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Low miR-133a expression is a predictor of outcome in patients with esophageal squamous cell cancer

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Abstract. – OBJECTIVE: Identification and development of new biomarkers could be beneficial for diagnosis and prognosis of esophageal squamous cell cancer (ESCC) patients. The aim of this study was to examine the clinical significance of miR-133a expression in tissues from ESCC patients.

PATIENTS AND METHODS: Quantitative real-time reverse transcriptive-PCR (qRT-PCR) was performed to examine miR-133a expression levels in 126 ESCC tissues. Survival curves were plotted using the Kaplan-Meier method and differences in survival rates were analyzed using the log-rank test. Univariate and multivariate Cox regression analyses were conducted to determine whether miR-133a was an independent predictor of survival.

RESULTS: We found that the levels of miR-133a in ESCC tissues exhibited lower than that in matched normal tissues ($p < 0.01$). Low miR-133a expression was positively associated with T stage of ESCC ($p = 0.013$) and tumor length ($p = 0.007$). Moreover, low levels of miR-133a was associated with lower overall survival (OS) ($p = 0.001$) and disease-free survival (DFS) ($p = 0.001$). According to multivariate analysis, miR-133a level was confirmed to be an independent prognostic factor for worse survival.

CONCLUSIONS: MiR-133a may represent a prognostic biomarker and therapeutic target in esophageal squamous cell cancer prognosis and treatment.

Key Words:
Mir-133a, ESCC, Prognosis, Marker.

Introduction

Esophageal cancer (EC) is the eighth most common malignancies and the sixth leading cause of cancer death in the world[1]. Esophageal squamous cell cancer (ESCC) is the most frequent subtype of esophageal cancer in China[2]. Tumor metastasis is mostly responsible for ESCC mortality, but the molecular mechanism of metastatic dissemination remains obscure. Therefore, exploring novel biomarkers related to ESCC metastasis is required for monitoring the progression of the disease, and improve early detection and prognosis evaluation.

MicroRNAs (miRNAs) are highly conserved, single-stranded and non-coding RNAs of about 22 nucleotides (nt) in length and that negatively regulate gene expression at the posttranscriptional level[3-5]. miRNAs are involved in a wide range of important biological processes including cell proliferation, differentiation, apoptosis, and stress resistance[6,7]. More and more evidences reveals that aberrant expression of miRNAs occurs in many kinds of malignant tumors, some of which serve as tumor oncogenes or suppressor genes[8,9].

microRNA-133a (miR-133a) is a multicopy gene with two copies distributed in chromosome 18 and chromosome 20[10]. miR-133a has been validated as a tumor suppressor in ovarian cancer[11], bladder cancer[12], gastric cancer[13]. However, the clinical significance of miR-133a in ESCC has not been investigated.

Patients and Methods

Patients and Tissue Samples

126 patients with ESCC, obtained from Department of Gastroenterology, Jining No. 1 People's Hospital during 2006-2014, were enrolled in this study. For the use of these clinical materials for research purposes, written informed consent from all patients and approval from the Institutional Research Ethics Committee were obtained. All patients recruited to this study did not

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receive any pre-operative treatments. Details of clinical and pathological characteristics of the patients are summarized in Table I.

Isolation of Total RNA and Real-time PCR Analysis

MiR-133a expression level in ESCC tissues and paired adjacent normal tissues have been measured by reverse transcription and real-time PCR (RT-PCR). Total RNA was extracted from tissues using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. QRT-PCR was performed using the miScript Reverse Transcription and miScript SYBR Green PCR Kit, according to the manufacturer's protocol (Biosystems, Foster City, CA, USA). Moreover, the relative amount of miRNAs was normalized with respect to U6 RNA. The relative quantitative value was expressed by the 2^{-Ct} method. Each experiment was performed in triplicates and repeated three times.

Statistical Analysis

Data was analyzed using SPSS 13.0 (statistical package for the Social Sciences Version 13.0, SPSS Inc., Chicago, IL, USA). Paired Student's *t*-test was conducted to compare miR-133a expression in paired clinical samples. The association between tissue miR-133a expression level and clinicopathological parameters of ESCC was evaluated by Chi-square test. A survival analysis was performed with the Kaplan-Meier method, and the log-rank test was used to compare survival times between groups. The Cox proportional hazards regression model was employed for univariate and multivariate analyses to estimate the prognostic factors for survival prediction. A difference was considered significant when $p < 0.05$.

Table I. Association between miR 133a expression level and clinical characteristics.

Clinical characteristics	miR-133a expression		
	Low, n (%)	High, n (%)	*p*-value
Age			0.708
> 55	30 (46%)	27 (43.5%)	
≤ 55	34 (54%)	35 (56.5%)	
Gender			0.408
Male	41 (64%)	44 (71%)	
Female	23 (36%)	18 (29%)	
Smoking			0.381
Never or light	38 (59.3%)	32 (51.6%)	
Heavy	26 (40.7%)	30 (48.4%)	
Drinking			0.490
Ever or light	42 (65.6%)	37 (59.7%)	
Heavy	22 (34.4%)	25 (40.3%)	
Differentiation			0.772
Well	17 (26.6%)	15 (24.2%)	
Moderate	31 (48.4%)	34 (54.9%)	
Poor	16 (25%)	13 (20.9%)	
T stage			0.013
T1	9 (14.1%)	25 (40.3%)	
T2	17 (26.6%)	16 (25.8%)	
T3	18 (28.1%)	13 (21%)	
T4	20 (31.2%)	8 (12.9%)	
N stage			0.362
N0	8 (12.5%)	11 (17.7%)	
N1	18 (28.1%)	21 (33.9%)	
N2	18 (28.1%)	16 (25.8%)	
N3	20 (31.3%)	14 (22.6%)	
TNM stage			0.517
I	7 (10.9%)	5 (8%)	
II	24 (37.5%)	21 (33.9%)	
III	33 (51.6%)	36 (58.1%)	
Tumor length			0.007
> 4 cm	26 (40.6%)	40 (64.5%)	
≤ 4 cm	38 (59.3%)	22 (35.5%)	

Results

MiR-133a was Decreased in ESCC Tissues

To determine the expression of miR-133a in ESCC, 126 paired human ESCC and paracancerous tissues were subjected to qRT-PCR. Results showed that expression of miR-133a in ESCC patients was significantly downregulated compared to normal paracancerous tissues ($p < 0.01$, Figure 1).

The Association between Tissue miR-133a Expression and Clinical Parameters of ESCC

To investigate the clinical significance of miR-133a expression in ESCC, We further analyzed the association between miR-133a expression levels and clinicopathological characteristics of ESCC. As shown in Table I, the Chi-square test showed that miR-133 expression was associated with T stage ($p = 0.013$, and tumor length ($p = 0.007$). However, miR-133a expression was not found to be associated with age, gender, smoking, drinking, N stage, TNM stage, and differentiation stage (all $p > 0.05$).

Relationship between miR-133a and OS and DFS of ESCC Patients

To explore the prognostic value of miR-133a for ESCC, we performed Kaplan-Meier analysis to evaluate the association between miR-133a expression level and clinical information. In these ESCC patients, the data showed that the low expressions of miR-133a were correlated with shorter OS ($p = 0.001$, Figure 2) and DFS ($p = 0.001$, Figure 3). Univariate analyses were performed to evaluate the expression of miR-133a and other clinicopathologic features on the prognosis of ESCC patients. The result revealed that the OS and DFS of patients with ESCC were associated with T stage, N stage, tumor length and miR-133a expression ($p = 0.013$, 0.029, 0.038, 0.001, Table II). Furthermore, multivariate analysis confirmed that miR-133a was an independent prognostic factor for worse survival.

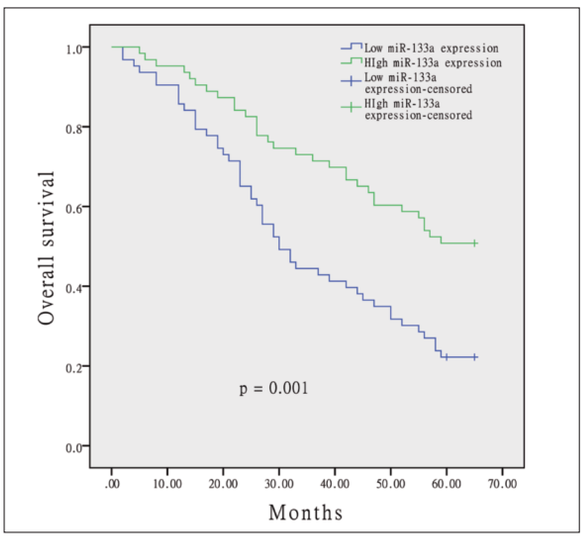

Figure 2. Kaplan-Meier curves of the overall survival of 126 ESCC patients.

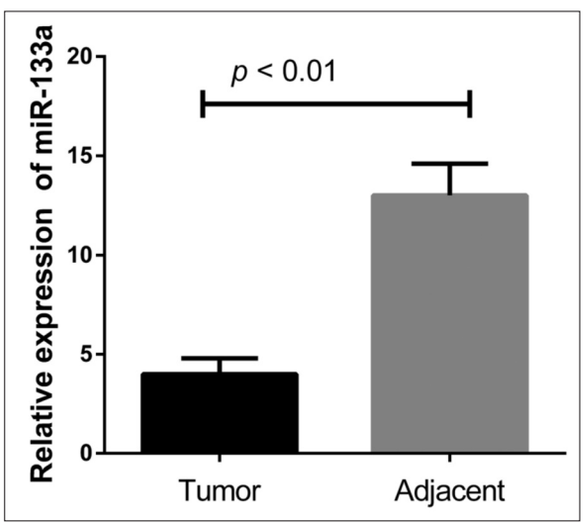

Figure 1. The relative expression level of miR-133a between primary ESCC tissues and corresponding adjacent tissues.

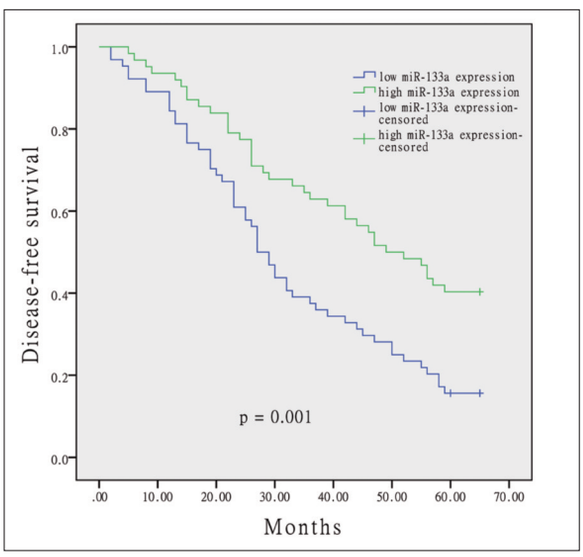

Figure 3. Kaplan-Meier curves of the disease-free survival of 126 ESCC patients.

Table II. Univariate and multivariate analysis with a Cox proportional hazards model between clinicopathological factors by Cox regression model.

Variable	OS univariate analysis *p*-value	OS multivariate analysis HR (95% CI)	OS multivariate analysis *p*-value	DFS univariate analysis *p*-value	DFS multivariate analysis HR (95% CI)	DFS multivariate analysis *p*-value
Gender	0.371	0.546 (0.331-1.476)	0.352	0.712	0.841 (0.429-1.566)	0.412
Age	0.662	1.332 (0.681-2.159)	0.572	0.554	1.235 (0.581-1.774)	0.649
Smoking	0.833	0.852 (0.659-1.819)	0.774	0.846	0.762 (0.663-1.749)	0.723
Drinking	0.719	1.226 (0.581-2.237)	0.641	0.658	1.239 (0.674-2.349)	0.428
T stage	0.026	1.428 (1.114-2.369)	0.003	0.021	1.318 (1.129-1.984)	0.016
N stage	0.015	1.170 (0.892-1.994)	0.029	0.045	1.663 (1.127-1913)	0.039
Differentiation	0.885	1.129 (0.683-1.834)	0.659	0.769	0.923 (0.664-1.659)	0.783
Tumor length	0.011	1.673 (1.149-3.337)	0.038	0.022	1.539 (1.048-2.997)	0.043
miR-133a	0.002	0.368 (0.164-0.773)	0.001	0.001	0.553 (0.236-0.823)	0.001

Discussion

Improvements in genomics, proteomics, and metabolomics technologies have been made in exploring key molecular events during lung ESCC[14]. More and more researches focused on potential biomarker to predict the diagnosis and prognosis of ESCC patients[15,16]. Compared with other biomarkers, miRNAs are considered to be ideal biomarkers because they are easy to determine, stable and strongly associated with prognosis of patients[17]. The low expression of miR-133a in ESCC has been known for a long time, but its underlying potential to predict clinical outcome of patients with this disease remain elusive. In the present study, we focus on miR-133a.

MicroRNA-133a (miR-133a), together with miR-133b, belongs to the miR-133 family[18]. Recently, growing pieces of evidence have shown that miR-133a acts as a tumor suppressor. Zhang et al[19] found that microRNA-133a was downregulated in HCC tissues and was associated with tumor differentiation, TNM stage, and lymph node metastasis. Furthermore, they found that miR-133a could suppress proliferation, colony formation, migration, and invasion by targeting IGF-1R. Wang et al[20] found that miR-133a was decreased in colorectal cancer and was correlated with a poor prognosis in colorectal cancer patients. Similar results were observed in non-small cell lung cancer[21]. For ESCC, Li et al[22] showed that the expression of miR-133a was markedly decreased in ESCC tissues. Moreover, they found that MiR-133a suppresses the migration and invasion of esophageal cancer cells by targeting the EMT regulator SOX4. These results suggested that miR-133a might be a novel miRNA that is important in human malignancies.

In the present study, as results of our analysis, there are four points of findings. Firstly, miR-133a was significantly downregulated in ESCC tissue samples compared with adjacent normal tissues. Secondly, low miR-133a expression was positively associated with T-stage of ESCC and tumor length. Thirdly, the results of Kaplan-Meier curves low levels of miR-133a were associated with lower OS and DFS. Finally, multivariate analyses demonstrated that status of miR-133a was an independent prognostic factor for both DFS and OS of ESCC patients. Our data showed that miR-133a might serve as a prognostic marker.

Conclusions

Our data demonstrated that the expression of miR-133a was downregulated in ESCC, and was associated with OS as well as DFS, suggesting that the upregulation of miR-133a might function as a novel molecular marker to predict the aggressive tumor progression and unfavorable prognosis of ESCC patients.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Yuequan J, Shifeng C, Bing Z. Prognostic factors and family history for survival of esophageal squamous cell carcinoma patients after surgery. Ann Thorac Surg 2010; 90: 908-913.
2) Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010; 127: 2893-2917.
3) Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.
4) Yang F, Liu Q, Hu CM. Epstein-Barr virus-encoded LMP1 increases miR-155 expression, which promotes radioresistance of nasopharyngeal carcinoma via suppressing UBQLN1. Eur Rev Med Pharmacol Sci 2015; 19: 4507-4515.
5) Yates LA, Norbury CJ, Gilbert RJ. The long and short of microRNA. Cell 2013; 153: 516-519.
6) Calin GA, Croce CM. Microrna signatures in human cancers. Nat Rev Cancer 2006; 6: 857-866.
7) Li ZB, Li ZZ, Li L, Chu HT, Jia M. MiR-21 and miR-183 can simultaneously target SOCS6 and modulate growth and invasion of hepatocellular carcinoma (HCC) cells. Eur Rev Med Pharmacol Sci 2015; 19: 3208-3217.
8) Chen CZ. MicroRNAs as oncogenes and tumor suppressors. N Engl J Med 2005; 353: 1768-1771.
9) Fu Y, Shao ZM, He QZ, Jiang BQ, Wu Y, Zhuang ZG. Hsa-miR-206 represses the proliferation and invasion of breast cancer cells by targeting Cx43. Eur Rev Med Pharmacol Sci 2015; 19: 2091-2104.
10) Yu H, Lu Y, Li Z, Wang Q. microRNA-133: expression, function and therapeutic potential in muscle diseases and cancer. Curr Drug Targets 2014; 15: 817-828.
11) Guo J, Xia B, Meng F, Lou G. miR-133a suppresses ovarian cancer cell proliferation by directly targeting insulin-like growth factor 1 receptor. Tumour Biol 2014; 35: 1557-15564.
12) Yoshino H, Chiyomaru T, Enokida H, Kawakami K, Tatarano S, Nishiyama K, Nohata N, Seki N, Nakagawa M. The tumour-suppressive function of miR-1 and miR-133a targeting TAGLN2 in bladder cancer. Br J Cancer 2011; 104: 808-818.
13) Gong Y, Ren J, Liu K, Tang LM. Tumor suppressor role of miR-133a in gastric cancer by repressing IGF1R. World J Gastroenterol 2015; 21: 2949-2958.
14) Kim VN. MicroRNA biogenesis: coordinated cropping and dicing. Nat Rev Mol Cell Biol 2005; 6: 376-385.
15) Cong J, Liu R, Wang X, Wang J, Wang H, Hou J. Low miR-498 expression levels are associated with poor prognosis in ovarian cancer. Eur Rev Med Pharmacol Sci. 2015; 19: 4762-4765.
16) Lei QQ, Liu JW, Zheng H. Potential role of anti-p53 antibody in diagnosis of lung cancer: evidence from a bivariate meta-analysis. Eur Rev Med Pharmacol Sci 2013; 17: 3012-3008.
17) Bartels CL, Tsongalis GJ. MicroRNAs: novel biomarkers for human cancer. Clin Chem 2009; 55: 623-631.
18) Luo J, Zhou J, Cheng Q, Zhou C, Ding Z. Role of microRNA-133a in epithelial ovarian cancer pathogenesis and progression. Oncol Lett 2014; 7: 1043-1048.
19) Zhang W, Liu K, Liu S, Ji B, Wang Y, Liu Y. MicroRNA-133a functions as a tumor suppressor by targeting IGF-1R in hepatocellular carcinoma. Tumour Biol 2015; 36: 9779-9788.
20) Wang LL, Du LT, Li J, Liu YM, Qu AL, Yang YM, Zhang X, Zheng GX, Wang CX. Decreased expression of miR-133a correlates with poor prognosis in colorectal cancer patients. World J Gastroenterol 2014; 20: 11340-11346.
21) Wang Y, Li J, Chen H, Mo Y, Ye H, Luo Y, Guo K, Mai Z, Zhang Y, Chen B, Zhou Y, Yang Z. Downregulation of miR-133a as a poor prognosticator in non-small cell lung cancer. Gene 2016; 59: 333-337.
22) Li S, Qin X, Li Y, Zhang X, Niu R, Zhang H, Cui A, An W, Wang X. MiR-133a suppresses the migration and invasion of esophageal cancer cells by targeting the EMT regulator SOX4. Am J Transl Res 2015; 7: 1390-1403.