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Reduced miR-485-5p expression predicts poor prognosis in patients with gastric cancer

L.-L. JING, X.-M. MO

Laboratory of Stem Cell Biology and Department of Pediatric Surgery, State Key Laboratory of Biotherapy and Collaborative Innovation Center for Biotherapy, West China Hospital, Sichuan University, Chengdu, China

Abstract. – OBJECTIVE: The present study was designed to explore expression and prognostic value of miR-485-5p in patients with gastric cancer.

PATIENTS AND METHODS: We determined the expression level of miR-485-5p in 132 cases of paired GC and adjacent non-tumor tissues by quantitative real-time PCR (qRT-PCR). The relevance of miR-485-5p expression to the clinicopathological factors was assessed. Overall survival (OS) was examined using Kaplan-Meier curves and the Cox proportional hazards regression model.

RESULTS: The expression of miR-485-5p was significantly down-regulated in GC tissues compared with adjacent normal tissues ($p < 0.01$). MiR-485-5p expression was positively correlated with larger tumor size ($p = 0.003$), deeper invasion depth, ($p = 0.005$), positive lymph node metastasis ($p = 0.039$), advanced tumor-node-metastasis (TNM) stage ($p = 0.017$). Patients survival analysis showed that a clear positive correlation between miR-485-5p expression level and survival time of gastric cancer patients ($p < 0.001$). Multivariate analyses confirmed that a low level of miR-485-5p expression was an independent predictor of poor prognosis in GC patients.

CONCLUSIONS: Expression level of miR-485-5p serves as a novel biomarker for the overall survival of patients with gastric cancer.

Key Words:
MiR-485-5p, Gastric cancer, Prognosis, Overall survival.

Introduction

Gastric cancer ranks as the second leading cause of cancer death worldwide[1]. Nearly half of gastric cancer occurs in China with an overall 5-year survival rate of approximately 20%[2]. Although the clinical outcome of GC has been gradually improved due to recent advancement of diagnosis and treatment. However, 5-year survival rate of GC patients is still not satisfied[3-5]. More and more reports showed that many oncogenes and tumor suppressor genes are closely correlated with GC[6-8]. Early diagnosis and prognostic evaluation of GC are crucial for timely and appropriate treatment. Thus, it is necessary to search for novel markers for GC, which can be used to detect GC at an early stage and predict the prognosis of this deadly disease.

MiRNAs are a family of small (approximately 22 nucleotides in length), single-stranded, endogenous non-coding RNAs which regulate gene expression post-transcriptionally[9,10]. Many studies confirmed that MiRNAs are involved in crucial biological processes, including development and differentiation[11,12]. Recently, accumulating evidences suggest that there are correlations between miRNAs expression and clinical recurrence, and survival[13]. MiRNAs can regulate various protein-coding genes, including tumor suppressor genes or oncogenes[14,15]. Emerging studies have revealed that miRNA is a promising biomarker associated with clinical outcomes in non-small cell lung cancer[16,17].

miR-485-5p is a recently identified cancer-related miR. Up to date, little studies were reported about the effect of miR-485-5p in human cancer. Previous studies reported that miR-485-5p was significantly down-regulated in gastric cancer, breast cancer, and ovarian epithelial cancer. Over-expression of miR-485-5p suppressed breast cancer cell proliferation, metastasis and promoted cell apoptosis[18-20]. Those results informed that miR-485-5p may function as a potential tumor suppressor. However, the prognostic value of miR-485-5p in GC patients is unknown. In the present study, qRT-PCR was performed to explore the expression of miR-485-5p in GC. The association between miR-485-5p and clinical characteristics and the prognosis were subsequently analyzed.

Corresponding Author: Xianming Mo, MD; e-mail: xianmin_h@163.com

Patients and Methods

Patients and Tissue Samples

This study was approved by the Research Ethics Committee of West China Hospital. Written informed consent was obtained from all of the patients.

A total of 132 primary GC tissues were collected from the Department of Digestive Surgery, West China Hospital, Sichuan University between 2007 and 2014. The diagnosis of all samples were histopathologically confirmed by two pathologists. The remaining tissues were snap-frozen in liquid nitrogen and then stored at -80°C for RNA extraction and other biological molecular experiments. All patients were not treated by chemotherapy, and other adjuvant therapies before surgeries. Patient characteristics are shown in Table I. OS time was calculated from the date of the initial surgery to death.

Real-time Quantitative PCR

Total RNA was extracted from fresh tissues using Trizol (Invitrogen Corp, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA was diluted in RNase-free water and stored at -80°C before use. TaqMan-based real-time reverse transcription-polymerase chain reaction (RT-PCR) assays was performed by using the ABI 7300 HT Sequence Detection system (Applied Biosystems, Foster City, CA, USA). The primers were designed and synthesized by Shengong Company (Pudong, Shanghai, China). U6 was used for miRNA template normalization. All reactions were run in triplicates. Relative quantification of miRNA expression was calculated with the $2^{-\Delta\Delta}$Ct method.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics Version 16 (SPSS Inc, Chicago, IL, USA). Data were expressed as mean ± SD. Paired Student's t-test was conducted to compare miR-485-5p expression in paired clinical samples. Overall survival of patients was estimated by the Kaplan-Meier method. COX regression model was used to analyze the influence of related factors on the survival time of patients with GC. A p-value < 0.05 was considered statistically significant.

Table I. Correlation between the expression of miR-485-5p and clinicopathological parameters in gastric cancer patients.

Characteristics	Low miR-485-5p expression	High miR-485-5p expression	*p* value
Age			0.717
≥60	41 (63.1%)	43 (65.2%)	
<60	25 (36.9%)	23 (34.8%)	
Gender			0.278
Male	39 (59.1%)	45 (68.2%)	
Female	27 (40.9%)	21 (31.8%)	
Differentiation			0.140
Well-moderate	18 (27.2%)	26 (39.4%)	
Poor	48 (62.8%)	40 (60.6%)	
Lauren type			0.112
Intestinal	34 (51.5%)	43 (65.2%)	
Diffuse and mixed	33 (48.5%)	23 (34.8%)	
Tumor size			0.003
≥5 cm	48 (72.7%)	31 (47%)	
<5 cm	18 (27.3%)	35 (53%)	
Invasion depth			0.005
T1, T2	21 (31.8%)	37 (56.1%)	
T3, T4	45 (68.2%)	29 (43.9%)	
TNM stage			0.017
I/II	16 (24.2%)	29 (43.9%)	
III	50 (75.8%)	37 (56.1%)	
Lymphatic metastasis			0.039
Negative	15 (22.7%)	26 (39.4%)	
Positive	51 (77.3%)	40 (60.6%)	

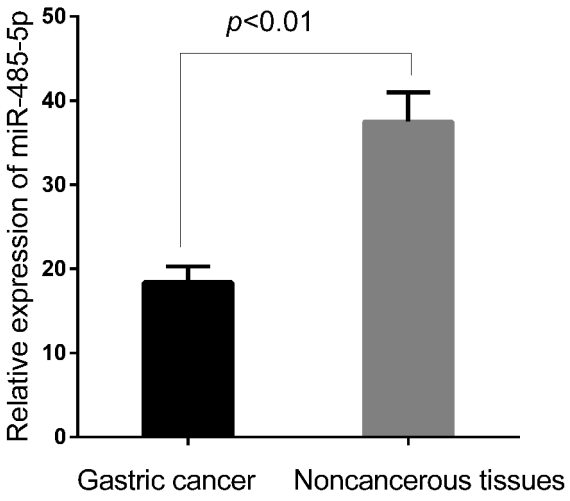

Figure 1. Comparison of miR-485-5p expression levels between gastric cancer tissues and the corresponding non-cancerous tissues.

Results

The Expression Level of miR-485-5p in Human GC Tissues

The relative expression levels of miR-485-5p in gastric cancer tissues and corresponding adjacent noncancerous tissues were shown in Figure 1. As shown, we found lower expression levels of miR-485-5p in GC tissues than in non-tumor tissues. Furthermore, the 132 patients were divided into a high expression group (n = 66) and a low expression group (n = 66), according to the median expression level of miR-485-5p.

Association Between Clinicopathological Features and miR-485-5p Expression Levels in GC Tissues

To explore the correlation between miR-485-5p expression and clinicopathological characteristics, we divided the 132 GC patients into high expression group (n = 66) and low expression group(n = 66). As shown in Table I, the Chi-square test showed that miR-485-5p expression was associated with tumor size, tumor depth, lymph node metastasis, and clinical stage (p = 0.003, 0.005,0.030 and 0.017, respectively). However, no significant differences about other characteristics of patients were found.

Downregulation of miR-485-5p Predicts Poor Biochemical Recurrence-free Survival

We performed survival analysis to evaluate whether miR-485-5p expression levels can predict prognosis of GC patients. The overall survival (OS, Figure 2, $p < 0.001$) of GC patients with low miR-485-5p expression was significantly shorter than those with high miR-485-5p expression. Univariate proportional hazard model showed that miR-485-5p expression ($p < 0.001$), tumor size ($p = 0.004$), tumor depth ($p = 0.018$), lymph node metastasis ($p = 0.031$), and TNM stage ($p < 0.001$) were prognostic predictors. Furthermore, multivariate analysis showed that low miR-485-5p expression was a significant and independent indicator of poor prognosis for patients with GC, as shown in Table II.

Discussion

Gastric cancer (GC) is the third most common cause of cancer death[21]. It is of great clinical value to identify novel molecular targets that could improve the outcome of this lethal disease. Recently, numerous researches reported the association between miRNAs and tumors. Aberrant expression of different miRNAs has been observed in GC. For instance, the expression level of miR-92a was upregulated in GC tissues and associated with local invasion and TNM stage[22]. The expression level of miR-185 was strongly downregulated in gastric cancer and associated with clinical stage and the presence of lymph node metastases[23]. Low miR-153 expression and high miR-221 expression predicted poor prognosis of human gastric cancer[24,25]. Furthermore, Hu et al[26] found

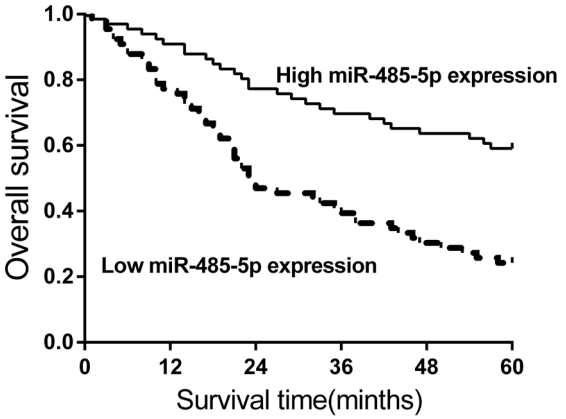

Figure 2. Kaplan-Meier analysis of 5-year overall survival in 132 gastric cancer patients in relation to miR-485-5p expression level. Patients in the low expression group had significantly poorer prognosis than those in high expression group ($p < 0.001$, log-rank test).

Table II. Univariate and multivariate analysis of overall survival in 132 gastric cancer patients.

	Univariate analysis		Multivariate analysis	
Variables	**RR**	***p*-value**	**RR**	***p*-value**
Age	0.81	0.321	1.52	0.129
Gender	1.66	0.191	1.31	0.316
Differentiation	1.89	0.116	1.71	0.091
Lauren type	1.81	0.124	1.57	0.177
Tumor size	3.42	0.004	1.83	0.092
Invasion depth	3.11	0.018	4.52	0.007
Lymphatic metastasis	2.98	0.031	3.35	0.023
TNM stage	4.32	<0.001	3.15	<0.001
miR-485-5p expression	4.11	<0.001	2.77	0.012

that miR-34a could inhibit tumor invasion and metastasis in gastric cancer by targeting Tgif2. Jian et al[27] observed that the downregulation of microRNA-193-3p inhibited tumor proliferation migration by regulating PTEN gene. Taken together, these results suggested that high miRNAs expression may function as a novel prognostic biomarker.

In the present study, we found that miR-485-5p expression was lower in gastric cancer tissues than that in the normal controls. It has been proved that miR-485-5p expression is significantly associated with aggressive clinicopathological features. Furthermore, the results of Kaplan-Meier analysis showed that patients with low expression levels of miR-485-5p had significantly shorter overall survivals compared to that with high miR-485-5p expression. At last, the multivariate and univariate analysis revealed that miR-485-5p expression was an independent prognostic factor. To our knowledge, our present study firstly reports the association between miR-485-5p and clinical significance

So far, little studies about the effect of miR-485-5p in human cancer were reported. Recently, Kang et al[20] found that upregulation of miR-485-5p expression could inhibit gastric cancer cell growth in vitro and in vivo. They also confirmed that flotillin-1 (Flot1) as a direct target of miR-485-5p. Guo et al[28] found that overexpression of miR-485-5p mimics could inhibit cell proliferation and invasion in hepatocellular carcinoma by targeting stanniocalcin 2. Moreover, Sun et al[29] indicated that miR-485-5p repressed HCC invasive and metastatic capacities by targeting EMMPRIN expression. Taken together, these researches indicated that miR-485-5p may function as a tumor suppressor. Further prospective studies are needed to explore the molecular mechanisms underlying.

Conclusions

The present work showed that the expression level of miR-485-5p was significantly decreased in GC tissues compared with adjacent normal tissues. The low miR-485-5p expression level was significantly associated with a shorter overall survival time of GC patients. Our results suggested that miR-485-5p may function as a novel biomarker for patient with poor overall survival in GC.

Conflicts of interest

The authors declare no conflicts of interest.

References

1) 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.
2) Chen W, Zheng R, Zeng H, Zhang S, He J. Annual report on status of cancer in China, 2011. Chin J Cancer Res 2015; 27: 2-12.
3) Li X, Wang F, Qi Y. MiR-126 inhibits the invasion of gastric cancer cell in part by targeting Crk. Eur Rev Med Pharmacol Sci 2014; 18: 2031-2037.
4) Beste LA, Ioannou GN, Yang Y, Chang MF, Ross D, Dominitz JA. Improved surveillance for hepatocellular carcinoma with a primary care-oriented clinical reminder. Clin Gastroenterol Hepatol 2015; 13: 172-179.
5) Katada T, Ishiguro H, Kuwabara Y, Kimura M, Mitui A, Mori Y. microRNA expression profile in undifferentiated gastric cancer. Int J Oncol 2009; 34: 537-542.
6) Yin WZ, Li F, Zhang L, Ren XP, Zhang N, Wen JF. Down-regulation of MicroRNA-205 promotes gastric cancer cell proliferation. Eur Rev Med Pharmacol Sci 2014; 18: 1027-1032.
7) Liu HY, Gao YJ, Song DF, Liu T, Feng Y. Correlation between microRNA-421 expression level and prognosis of gastric cancer. Int J Clin Exp Pathol 2015; 8: 15128-15132.

8) Tan Z, Jiang H, Wu Y, Xie L, Dai W, Tang H, Tang S. miR-185 is an independent prognosis factor and suppresses tumor metastasis in gastric cancer. Mol Cell Biochem 2014; 386: 223-231.
9) Yang J, Zeng Y. Identification of miRNA-mRNA crosstalk in pancreatic cancer by integrating transcriptome analysis. Eur Rev Med Pharmacol Sci 2015; 19: 825-834.
10) Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.
11) Fan YH, Ye MH, Wu L, Lv SG, Wu MJ, Xiao B, Liao CC, Ji QK, Chai Y, Zhu XG. Overexpression of miR-98 inhibits cell invasion in glioma cell lines via downregulation of IKKε. Eur Rev Med Pharmacol Sci 2015; 19: 3593-3604.
12) Li J, Dong G, Wang B, Gao W, Yang Q. miR-543 promotes gastric cancer cell proliferation by targeting SIRT1. Biochem Biophys Res Commun 2016; 469: 15-21.
13) Hurst DR, Edmonds MD, Welch DR. Metastamir: the field of metastasis-regulatory microRNA is spreading. Cancer Res 2009; 69: 7495-7498.
14) Zhang WH, Gui JH, Wang CZ, Chang Q, Xu SP, Cai CH. The identification of miR-375 as a potential biomarker in distal gastric adenocarcinoma. Oncol Res 2012; 20: 139-147.
15) Li N, Zhang QY, Zou JL, Li ZW, Tian TT, Dong B, Liu XJ, Ge S, Zhu Y, Gao J, Shen L. miR-215 promotes malignant progression of gastric cancer by targeting RUNX1. Oncotarget 2015; 7: 4817-4828.
16) Zhang HP, Sun FB, Li SJ. Serum miR-200c expression level as a prognostic biomarker for gastric cancer. Genet Mol Res 2015; 14: 15913-15920.
17) Zhuang K, Han K, Tang H, Yin X, Zhang J, Zhang X, Zhang L. Up-regulation of plasma miR-23b is associated with poor prognosis of gastric cancer. Med Sci Monit 2016; 22: 356-361.
18) He N, Zheng H, Li P, Zhao Y, Zhang W, Song F,Chen K. miR-485-5p binding site SNP rs8752 in HPGD gene is associated with breast cancer risk. PLoS One 2014; 9: e102093.
19) Kim TH, Kim YK, Kwon Y, Heo JH, Kang H, Kim G, An HJ. Deregulation of miR-519a, 153, and 485-5p and its clinicopathological relevance in ovarian epithelial tumours. Histopathology 2010; 57: 734-743.
20) Kang M, Ren MP, Zhao L, Li CP, Deng MM. miR-485-5p acts as a negative regulator in gastric cancer progression by targeting flotillin-1. Am J Transl Res 2015; 7: 2212-2222.
21) Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.
22) Ren C, Wang W, Han C, Chen H, Fu D, Luo Y, Yao H, Wang D, Ma L, Zhou L, Han D, Shen M. Expression and prognostic value of miR-92a in patients with gastric cancer. Tumour Biol 2016; 6: 247-255.
23) Tan Z, Jiang H, Wu Y, Xie L, Dai W, Tang H, Tang S. miR-185 is an independent prognosis factor and suppresses tumor metastasis in gastric cancer. Mol Cell Biochem 2014; 386: 223-231.
24) Zhang Z, Sun J, Bai Z, Li H, He S, Chen R, Che X. MicroRNA-153 acts as a prognostic marker in gastric cancer and its role in cell migration and invasion. Onco Targets Ther 2015; 8: 357-364.
25) Liu K, Li G, Fan C, Diao Y, Wu B, Li J. Increased Expression of MicroRNA-221 in gastric cancer and its clinical significance. J Int Med Res 2012; 40: 467-474.
26) Hu Y, Pu Q, Cui B, Lin J. MicroRNA-34a inhibits tumor invasion and metastasis in gastric cancer by targeting Tgif2. Int J Clin Exp Pathol 2015; 8: 8921-8928.
27) Jian B, Li Z, Xiao D, He G, Bai L, Yang Q. Downregulation of microRNA-193-3p inhibits tumor proliferation migration and chemoresistance in humangastric cancer by regulating PTEN gene. Tumour Biol 2016; 11: 231-238.
28) Guo GX, Li QY, Ma WL, Shi ZH, Ren XQ. MicroRNA-485-5p suppresses cell proliferation and invasion in hepatocellular carcinoma by targeting stanniocalcin 2. Int J Clin Exp Pathol 2015; 8: 12292-12299
29) Sun X, Liu Y, Li M, Wang M, Wang Y. Involvement of miR-485-5p in hepatocellular carcinoma progression targeting EMMPRIN. Biomed Pharmacother 2015; 72: 58-65.