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Original Article

MicroRNA-155 expression as a prognostic factor in patients with gallbladder carcinoma after surgical resection

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Abstract: Background: MicroRNA-155 (miR-155) is over-expressed in both hematopoietic malignancies and solid tumors. In the present study, we investigated the clinical significance of miR-155 in gallbladder carcinoma among Chinese population. Methods: Tissue specimens were collected from 133 patients who had undergone surgical resection at Shandong Provincial Hospital, Shandong University between May 2008 and April 2014. We profiled miR-155 expression in the gallbladder carcinoma tissues and normal gallbladder tissues by qRT-PCR. The Kaplan-Meier method was used to analyze the 5-year survival rate. Results: The expression levels of miR-155 were significantly higher in gallbladder carcinoma tissues than that in normal gallbladder tissues (P<0.001). High miR-155 expression was significantly associated with TNM stage (P=0.003), lymph node status (P=0.042), liver metastasis (P=0.010), and differentiated degree (P<0.001). We found that gallbladder carcinoma patients with high miR-155 expression level had distinctly shorter overall survival than patients with low miR-155 expression level (P=0.03). Multivariate analysis revealed that miR-155 expression level was independent prognostic factors for overall survival (HR=2.394, 95% CI: 1.568-10.034; P=0.009). Conclusion: High miR-155 expression is a prognostic indicator for poor prognosis of patients with gallbladder carcinoma among Chinese population.

Keywords: microRNA-155, expression, prognostic factor, gallbladder carcinoma

Introduction

Gallbladder carcinoma is the most common malignancy of the bile duct, and it is the sixth most common cause of cancer-related death worldwide [1]. Gallbladder carcinoma is insensitive to radiotherapy and chemotherapy, and the recurrence rate is high. The 5-year survival rate for gallbladder carcinoma patients was less than 10%, with the overall mean survival time of 6 months [2, 3]. Thus, the investigation about the prognostic factors for gallbladder carcinoma is especially important.

MicroRNAs (miRNAs) are small (20-25 nts) non-coding, single-stranded RNAs that post-transcriptionally regulate the gene expression by binding to 3' untranslated regions (3'-UTRs) of the target mRNAs [4]. miRNAs are able to regulate the gene expression in cell development, proliferation and differentiation, apoptosis, immune response, inflammation, viral infection, as well as in pathological states as autoimmune, cancerous and other diseases [5, 6]. Moreover, due to the stability in clinical specimens and its altered expression pattern in cancer, miRNAs can be used as biomarkers for diagnosis and prognosis of cancer [7].

MicroRNA-155 (miR-155) is over-expressed in both hematopoietic malignancies and solid tumors, such as leukemia, lung cancer, breast cancer, cervical cancer, thyroid carcinoma, hepatocellular carcinoma (HCC), gastric cancer, pancreatic ductal adenocarcinoma, colorectal cancer, bladder cancer, and renal cell carcinoma (RCC) [8-15]. Previously, Kono et al found that miR-155 was significantly over expressed in gallbladder carcinoma when compared with that in normal gallbladders (P=0.04). The high expression level of miR-155 in gallbladder carcinoma was significantly associated with the

Table 1. Correlation between the expression level of miR-155 with clinicopathological features

Characteristic	Case number	miR-155 expression		*P* value
		High (n=68)	Low (n=65)	
Age (y)				
<55	61	29	32	0.489
≥55	72	39	33	
Gender				
Male	79	36	43	0.158
Female	54	32	22	
TNM stage				
I+II	92	39	53	0.003
III+IV	41	29	12	
Lymph node status				
Yes	24	17	7	0.042
No	109	51	58	
Liver metastasis				
Yes	22	17	5	0.010
No	111	51	60	
Differentiated degree				
Well	88	35	53	<0.001
Moderate/poor	45	33	12	

presence of lymph node metastasis (P=0.01) and a poor prognosis (P=0.02). In vitro assays showed that aberrant expression of miR-155 significantly enhanced gallbladder carcinoma cell proliferation and invasion [16]. However, the clinical significance of miR-155 in gallbladder carcinoma among Chinese population has not been studied.

Material and methods

Patient specimens

Experiments were performed in compliance with the Chinese laws and guidelines concerning the patients' informed consent. The use of the human specimens was approved by the Shandong Provincial Hospital Institutional Review Board. Hospital and all participants signed written informed consent form. Tissue specimens were collected from 133 patients who had undergone surgical resection at the Department of General Surgery, Shandong Provincial Hospital, Shandong University between May 2008 and April 2014. None of them received any preoperative radiochemotherapy. All diagnoses were based on clinical findings, tumor morphological criteria, and immunohistochemical staining. The patients' medical records were reviewed to obtain data including age, gender, tumor differentiation, and TNM stage. Patient characteristics are listed in **Table 1**.

RNA isolation and qRT-PCR

Total RNA was extracted from gallbladder tissues using the mirVana miRNA extraction kit (Ambion, Austin, TX) according to the manufacturer's protocol. The cDNA template was amplified by real-time PCR using the SYBR Premix Dimmer Eraser kit (TaKaRa). Real-time PCR reactions were performed by the ABI7900 system (Applied Biosystems). U6 was used as the endogenous control to normalize expression levels. The mean Ct values of each sample were determined from duplicate reactions and then normalized against the corresponding U6 Ct values, calculated as delta Ct ($\Delta Ct = Ct_{miR\text{-}155} - Ct_{U6}$).

Statistical analysis

Count data were analyzed by Chi square test. Measurement data were expressed as the mean ± standard deviation (SD) and analyzed by t test. The Kaplan-Meier method was used to analyze the 5-year survival rate. The log-rank was employed in univariate survival analysis. Cox proportional hazard model was used for multivariate factor analysis. A value of $P<0.05$ was regarded as significantly different. Analyses were performed using the SPSS statistical software program for Windows (SPSS Inc., Chicago, IL).

Results

The expression of miR-155 in the gallbladder carcinoma tissues and the control tissues

We profiled miR-155 expression in the 133 pairs of gallbladder carcinoma tissues and normal gallbladder tissues by qRT-PCR. The expression levels of miR-155 were significantly higher in gallbladder carcinoma tissues than that in normal gallbladder tissues (P<0.001, shown in **Figure 1**). The median miR-155 expression level of all gallbladder carcinoma tissues was utilized to divide gallbladder carcinoma patients into two groups. 68 patients were assigned to the high-expression group,

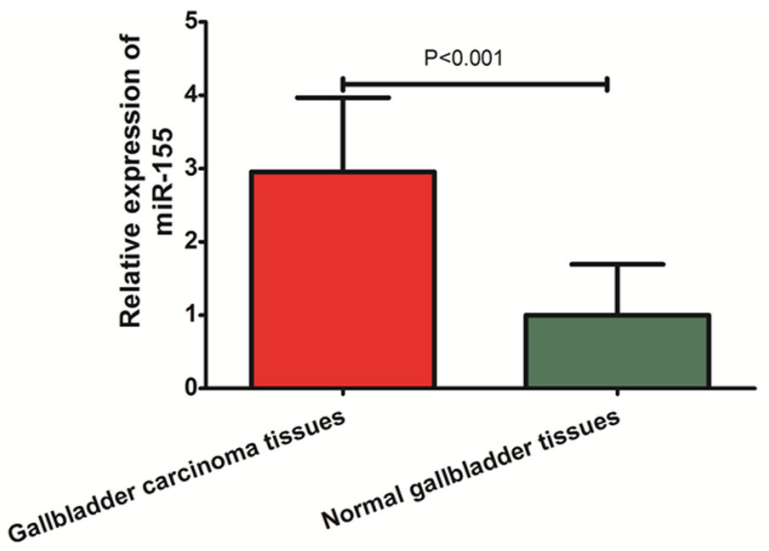

Figure 1. miR-155 expression level in gallbladder carcinoma tissues and normal gallbladder tissues.

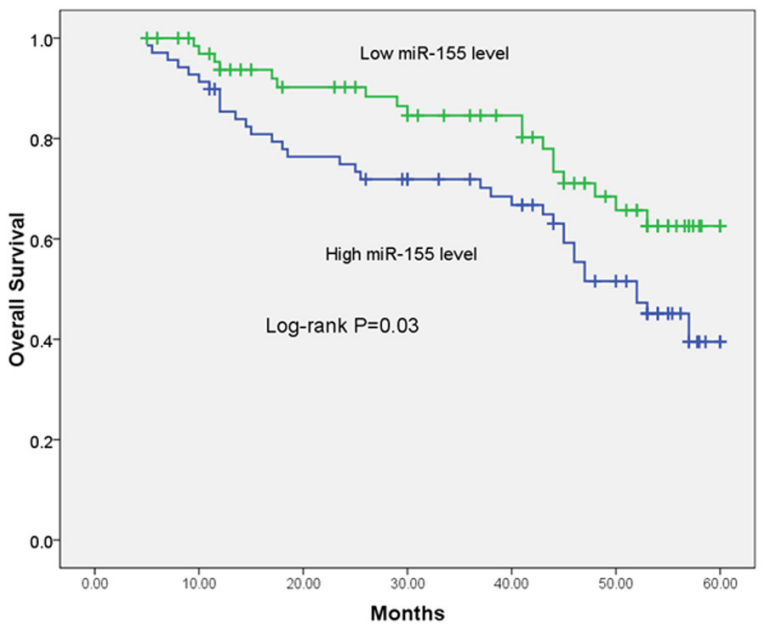

Figure 2. Kaplan-Meier plots of overall survival in patients with gallbladder carcinoma and with low and high level of miR-155.

and the remaining 65 patients were assigned to the low-expression group.

miR-155 expression level and clinicopathological features

We next analyzed the association between the miR-155 expression and various clinicopathological factors of the gallbladder carcinoma patients. High miR-155 expression was significantly associated with advanced TNM stage (P=0.003), lymph node status (P=0.042), liver metastasis (P=0.010), and differentiated degree (P< 0.001).

However, there was no significant association between miR-155 expression and other clinicopathological factors, including gender and age (all P>0.05, shown in **Table 1**).

Correlations of miR-155 expression with patient survival

Kaplan-Meier method and log-rank test were used to evaluate the differences of overall survival between low-expression group and high-expression group. We found that gallbladder carcinoma patients with high miR-155 expression level had distinctly shorter overall survival than patients with low miR-155 expression level (P=0.03, shown in **Figure 2**). Multivariate analyses were utilized to evaluate whether the miR-155 expression level and various clinicopathological features were independent prognostic parameters of patient outcomes. Multivariate analysis revealed that miR-155 expression level was independent prognostic factors for overall survival (HR=2.394, 95% CI: 1.568-10.034; P= 0.009, shown in **Table 2**).

Discussion

Gallbladder carcinoma is usually diagnosed at advanced stage due to absence of specific symptoms [17]. Despite recent advances in its diagnostic techniques and therapeutic managements that might give hope on consequent disease remission, prognosis of patients with gallbladder cancer remains poor [18]. The

Table 2. Multivariate analysis for prognostic factors of gallbladder carcinoma

Characteristic	HR	95% CI	*P* value
Age	1.352	0.449-2.038	0.492
Gender	0.458	0.239-3.102	0.691
TNM stage	2.391	1.283-9.995	0.005
Lymph node status	2.383	1.201-7.925	0.011
Liver metastasis	1.291	1.001-6.192	0.014
Differentiated degree	2.102	0.993-7.927	0.052
miR-155 expression	2.394	1.568-10.034	0.009

5-year survival rates of advanced-staged gallbladder carcinoma patients ranges from 20% to 40% [19]. It is therefore of paramount importance to elucidate its molecular biology, genetic causes, and cellular origin in order to develop novel therapeutic strategies to improve clinical outcome of patients with gallbladder carcinoma [20].

It has been predicted that miRNAs could regulate approximately 60% of human genes, including many oncogenes and tumor suppressor genes [21]. miRNAs are expressed in a tissue-specific manner and play important roles in the regulation of a large number of essential biological functions that are critical to normal development, including cell proliferation, differentiation, apoptosis, metabolism and immune response [21]. Therefore, the deregulation of their expression may have negative effect on normal cell growth, contributing to the development of diseases such as diabetes, immuno- or neurodegenerative disorders and cancer. Many studies have demonstrated that the loss and gain of function of specific miRNAs may be key events in oncogenesis. Many miRNAs are currently under investigation as diagnostic and prognostic biomarkers, therapeutic targets and as markers of cancer subtypes [22].

miR-155 can act as a multifunctional miR, with roles in hematopoiesis, inflammation, immunity, viral infection, cardiovascular disease, and neoplastic diseases [23, 24]. miR-155 is reportedly involved in the tumorigenesis processes of various cancers, and its expression level correlates with poor outcome. For example, Wang et al found that the progression-free survival (PFS) was significantly lower for patients with bladder cancer who had a high expression level of miR-155 (5-year survival rate, 23.0%) than those with a low miR-155 expression level (5-year survival rate, 48.9%; P<0.001) [25]. Lv et al examined the potential usefulness of serum miR-155 as a biomarker for diagnosis and prognosis in colorectal cancer (CRC). They found that miR-155 was a useful marker for discriminating cases from healthy controls, with an area under the ROC curve (AUC) of 0.776 (95% confidence interval (CI) 0.714 to 0.837, P<0.001). Kaplan-Meier analysis with the log-rank test indicated that high serum miR-155 expression had a significant impact on overall survival (38.2 vs. 69.9%; P<0.001) and progression-free survival (34.8 vs. 66.0%; P<0.001) [15]. In the study by Sun et al, the expression levels of miR-155 were significantly higher in glioma tissues than that in normal brain tissues (P<0.001), which was associated with high pathological grade (P<0.001) and low Karnofsky Performance Status score (P=0.022). As a result of Kaplan-Meier survival and Cox regression analyses, overall survival rates and progression-free survival were significantly poorer in high-expression group relative to low-expression group (both P<0.001) [26].

Previously, Kono et al found that miR-155 was significantly overexpressed in gallbladder carcinoma when compared with that in normal gallbladders (P=0.04). The high expression level of miR-155 in gallbladder carcinoma was significantly associated with the presence of lymph node metastasis (P=0.01) and a poor prognosis (P=0.02). In vitro assays showed that aberrant expression of miR-155 significantly enhanced gallbladder carcinoma cell proliferation and invasion [16]. However, the clinical significance of miR-155 in gallbladder carcinoma among Chinese population has not been studied. In the present study, we profiled miR-155 expression in the 133 pairs of gallbladder carcinoma tissues and normal gallbladder tissues by qRT-PCR. The expression levels of miR-155 were significantly higher in gallbladder carcinoma tissues than that in normal gallbladder tissues. We next analyzed the association between the miR-155 expression and various clinicopathological factors of the gallbladder carcinoma patients. High miR-155 expression was significantly associated with advanced TNM stage, lymph node status, liver metastasis, and differentiated degree. Kaplan-Meier method and log-rank test were used to evaluate the differences of overall survival between low-expression group and high-expression

group. We found that gallbladder carcinoma patients with high miR-155 expression level had distinctly shorter overall survival than patients with low miR-155 expression level. Multivariate analyses were utilized to evaluate whether the miR-155 expression level and various clinicopathological features were independent prognostic parameters of patient outcomes. It revealed that miR-155 expression level was independent prognostic factors for overall survival. In conclusion, our findings reveal that miR-155 expression might be an independent prognostic factor and a therapeutic target for gallbladder carcinoma.

Disclosure of conflict of interest

None.

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