moderate)	3.56 (1.82–6.96)	<0.001	2.61 (1.31–519)	0.006
Lymph node metastasis (present vs absent)	3.22 (1.74–5.97)	<0.001	1.38 (0.62–3.09)	0.431
TNM stage (III/ IV vs I/II)	6.01 (2.9–12.61)	<0.001	5.78 (2.16–15.5)	<0.001
hsa-miR-410 (high vs low)	2.71 (1.41–5.23)	0.003	2.20 (1.12–4.25)	0.021

Discussion

Detection of specific prognostic and predictive markers is essential in identifying clinical outcomes and to determine optimal therapeutic options in colorectal cancer. Recent evidence has demonstrated that abnormal miRNAs expression levels are involved in the process of carcinogenesis. Therefore, it is now widely accepted that miRNAs could serve as potential diagnosis and prognosis biomarkers in various types of cancers [9,18,19]. Among the miRNAs, miR-410 regulates multiple cellular functions, including angiogenesis, differentiation, and proliferation [19,20]. Therefore, miR-410 may have a key function in the tumorigenesis process in different types of human cancer.

Contradictory results regarding functions of miR-410 in tumorigenesis have been reported [10]. In breast cancer cells, it is indicated that miR-410 acts as a cell cycle regulator through negative regulation of pRb/E2 F pathway [21]. In a recent study, Chen and colleagues reported that miR-410 plays a tumour inhibitory role in glioma cells by regulating MET-induced AKT signal pathway [22]. Moreover, Guo et.al indicated a tumour suppressor function for miR-410 in pancreatic cancer by targeting AGTR1 [23]. Unlike the above-mentioned types of cancers, miR-410 plays an oncogenic role in liver cancer and colorectal cancer by silencing FHL1 and in lung cancer by targeting BRD7 [24]. These data support both tumour-promoting and -suppressive roles for miR-410 depending on different cancer types [10].

The ability of miR-410 to function as an oncogene in colorectal cancer development and progression has been demonstrated previously not only by its overexpression in colorectal cancer tissues/cell lines but also by its regulatory roles in cell proliferation and apoptosis through targeting FHL1, ITPKB and, Bak1 [11–13]. However, the clinical value of miR-410 expression in colorectal cancer is not yet established. We assessed miR-410 expression in colorectal cancer patients and the results were evaluated for clinicopathological associations. In line with previous studies by Liu et al. [12] and Wang et al. [11], we first found a higher miR-410 expression in malignant tissues compared with noncancerous tissues suggesting that miR-410 might be implicated in pathogenesis. So far, in addition to colorectal cancer, overexpression of miR-410 was demonstrated in various types of human cancers such as prostate [25], lung [24], and liver cancers [11] emphasizing the oncogenic role of the miR-410.

Furthermore, by assessing the clinicopathological relevance of miR-410 expression, we found that elevated miR-410 levels were significantly associated with positive lymph nodes metastasis and TNM stage, suggesting that up-regulation of miR-410 may be involved in the clinical progression. In addition, Kaplan-Meier analysis showed that up-regulation of miR-410 was significantly linked with poorer outcome. This

predictive role of high miR-410 expression on outcome was further confirmed by on the results obtained from univariate Cox regression analyses. Moreover, multivariate Cox regression analysis demonstrated that miR-410 up-regulation was independently associated with the poor outcome.

Up-regulation of miR-410 highlights its possible role in pathogenesis, and several distinct mechanisms have been described for the oncogenic function of miR-410 in this disease. Wang and colleagues indicated that dickkopf-related protein 1 (DKK-1) is a target of miR-410 [14] and that silencing of miR-410 in colorectal cancer cells inhibits cell proliferation and induces apoptosis. In another study, Wang and colleagues revealed that miR-410 is a negative regulator of FHL1 by targeting its mRNA directly or inducing its promoter methylation in colorectal cancer cells indirectly, thereby, influencing the growth and migration of colorectal cancer cells [11]. Liu et.al reported that miR-410 functions as an anti-apoptotic factor in colorectal cancer cells by targeting the pro-apoptotic protein Bak1 [12]. This suggests that miR-410 contributes to multistep tumorigenesis by regulating different tumorigenic processes. However, it is crucial to investigate the precise mechanisms through which miR-410 participates in colorectal cancer tumorigenesis to use this miRNA as a potential prognostic indicator and promising therapeutic target for patients with this disease.

Our data are an advance in biomedical science as it demonstrates that miR-410 overexpression colorectal cancer tissue is associated with the poorer outcome, and so could useful additional marker.

Summary table

What is known about this subject:
- miR-410 functions as a tumour suppressor in many cancers.
- Up-regulation of miR-410 has been previously reported in patients with colorectal cancer and in cell lines.

What this study adds:
- High miR-410 transcription level is related with TNM stage and lymph node metastasis.
- miR-410 up-regulation is linked to overall survival.

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Disclosure statement

The authors have no conflict of interest to declare.

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