Research Article

Cancer Epidemiology, Biomarkers & Prevention

Circulating Exosomal miR-27a and miR-130a Act as Novel Diagnostic and Prognostic Biomarkers of Colorectal Cancer

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Abstract

Background: Colorectal cancer is one of the most common cancers worldwide usually is associated with poor prognosis due to the advanced stage when diagnosed. This study aimed to investigate whether specific circulating exosomal miRNAs could act as biomarkers for early diagnosis of colorectal cancer.

Methods: A total of 369 peripheral blood samples were included in this study. In the discovery phase, circulating exosomal miR-27a and miR-130a were selected after synthetical analysis of two GEO datasets and TCGA database. The differential expression and diagnostic utility of miR-27a and miR-130a panel were validated using qRT-PCR and ROC curve analysis in subsequent training phase, validation phase, and external validation phase. The prognosis of circulating exosomal miR-27a and miR-130a were investigated using the Kaplan–Meier method.

Results: The expression of exosomal miR-27a and miR-130a in plasma significantly increased in colorectal cancer. The area under ROC curves (AUC) of miR-27a (miR-130a) were 0.773 (0.742) in the training phase, 0.82 (0.787) in the validation phase, and 0.746 (0.697) in the external validation phase. The combination of two miRNAs presented higher diagnostic utility for colorectal cancer (AUCs = 0.846, 0.898, and 0.801 for the training, validation, and external validation phases, respectively). Patients with colorectal cancer with high expression of circulating exosomal miR-27a or miR-130a underwent poorer prognosis.

Conclusions: We identified a circulating exosomal miRNAs panel for the detection of colorectal cancer.

Impact: The exosomal miR-27a and miR-130a panel in plasma may act as a noninvasive biomarker for early detection and predicting prognosis of colorectal cancer. *Cancer Epidemiol Biomarkers Prev; 27(7); 746–54. ©2018 AACR.*

Introduction

Colorectal cancer is the second most common cancer in females and the third most common cancer in males, with more than 50,000 cancer-related deaths every year worldwide (1). The prognosis of patients has been demonstrated to be associated with the tumor stage when diagnosed, with almost 90% of mortality could be prevented if diagnosed at early stage (2). Although the survival rates of patients with colorectal cancer have improved in recent years due to early detection of the disease via various colorectal cancer screening tests, such as fecal occult blood test (FOBT) and colonoscopy (3, 4), the lack of high sensitivity and particular specificity of FOBT, and the invasiveness and expensive cost of colonoscopy make both of them not suitable for screening the general population. Besides, carcinoembryonic antibody (CEA), carbohydrate antibody 19-9 (CA19-9) and carbohydrate antibody 72-4 (CA72-4), the widely used serum tumor biomarkers, are neither very sensitive nor specific (5). Therefore, novel noninvasive biomarkers in serum or plasma for diagnosis and prognosis are urgently in need.

Mounting efforts have been devoted to discover protein-based, DNA-based, RNA-based, or circulating tumor cell–based screening tests, and miRNA is one of the most promising molecular biomarkers for tumor early diagnosis and prediction of prognosis (6). miRNAs, a class of small noncoding RNA molecules, are common 19–25 nucleotides in length. miRNAs regulate gene expression at a posttranscriptional level through direct binding to the 3′-untranslated region (UTR) of target mRNAs, leading to the inhibition of mRNAs translation or mRNAs degradation (7). In contrast to most previous studies about miRNA biomarkers focused on tissue specimens, some researches have reported the potential diagnostic and prognostic role of circulating miRNAs (8, 9). Tumor-derived circulating miRNAs could exist in plasma or serum stably due to their ability of resisting to endogenous ribonuclease activity, extreme pH, and temperature (10). One explanation for this phenomenon is that miRNAs could be packaged into exosomes and then secreted to peripheral blood by cancer cells (11).

Exosomes, kinds of lipid vesicles with a diameter of 40 to 100 nm, were first discovered from sheep reticulocytes in 1983 (12). Exosomes are remarkably stable in body fluids, and could carry various contents from donor cells to recipient cells, including proteins, lipids, DNA, and RNA (12, 13). Emerging evidence has demonstrated that exosomes and their delivered miRNAs

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were recognized as novel potential biomarkers in various cancers, including colorectal cancer (14, 15). Matsumura and colleagues reported that exosomal miRNA in serum could serve as a novel biomarker of recurrence in human colorectal cancer (16); Tsukamoto and colleagues declared that circulating exosomal miR-21 is a promising biomarker in each tumor stage of colorectal cancer (17); Machida and colleagues demonstrated that miR-1246 and miR-4644 in salivary exosome are potential biomarkers for pancreatobiliary tract cancer (18). However, the information regarding the relationship between the circulating exosomal miRNA expression profiles and the diagnosis along with the prognosis of colorectal cancer is currently elusive. The aim of this study is to clarify the potential of circulating exosomal miRNAs as a biomarker for diagnosis and prognosis of patients with colorectal cancer.

Materials and Methods

Study population

All patients with colorectal cancer were recruited from Nanjing First Hospital affiliated to Nanjing Medical University. The surgical resected tumor specimens were confirmed through histopathologic analysis and the tumor stage was determined according to the tumor–node–metastasis (TNM) system. None of patients enrolled in this study had received therapy when specimens collected. Healthy controls were collected from volunteers participating in the physical examination. All participants in this study had signed the informed consent before starting the study. This study was approved by the Research and Ethical Committee of Nanjing First Hospital.

Study design

This study included 369 participants and four phases, shown in Supplementary Fig. S1. In the discovery phase, we conducted an integrated analysis of miRNAs significantly associated with prognosis of patients with colorectal cancer using one Gene Expression Omnibus (GEO) dataset and The Cancer Genome Atlas (TCGA) database. Then another GEO dataset (GSE39833) was utilized to investigate the expression profiles of miRNAs selected between healthy controls and patients with colorectal cancer as well as the diagnostic role of circulating exosomal miR-27a and miR-130a. In the training phase, the expression of exosmal miR-27a and miR-130a in plasma were explored by qRT-PCR in 80 samples (40 healthy subjects and 40 patients with colorectal cancer at stage I. These 80 samples were used to estimate diagnostic parameters of exosomal miR-27a and miR-130a in plasma. In the validation phase, the diagnostic parameters of miR-27a and miR-130a calculated in the training phase was further assessed in 120 subjects (40 patients with colorectal cancer at stage I, 20 patients with colorectal cancer at stage II, 14 patients with colorectal cancer at stage III, 6 patients with colorectal cancer at stage IV, and 40 healthy subjects). In the external validation phase, the diagnostic role of miR-27a and miR-130a was independently evaluated in additional 150 subjects (50 patients with colorectal cancer at stage I, 50 adenomas patients, and 50 healthy subjects). There was no significant difference in the factors of age, gender, and drinking status between three groups. The clinical characteristics of these subjects are shown in Table 1.

Transmission electron microscopy

The isolated exosomes were dissolved in PBS, and a drop of the suspension was placed on a sheet. A carbon-coated copper grid was floated on the drop for 10 seconds. The grid was then removed and excess liquid was drained with filter paper. The grid was put in contact with a drop of 2% uranyl acetate of phosphotungstic acid, and excess liquid was removed. The grid was allowed to dry for several minutes and was then examined using the electron microscope H-7600 (Hitachi High-Technologies Corporation).

Purification of exosomes and extraction of exosomal RNAs

Exosomes in plasma and medium were purified by Invitrogen Total Exosome Isolation Kit (Invitrogen) according to the manufacture's instruction. Briefly, proteinase K and plasma/medium specimens were mixed and incubated for 30 minutes at 4°C. Then, the precipitated exosomes were recovered by centrifugation at 10,000 × *g* for 5 minutes at room temperature, and resuspended in PBS. miRNeasy Mini Kit (Qiagen) was used to extract RNA from exosomes in plasma/medium according to the product protocol, and cel-miR-39 (Takara) was added into each sample at a final concentration of 10^{-4} pmol/μL acting as external reference. Total RNA was stored at −80°C for subsequent experiments.

Table 1. Clinical characteristics of the subjects included in this study

	Discovery phase		Training phase		Validation phase		External validation phase		
Variables	HC	Colorectal cancer	HC	Colorectal cancer	HC	Colorectal cancer	HC	Adenoma	Colorectal cancer
Number	11	88	40	40	40	80	50	50	50
Age (mean ± SD)	51.0 ± 2.9	48.1 ± 3.6	52.8 ± 5.8	54.2 ± 4.1	52.8 ± 5.8	51.4 ± 6.2	53.3 ± 8.7	55.8 ± 7.1	52.1 ± 9.2
Gender									
Male	8	67	25	24	25	53	32	31	34
Female	3	21	15	16	15	27	18	19	16
Drinking status									
Never			16	14	16	30	19	17	15
Ever			34	36	34	50	31	33	35
Tumor site									
Colon				25		49		34	36
Rectum				15		31		16	14
TNM stage									
I		20		40		40			50
II		20				20			
III		36				16			
IV		12				4			

Table 2. Clinicopathologic characteristics of patients with colorectal cancer

	High miR-130a	Low miR-130a	*P*	High miR-27a	Low miR-27a	*P*
Gender						
Male	34	46		38	42	
Female	26	24	0.290	26	24	0.618
Age						
≥64	27	41		29	39	
<64	33	29	0.122	35	27	0.116
Tumor site						
Colon	25	33		34	24	
Rectum	35	37	0.531	30	42	0.055
TNM stage						
I+II	46	64		49	61	
III+IV	14	6	0.020	15	5	0.012
Histologic grade						
Well+moderate	13	28		22	19	
Poorly	47	42	0.025	42	47	0.493
Lymphoma metastasis						
Yes	11	9		12	8	
No	49	61	0.221	52	58	0.295
Distant metastasis						
Yes	3	4		3	4	
No	57	66	0.857	61	62	0.729

Cell lines and culture conditions

The colonic mucosal epithelial cell (FHC) and colorectal cancer cell lines (HCT116, HCT8, SW480, SW620, and HT29) were purchased from ATCC and had been tested and authenticated through short tandem repeat (STR) method in 2017. All cell lines were cultured in DMEM supplemented with 100 μL FBS, 10 μL penicillin, and 10 μL streptomycin per milliliter media in humidified atmosphere containing 5% CO_2 at 37°C. All cell lines as prescribed above were obtained from the Chinese Academy Medical Science. For exosome isolation, 1×10^6 cells were seeded into six-well culture plates and cultured for 48 hours.

Eosomal miRNAs quantitation

Reverse transcription and qRT-PCR for exosomal miR-27a, miR-130a, and external reference miR-39 were performed using Hairpin-it miRNA RT-PCR Quantitation Kit (GenePharma) according the manufacturer's instructions. The reactions were initiated with denaturation at 95°C for 3 minutes, followed by 40 cycles of 95°C for 15 seconds and 62°C for 34 seconds. The relative expression levels of exosomal miR-27a and miR-130a were calculated with $2^{-\Delta\Delta C_t}$.

Cell transfection and mRNAs quantitation

A total of 5×10^5 SW480 cells were seeded into six-well plates and grown to 60% confluency in complete medium. Then cells were transfected with 100 nmol/L miR-27a inhibitor, miR-130a inhibitor, and corresponding negative control (NC), respectively, using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. After 48 hours transfection, total RNA was isolated from cells using Trizol LS reagent (Invitrogen). Real-time PCR for detecting mRNA expression were performed in 20 μL reactions following the SYBR Premix Ex TaqTM Kit (Takara) and GAPDH was used as internal control. Two micrograms of RNA was reverse transcribed and cDNA was synthesized using PrimeScript RT-PCR Kit (Takara). The primers of mRNAs were listed in Table 3. The relative expression levels of mRNAs were calculated with $2^{-\Delta\Delta C_t}$.

Statistical analysis

All data were presented as mean ± SD. Clinicopathologic characteristics among groups were compared by χ^2 test. The different expression of miRNAs among groups were determined using the Mann–Whitney unpaired test or paired *t* test. ROC curve and area under the ROC curve (AUC) were established for

Table 3. The primers of downstream genes of Wnt/β-catenin pathway (CD44, TCF4, cyclin D1, and c-myc) and TGFβ pathway (SMAD2 and SMAD4)

Gene names	Primer sequences	
CD44	Forward	5′-GCAGGTATGGGTTCATAGAAGG-3′
	Reverse	5′-GGTGTTGGATGTGAGGATGT-3′
TCF4	Forward	5′-CCACCCATTTCTTTGCTGAAC-3′
	Reverse	5′-CCCTGACTCTTAACACCAACTC-3′
Cyclin D1	Forward	5′-GGGTTGTGCTACAGATGATAGAG-3′
	Reverse	5′-AGACGCCTCCTTTGTGTTAAT-3′
c-myc	Forward	5′-TGAGGAGGAACAAGAAGATG-3′
	Reverse	5′-ATCCAGACTCTGACCTTTT-3′
SMAD2	Forward	5′-ACTAACTTCCCAGCAGGAA-3′
	Reverse	5′-GTTGGTCACTTGTTTCTCCA-3′
SMAD4	Forward	5′-AGTAACGATGCCTGTCTGA-3′
	Reverse	5′-TGAAGTCGTCCATCCAAT-3′
GAPDH	Forward	5′-GCACCGTCAAGGCTGAGAAC-3′
	Reverse	5′-ATGGTGGTGAAGACGCCAGT-3′

discriminating patients with colorectal cancer from healthy individuals. Cut-off value of the relative expression of exosomal miR-27a and miR-130a in plasma were determined by Youden index from ROC curves. The diagnostic models were constructed using binary logistic regression analysis. Survival curves were plotted using the Kaplan–Meier method and then compared with the log-rank test. All statistical analyses were carried out using SPSS 19.0 (IBM) and GraphPad 5.0 (GraphPad Software). A P value < 0.05 was considered statistically significant.

Results

Expression of tissular miR-27a and miR-130a were significantly associated with prognosis of patients with colorectal cancer

First, we found a GEO dataset (GSE33961) which conducted a comprehensive comparative analysis of miRNA expression in patients with colorectal cancer with long (≥5 years) or short (<5 years) time survival after surgery. Forty-six upregulated miRNAs and five downregulated miRNAs were found in patients with short time survival as compared with that in patients with long time survival (Fig. 1A–D). Next, we further validated these deregulated miRNAs in TCGA colon adenocarcinoma (COAD) dataset. Only eight miRNAs (let-7g, miR-27a, miR-31, miR-99a, miR-125b-1, miR-130a, miR-214, and miR-337) exhibited a consistent result that these deregulated miRNAs were significantly associated with prognosis of patients with colorectal cancer (Supplementary Fig. S2). Given the prognostic role of these eight miRNAs in colorectal cancer tissues, we wonder whether these deregulated miRNAs could be packaged into exosomes and secreted to plasma serving as noninvasive diagnostic biomarkers of colorectal cancer.

Circulating exosomal miR-27a and miR-130a act as a novel potential diagnostic biomarker for colorectal cancer

To investigate the diagnostic role of circulating exosomal miRNAs, another GEO dataset (GSE39833) was analyzed. Among eight selected miRNAs, only miR-27a and miR-130a were significantly deregulated (Supplementary Fig. S3). ROC curve analysis was performed to assess the diagnostic role of circulating exosomal miR-27a and miR-130a. When distinguishing healthy subjects from patients with colorectal cancer with stage I, the AUC was 0.868 [95% confidence interval (CI), 0.738–0.999] for miR-27a with 85% sensitivity and 90.91% specificity, 0.850 (95% CI, 0.711–0.989) for miR-130a with 75% sensitivity and 100% specificity, and 0.936 (95% CI, 0.854–1.019) for two miRNAs combination with 90% sensitivity and 90.91% specificity (Fig. 2A). When distinguishing healthy subjects from patients with colorectal cancer with various stages, the AUC was 0.866 (95% CI, 0.774–0.957) for miR-27a with 81.82% sensitivity and

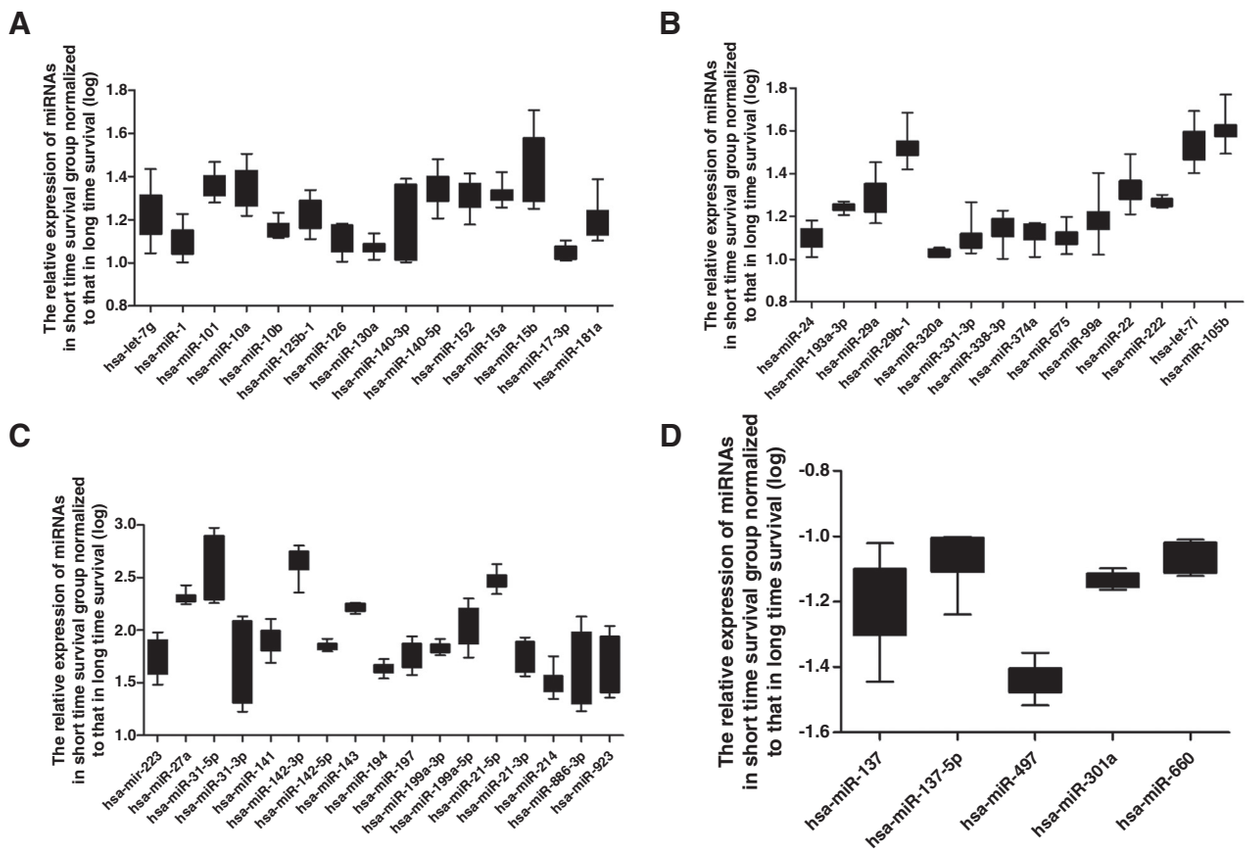

Figure 1.

Expression of tissular miRNAs significantly associated with prognosis of patients with colorectal cancer. **A–D,** Differential tissular miRNAs of patients with colorectal cancer with short survival time (<5 years) as compared with patients with long survival time (≥5 years) in GEO dataset. All P values <0.05.

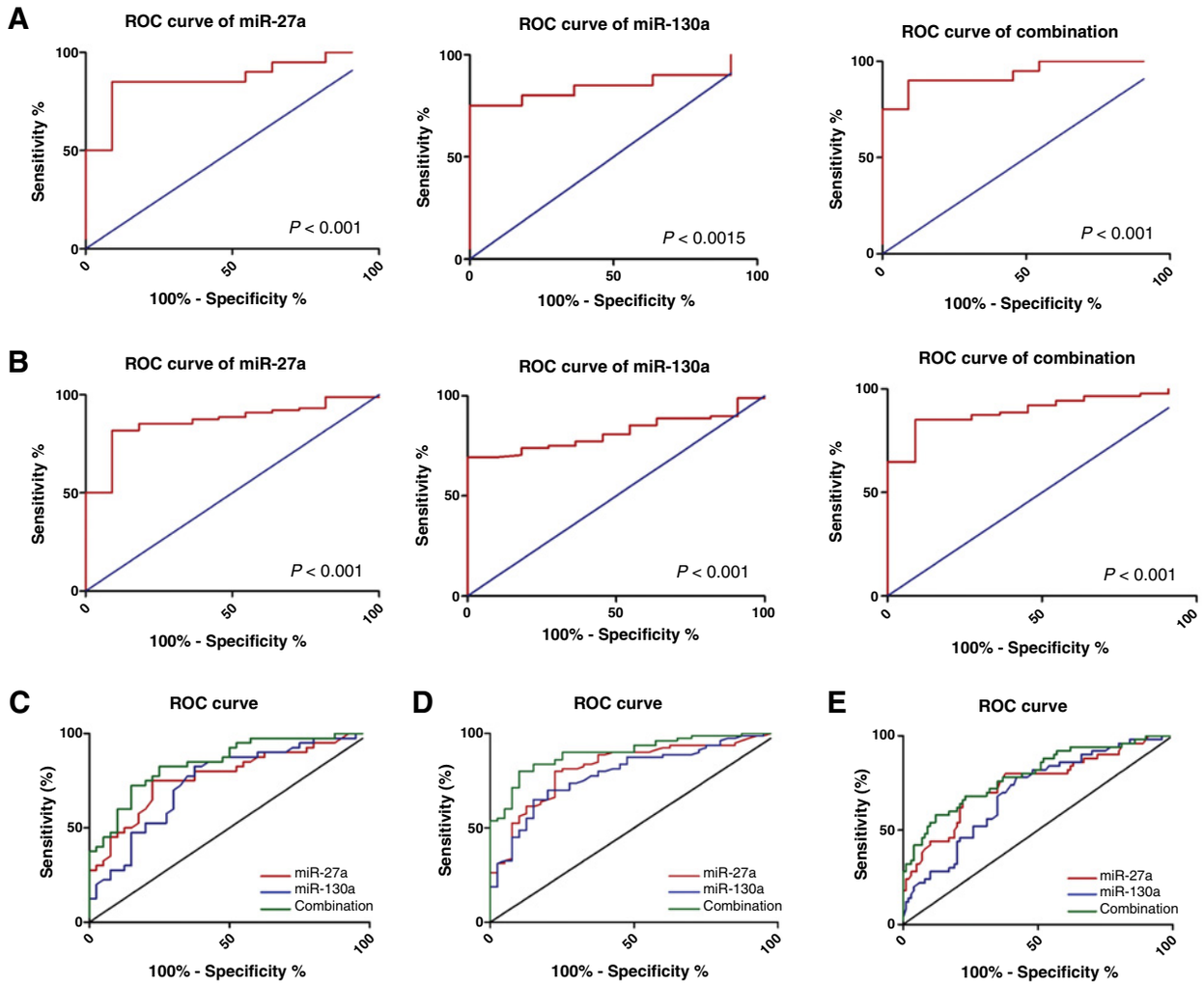

Figure 2.

Circulating exosomal miR-27a and miR-130a panel serves as novel diagnostic biomarker of colorectal cancer. **A,** ROC curves for the two-miRNA panel to discriminate patients with colorectal cancer with stage I from healthy controls in GSE39833. **B,** ROC curves for circulating exosomal miR-27a and miR-130a panel to discriminate patients with colorectal cancer with various stage from healthy controls in GSE39833. **C,** ROC curves for miR-27a and miR-130a panel to discriminate patients with colorectal cancer with stage I from healthy controls enrolled in these study. **D,** ROC curves for exosomal miR-27a and miR-130a panel to discriminate patients with colorectal cancer from healthy controls enrolled in these study. **E,** ROC curves for exosomal miR-27a and miR-130a panel in plasma to discriminate patients with colorectal cancer from patients with adenoma and healthy controls enrolled in these study.

90.91% specificity, 0.816 (95% CI, 0.73–0.901) for miR-130a with 69.32% sensitivity and 100% specificity, and 0.899 (95% CI, 0.854–1.019) for two miRNAs combination with 85.23% sensitivity and 90.91% specificity (Fig. 2B). Data from GSE39833 showed that circulating exosomal miR-27a and miR-130a were novel potential diagnostic biomarkers of colorectal cancer.

Performance of the exosomal miR-27a and miR-130a for early detection of colorectal cancer

To explore the potential diagnostic role of exosomal miR-27a and miR-130a in plasma, a cohort composed of 40 patients with colorectal cancer with stage I and 40 healthy controls was conducted. As shown in Supplementary Fig. S3, the relative expression of exosomal miR-27a and miR-130a in plasma were significantly higher in the patients with colorectal cancer with stage I than in healthy controls. ROC curve revealed that the AUC was 0.773 (95% CI, 0.669–0.876) for miR-27a with 75% sensitivity and 77.5% specificity, 0.742 (95% CI, 0.6331–0.8513) for miR-130a with 82.5% sensitivity and 62.5% specificity, and 0.846 (95% CI, 0.762–0.930) for two miRNAs combination with 82.5% sensitivity and 75% specificity (Fig. 2C).

Validation in patients with colorectal cancer with various stages

To investigate whether exosomal miR-27a and miR-130a in plasma could be applied to patients with colorectal cancer with different stages, the expression of miR-27a and miR-130a was tested in 40 healthy subjects, 40 patients with colorectal cancer with stage I, 20 patients with colorectal cancer with stage II, 14 patients with colorectal cancer with stage III, and six patients with colorectal cancer with stage IV. The relative expression of

exosomal miR-27a and miR-130a in plasma were significantly higher in the patients with colorectal cancer than in healthy controls (Supplementary Fig. S3). ROC curve revealed that the AUC was 0.82 (95% CI, 0.742–0.899) for miR-27a with 80% sensitivity and 77.5% specificity, 0.787 (95% CI, 0.704–0.871) for miR-130a with 70% sensitivity and 80% specificity, and 0.898 (95% CI, 0.844–0.953) for two miRNAs combination with 80% sensitivity and 90% specificity (Fig. 2D).

External validation in patients with adenoma

To further validate the role of circulating exosomal miR-27a and miR-130a for early detection of colorectal cancer, a larger cohort composed of 50 patients with colorectal cancer with stage I, 50 patients with adenoma and 50 healthy controls was conducted. As shown in Supplementary Figure S3, the relative expression of exosomal miR-27a and miR-130a were significantly higher in the plasma of patients with colorectal cancer than in that of the patients with adenoma and healthy controls. ROC curve revealed that the AUC was 0.746 (95% CI, 0.659–0.833) for miR-27a with 80% sensitivity and 77.5% specificity, 0.697 (95% CI, 0.61–0.784) for miR-130a with 70% sensitivity and 80% specificity, and 0.801 (95% CI, 0.712–0.87) for two miRNAs combination with 80% sensitivity and 90% specificity (Fig. 2E).

Identification of exosomes in plasma and medium

To verify the isolation of exosomes in plasma and medium, we examined transmission electron microscopy images of samples of plasma and medium after ultracentrifugation. In these samples, we captured images of round microvesicles with diameters of 40 to 100 nm (Supplementary Fig. S3).

Circulating exosomal miR-27a and miR-130a were tumor-derived miRNAs

We determined the identified miRNAs (miR-27a and miR-130a) as secretory miRNAs that excreted into the peripheral blood by cancer cells. Fifteen pairs of pre- and postoperation plasma samples were collected, and qRT-PCR discovered that the expression of circulating exosomal miR-27a and miR-130a were significantly decreased after surgical resection of colorectal cancer tissues (Fig. 3A). In addition, we found that exosomal miR-27a and miR-130a in medium of colorectal cancer cell lines (HCT8, HT29, HCT116, SW480, and SW620) were significantly higher than that of FHC (Fig. 3B). Results above demonstrated that circulating exosomal miR-27a and miR-130a were tumor-derived.

High expression of exosomal miR-27a and miR-130a in plasma were correlated with poor prognosis of patients with colorectal cancer

To assess the correlation between circulating exosomal miR-27a (miR-130a) and clinicopathologic characteristics of patients with colorectal cancer, the expression levels of miRNAs were categorized as high or low according to the median value of relative miRNA expression. Correlation regression analysis indicated that exosomal miR-27a was positively correlated with the TNM stage ($P = 0.012$), and exosomal miR-130a was positively correlated with the TNM stage ($P = 0.02$) and histologic grade ($P = 0.025$). However, no significant association was found between the expression of miRNAs and other parameters, such as age and gender (Table 2). In addition, we further analyzed whether there was an association between the expression of exosomal miR-27a (miR-130a) and clinical outcomes in patients with cancer using Kaplan–Meier survival analysis. Results indicated that high expression of exosomal miR-27a (HR = 2.74; 95% CI, 1.25–6.01; $P = 0.012$) and exosomal miR-130a (HR = 2.36; 95% CI, 1.07–5.23; $P = 0.034$) in plasma were novel potential predictors of poor overall survival shown in Figure 3C.

miR-27a and miR-130a regulate colorectal cancer progression by activating Wnt/β-catenin and TGFβ pathways

To validate whether these known signaling pathways were regulated by miR-27a and miR-130a, the mRNA expression levels of downstream genes of Wnt/β-cantenin pathway (CD44, TCF4, cyclin D1, and c-myc) and TGFβ pathway (SMAD2 and SMAD4) were investigated after colorectal cancer cells transfected with miRNA inhibitor or NC, respectively. After 48 hours transfection, the expression of miR-27a and miR-130a in miRNA inhibitor (miR-inhibitor) groups were significantly downregulated (Supplementary Fig. S4). Besides, we discovered that the expression of CD44, TCF4, cyclin D1, c-myc, SMAD2, and SMAD4 were significantly decreased in miR-27a inhibitor group compared with that in NC group, which means miR-27a could promote colorectal cancer progression through Wnt/β-catenin and TGFβ pathways (Fig. 3D). Similarly, the expression of CD44, TCF4, cyclin D1, and c-myc were significantly decreased in miR-130a inhibitor group compared with that in NC group, which means miR-130a could promote colorectal cancer progression through Wnt/β-catenin pathway (Fig. 3E).

Discussion

This study aimed to discover specific exosomal miRNAs in peripheral blood as noninvasive biomarkers for early detection and predicting prognosis of patients with colorectal cancer. Emerging evidences have reported that cancer cells secret exosomes containing miRNAs into the body fluids including peripheral blood to transport signals from donor cells to recipient cells (19–21). Our study demonstrated that exosomal miR-27a and miR-130a in plasma could be potential diagnostic and prognostic biomarkers of colorectal cancer.

In this study, integrated analysis of GEO database and TCGA database identified eight miRNAs that were significantly associated with the prognosis of patients with colorectal cancer. Considering the poor outcomes of patients with colorectal cancer mainly result from advanced stage when diagnosed, we decided to investigate whether circulating miRNAs could serve as noninvasive biomarkers for early diagnosis of colorectal cancer. Furthermore, exosomes also aroused our concern due to their stability in peripheral blood. Mounting reports have declared that exosomal miRNAs were potential promising biomarkers for cancers. Li and colleagues demonstrated that GPC1 exosome and its regulatory miRNAs are specific markers for the detection and target therapy of colorectal cancer (22); Hornick and colleagues reported that serum exosome miRNAs act as minimally invasive early biomarkers of acute myelocytic leukemia (AML; ref. 23). In addition, Liu and colleagues identified that circulating exosomal microRNAs could function as prognostic biomarkers for non–small cell lung cancer (NSCLC; ref. 24).

After analysis of another GEO dataset (GSE39833), we found that among these eight miRNAs selected, only circulating exosomal miR-27a and miR-130a were significantly deregulated between healthy controls and patients with colorectal cancer. The

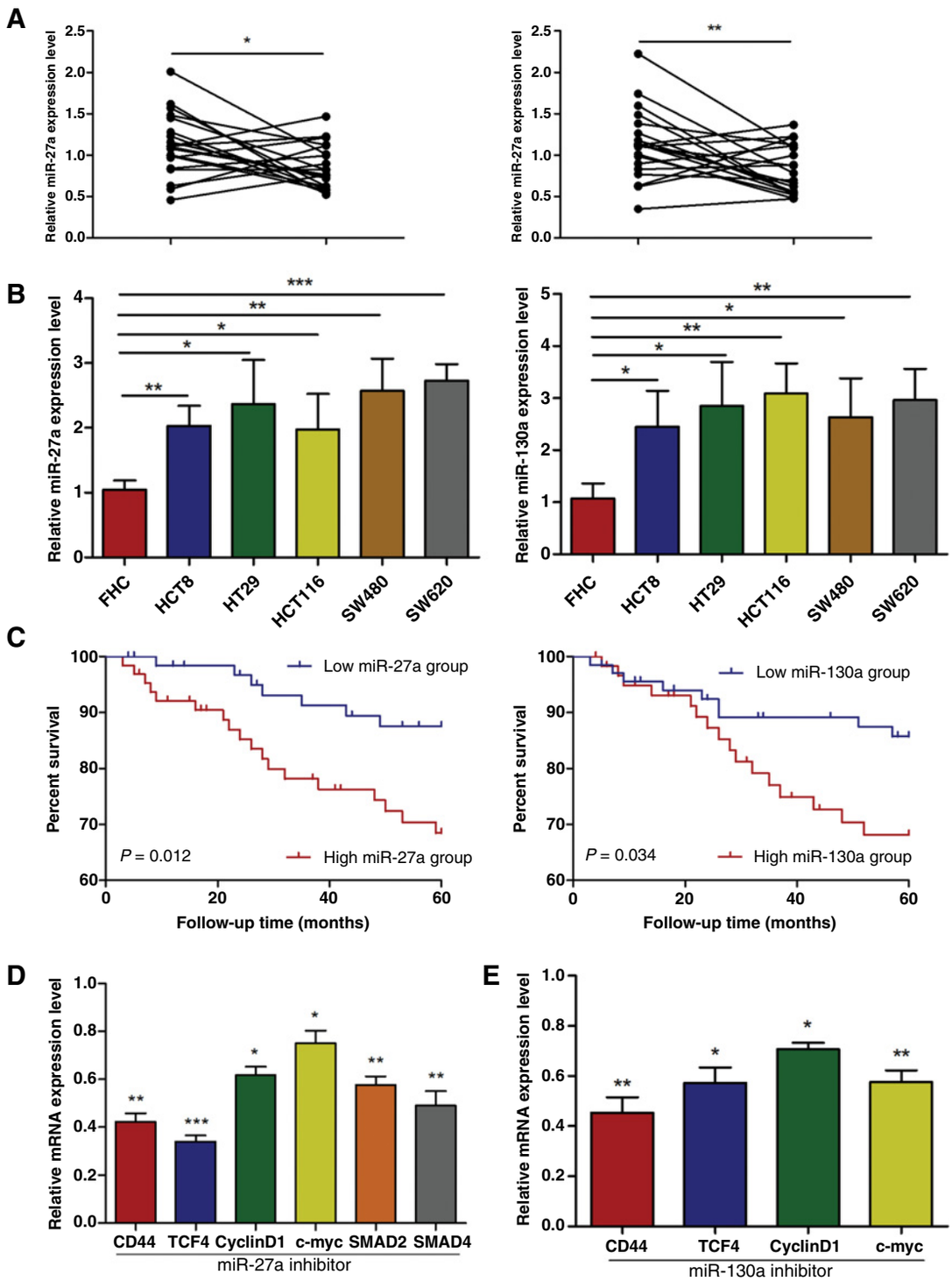

Figure 3.

Tumor derived miR-27a and miR-130a were significantly associated with the prognosis of patients with colorectal cancer and Wnt/β-catenin and TGFβ pathways. **A,** The expression of circulating exosomal miR-27a and miR-130a were significantly decreased after surgical resection of colorectal cancer tissues. **B,** Exosomal miR-27a and miR-130a in medium of colorectal cancer cell lines were significantly higher than that of FHC. **C,** Circulating exosomal miR-27a and miR-130a were significantly associated with the prognosis of patients with colorectal cancer. **D,** miR-27a inhibition remarkably suppressed the expression of downstream genes of Wnt/β-catenin and TGFβ pathways. **E,** miR-130a inhibition significantly suppressed the expression of downstream genes of Wnt/β-catenin pathways.

miR-27a and miR-130a have been shown to be significantly associated with diagnosis of various types of cancers, such as osteosarcoma (25), breast cancer (26), lung adenocarcinoma (27), gastric cancer (28), and pancreatic cancer (29). However, circulating exosomal miR-27a and miR-130a as noninvasive biomarkers for colorectal cancer have not been reported. Data from GSE39833 showed that both circulating exosomal miR-27a and miR-130a were potential diagnostic biomarkers for colorectal cancer, which was validated in our subsequent experiments.

Liu and colleagues revealed that miR-27a promotes proliferation, migration, and invasion of colorectal cancer by targeting FAM172A (30). Chen and colleagues demonstrated that miR-130a is upregulated in colorectal cancer and promotes cell growth and motility by directly targeting forkhead box F2 (FOXF2; ref. 31). Liang and colleagues identified that miR-27a targets RXRα to promote colorectal cancer cells progression by activating Wnt/β-catenin pathway (32). Although, miR-27a and miR-130a have been reported to be onco-miRNAs in colorectal cancer, whether circulating exosomal miR-27a and miR-130a could be used to predict the prognosis of colorectal cancer patients are still elusive. Here, we found that exosomal miR-27a and miR-130a in plasma were novel potential prognostic biomarkers of colorectal cancer, and positively associated with TNM stage and histologic grade. RXRa, SMAD2, SMAD4, and SFRP1 were reported as targets of miR-27a, and these targets are related with known Wnt/β-catenin and TGFβ pathways (32–34). Nkd2, which is significantly associated with Wnt/β-catenin pathway, was demonstrated to be a target of miR-130a (35). In our study, we validated that miR-27a and miR-130a promote colorectal cancer progression by activating Wnt/β-cantenin and TGFβ pathways.

Because of the first discovery of circulating exosomal miR-27a and miR-130a acting as diagnostic and prognostic biomarkers of colorectal cancer, we would like to know whether miR-27a and miR-130a were tumor-derived. After collection and analysis of exosomes in pre- and postoperation plasma from same patients with colorectal cancer, we found that the expression of exosomal miR-27a and miR-130a decreased after operation. After collection and analysis of exosomes in the medium of FHC and cancer cell lines, we found that the expression of exosomal miR-27a and miR-130a increased in the medium of colorectal cancer cell lines when compared with that of FHC. Although Carolina and colleagues demonstrated that T lymphoblasts could selectively secret miRNAs to exosomes through binding to specific motifs of hnRNPA2B1 (36), the mechanisms through which cancer cells secreted exosomes containing specific miRNAs need to be further explored, and studies clarifying the relationship between exosomal miR-27a/miR-130a and intercellular signaling in colorectal cancer are required.

There are several limitations in our study. First, the sample size included was relatively small. Second, Karolina and colleagues reported that there was an association of miR-27a and patients with metabolic syndrome (37), and Sun and colleagues reported that adipose tissue could secreted miR-27a to promote liver cancer by targeting FOXO1 (38), which means miR-27a is significantly associated with metabolic diseases and cancers. Although there was no patients with colorectal cancer with metabolic syndrome or patients with type 2 diabetes enrolled in our study, further studies with a large number of subjects ought to consider these cofounding factors. Third, since Tsukamoto and colleagues declared that circulating exosomal miR-21 is a promising biomarker in each tumor stage of colorectal cancer (17), the diagnostic and prognostic value of exosomal miR-27a, miR-130a, and miR-21 deserve to be investigated.

In summary, the expression of exosomal miR-27a and miR-130a in plasma were significantly upregulated in colorectal cancer patients compared with that in patients with adenoma and healthy subjects. We concluded that circulating exosomal miR-27a and miR-130a were powerful and promising, noninvasive biomarkers for the early detection and prognosis of patients with colorectal cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: X. Liu, L. Sun, S. Wang
Development of methodology: X. Liu, B. Pan
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K. Zeng, X. Hu
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K. Zeng
Writing, review, and/or revision of the manuscript: S. Wang
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): X. Chen, K. Zeng, X. Hu, T. Xu, M. Xu
Study supervision: L. Sun

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