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Research paper

Down-regulation of miR-133a as a poor prognosticator in non-small cell lung cancer

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ABSTRACT

miR-133a has been demonstrated to play an important role in tumor progression. The aim of present study was to analyze the correlation between miR-133a expression level and clinicopathologic features and its prognostic significance in non-small cell lung cancer (NSCLC). The expression of miR-133a in 104 pairs of human lung cancer tissues and adjacent normal lung tissues were analyzed by qRT-PCR. Here we show that miR-133a was down-regulated in NSCLC. The levels of miR-133a were negatively correlated with the status of N classification (N0–N1 vs. N2–N3, $P = 0.000$), clinical stage (I–II vs. III–IV, $P = 0.010$) and MMP-14 expression (High vs. Low, $P = 0.012$). The patients with low miR-133a expression had shorter survival time than those with high miR-133a expression. Multivariate analysis indicated that the level of miR-133a expression was an independent prognostic indicator ($P = 0.012$) for the survival of patients with NSCLC. In conclusion, decreased expression of miR-133a might be a potential unfavorable prognostic factor for patients with NSCLC, and further studies would be needed to prove our findings.

1. Introduction

Lung cancer is the leading cause of cancer-related mortality both in men and women worldwide. In China, lung cancer morbidity and mortality have increased rapidly over the past 30 years (She et al., 2013). Non-small cell lung cancer (NSCLC) is the predominant histological subtype, accounts for nearly 83% of all cases of lung cancer. Major therapies for lung cancer now include surgery, radiation therapy, chemotherapy, and/or targeted therapies. Although advances in early diagnosis and therapy, the prognosis of lung cancer is still unfavorable. Only 15% of lung cancers are diagnosed at a localized stage, for which the 5-year survival rate is 54%. More than half (57%) are diagnosed at a distant stage, for which the 1- and 5-year survival is 26% and 4%, respectively (Society, 2015). Tumor markers of lung cancer could potentially conducive to earlier diagnosis as well as playing a part in assessing tumor response to therapy. Nowadays, accumulative studies have demonstrated the diagnostic and prognostic potential of new biomarkers for lung cancer, such as miR-155 (Xie et al., 2015), miR-1290 (Mo et al., 2015), CDK4 (Wu et al., 2011), MMP-14 (Wang et al., 2014d). They play crucial roles in the growth, motility or metastasis of lung cancer. Therefore, the significance of detecting novel biomarkers for NSCLC should be emphasized.

MicroRNAs (miRNAs) are a class of small non-coding RNAs which plays an important role in various biological processes by post-transcriptional regulation. Mechanistically, miRNAs bind to their target mRNAs and cause protein synthesis inhibition or mRNA degradation (Fabian et al., 2010). Previous researches demonstrated that miRNAs has a great clinical significance as diagnostic and prognostic biomarkers to evaluate tumorigenesis, progression, and response to treatment in cancer patients. For instance, tissue miR-153 (Chen et al., 2015), miR-148b (Ge et al., 2015), miR-150 (Yin et al., 2015), miR-224 (Zhu et al., 2014), have been identified as diagnostic factors for NSCLC.

MiR-133a was initially identified during the development and differentiation of skeletal muscle (Chen et al., 2006) and cardiac muscle (Care et al., 2007; Liu and Olson, 2010). MiR-133a has two subtype, including miR-133a-1 and miR-133a-2, which are located on chromosomes 18q11.2, 20q13.33 (Mitchelson and Qin, 2015). In renal cell carcinoma, miR-133a could inhibit cell proliferation and invasion by regulating target gene transgelin-2 (TAGLN2) (Kawakami et al., 2012a). In gastric

Abbreviation: NSCLC, non-small cell lung cancer; CDK4, cyclin-dependent kinase 4; MMP-14, matrix metalloproteinase-14; IGF-1R, insulin-like growth factor 1; TGFBR1, transforming growth factor, beta receptor 1; EGFR, epidermal growth factor receptor; lung-SCC, lung squamous cell carcinoma; ARPC5, actin-related protein 2/3 complex subunit 5; GSTP1, glutathione S-transferase P; HCC, hepatocellular carcinoma; ESCC, esophageal squamous cell carcinoma.

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cancer, miR-133a was found decreased expression in cancer tissue. Overexpression of miR-133a could suppressed proliferation and invasion, but promote apoptosis of gastric cancer cells through targeting FSCN1 gene (Lai et al., 2015). We previously found that miR-133a suppressed NSCLC cell proliferation, migration, and invasion by targeting MMP-14 (Xu and Wang, 2013). In addition, miR-133a was demonstrated significant down-regulation in NSCLC tissue relative to normal or adjacent lung tissue (Lan et al., 2015, Wang et al., 2014b). Low expression of miR-133a has shown poor prognosis in several cancers, including breast cancer (Wu et al., 2012a), colorectal cancer (Wang et al., 2014a) and osteosarcoma (Mirghasemi et al., 2015a), however, little is known about the accurate significance of miR-133a in NSCLC patients.

In the present study, we detected the expression of miR-133a in NSCLC patients and normal tissues firstly. Furthermore, the correlation of miR-133a expression with clinicopathologic features and prognosis of NSCLC patients was explored in order to clarify the role of miR-133a in the NSCLC pathogenesis. Our study will provide a better understanding of NSCLC pathogenesis.

2. Materials and methods

2.1. Clinical specimens

This study included 104 paired primary NSCLC and normal lung tissues that were obtained from patients undergoing surgical resection between March 2008 and April 2012 at the Affiliated Hospital of Guangdong Medical College, China. All samples were flash-frozen in liquid nitrogen and stored at −80 °C until further molecular analysis. In these 104 lung cancer cases, there were 69 males and 35 females with a median age of 58 years (range 33–79 years). The clinical follow-up time of patients ranged from 3 to 82 months. Prior consents from the patients and approval from the Institutional Ethics Committee of the Affiliated Hospital of Guangdong Medical School were obtained before the use of these clinical samples. The histopathological diagnosis of all samples was respectively diagnosed by two pathologists. The clinicopathological characteristics were summarized in Table 1.

2.2. RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

The tissue samples were preserved at −80 °C prior to RNA isolation. Total RNA was extracted from fresh tissue, with RNAiso Plus (Takara, Japan). The isolated total RNA was reverse transcribed using the One Step PrimeScript miRNA cDNA Synthesis Kit (Takara, Japan) according to the manufacturer's protocols. Relative expression was calculated via the comparative cycle threshold (Ct) method. The expression levels of miRNAs in tissue were normalized to U6 small RNA. The forward primers for miR-133a and U6 were 5′-GTGCATTTGGTCCCCTTCA-3′ and 5′-CTCGCTTCGGCAGCACA-3′, respectively. The Uni-miR qPCR primer was included in the kit. qRT-PCR was performed using SYBR Premix Ex Taq™ II (Takara, Japan) on a LightCycler (Roche Diagnostics, USA). The PCR conditions were 30 s at 95 °C, followed by 40 cycles at 95 °C for 5 s and 60 °C for 20 s. Relative quantification of miRNA expression was calculated by using the $2^{-\Delta\Delta Ct}$ method. All qRT-PCR reactions were performed in triplicate.

2.3. Statistical analysis

The statistical analyses were performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). A paired Student's *t*-test was performed to compare the difference of miRNA level between NSCLC and paired normal tissue. Correlations between clinical characteristics and miR-133a expression were evaluated using the Chi squared test. We used Kaplan-Meier method and log-rank test to analyze the association between miR-133a expression level and the survival of NSCLC patients.

Table 1
Correlation between the clinicopathologic characteristics and expression of miR-133a protein in lung cancer.

Characteristics	Number of patients	miR-133a (relative mean level[a])		*P* value
		High expression	Low expression	
Gender				
Female	35	12 (0.67 ± 0.14)	23 (0.24 ± 0.15)	0.298
Male	69	31 (0.61 ± 0.21)	38 (0.25 ± 0.13)	
Age (years)				
≥60	48	22 (0.66 ± 0.30)	26 (0.21 ± 0.11)	0.390
<60	56	21 (0.64 ± 0.22)	35 (0.26 ± 0.09)	
Smoking				
No	49	20 (0.56 ± 0.13)	29 (0.29 ± 0.14)	0.918
Yes	55	23 (0.71 ± 0.18)	32 (0.21 ± 0.10)	
Pathology classification				
Squamous cell	48	18 (0.65 ± 0.11)	30 (0.21 ± 0.09)	0.461
Adenocarcinoma	56	25 (0.61 ± 0.24)	31 (0.30 ± 0.12)	
Differentiated degree				
High	36	15 (0.54 ± 0.21)	21 (0.21 ± 0.07)	0.883
Middle	22	10 (0.60 ± 0.29)	12 (0.15 ± 0.14)	
Low	46	18 (0.61 ± 0.18)	28 (0.19 ± 0.16)	
T classification				
T1–T2	62	28 (0.59 ± 0.19)	34 (0.28 ± 0.16)	0.337
T3–T4	42	15 (0.65 ± 0.28)	27 (0.25 ± 0.17)	
N classification				
N0–N1	63	37 (0.74 ± 0.21)	26 (0.26 ± 0.09)	0.000*
N2–N3	41	6 (0.58 ± 0.11)	35 (0.19 ± 0.10)	
Distant metastasis				
Yes	6	2 (0.65 ± 0.27)	4 (0.22 ± 0.16)	1.000
No	98	41 (0.60 ± 0.14)	57 (0.30 ± 0.15)	
Clinical stage				
I–II	57	30 (0.73 ± 0.26)	27 (0.28 ± 0.18)	0.010*
III–IV	47	13 (0.59 ± 0.22)	34 (0.17 ± 0.08)	
MMP-14 expression				
High	54	16 (0.56 ± 0.28)	38 (0.21 ± 0.07)	0.012*
Low	50	27 (0.70 ± 0.27)	23 (0.30 ± 0.12)	

[a] Mean ± SD.
* $P < 0.05$.

Cox survival analyses were applied to assess the hazard ratios (HRs). A *P* value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. The expression level of miR-133a in NSCLC tissue compared to normal tissue

The miR-133a expression level in 104 pairs of NSCLC cancerous and normal tissue was detected by qRT-PCR. Mean miR-133a levels was significantly lower in NSCLC tissues compared to normal lung tissues (0.40 ± 0.24 vs. 0.88 ± 0.23, $P < 0.001$) (Fig. 1).

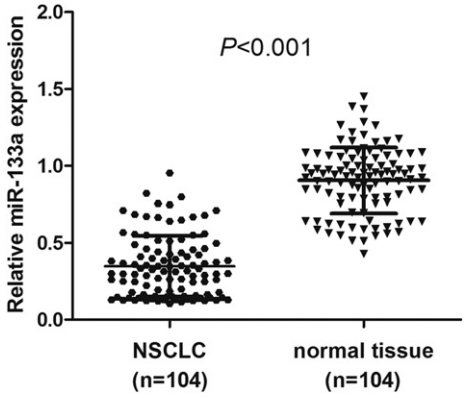

Fig 1. The expression level of miR-133a in 104 pairs of cancerous and matched normal tissue samples.