Rab25 Acts as a Promising Novel Bio-Marker in the Prognosis of Patients with Hepatocellular Carcinoma

Zhong Dai
Cancer Hospital of Huanxing, Chaoyang District
Ke-Qing Yao
Cancer Hospital of Huanxing, Chaoyang District
Xing-Sheng Hu (hgfddsz@126.com)
Cancer Hospital ,Chinese Academy of Medical Sciences & Peking Union Medical College
https://orcid.org/0000-0001-6268-889X
Yi-Qun Li
Chinese Academy of Medical Sciences & Peking Union Medical College
Yu-Tao Liu
Chinese Academy of Medical Sciences & Peking Union Medical College
Shu-Luan Li
Cancer Hospital of Huanxing, Chaoyang District
Xue Ji
Cancer Hospital of Huanxing, Chaoyang District

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Abstract

Background: *Rab25* was indicated to be involved in several human tumors. However, the clinical significance of *Rab25* in hepatocellular carcinoma (HCC) was still unclear. The purpose of this study was to investigate the expression and prognostic value of *Rab25* in HCC.

Methods: The relative mRNA expression levels of *Rab25* in HCC tissues and adjacent normal tissues were detected by quantitative real-time polymerase chain reaction (qRT-PCR). Chi-square test was used to analyze the relationship between *Rab25* expression and clinical characteristics of patients. The prognostic value of *Rab25* in HCC was estimated through Kaplan-Meier method and cox regression analysis.

Results: *Rab25* gene expression level was significantly higher in HCC tissues than that in normal tissues (P<0.001). Importantly, the increased *Rab25* expression was closely associated with TNM stage (P=0.024), metastasis (P=0.022) and invasion classification (P=0.039). Moreover, patients with high *Rab25* expression tended to have obviously shorter overall survival than those with low expression of *Rab25* (log rank test, P<0.001) via Kaplan-Meier analysis. Univariate and multivariate cox regression analyses revealed that *Rab25* was an independent prognostic factor of HCC.

Conclusions: *Rab25* is up-regulated in HCC and contributes to the progression of this tumor. What's more, *Rab25* may be a potential bio-marker for the prognosis of HCC.

Background

Currently, hepatocellular carcinoma (HCC) is a most frequent malignancy worldwide [1]. HCC is one of the major causes of incidence and mortality rates among malignant tumors [2]. The risk factors for the progression of HCC consist of chronic hepatitis virus infection and liver cirrhosis [3]. Although surgical resection is the most common therapies for HCC, the death rate is still high due to the frequent recurrence and high metastasis [4]. In addition, most HCC patients are diagnosed with advanced stages and the current treatments are not effective for them [5]. Because of the above-mentioned reasons, the prognosis of HCC is poor and the 5-year overall survival is very low [6]. Therefore, it is important to find novel bio-markers for the prognosis and targets for the therapy of HCC.

Rab GTPases family plays crucial roles in regulating vesicle transportation [7]. Previous studies had demonstrated that several members of Rab GTPases were involved in some processes, including the progression of human tumors [8, 9]. *Rab25* is a member of the *Rab11* family and located on human chromosome 1q22. *Rab25* gene encodes an intracellular transport protein that participates in the regulation of signal transduction and pathways, including cell proliferation, differentiation and migration of cancers [10]. The expression of *Rab25* was considered to be ubiquitous and enriched in epithelial cells. A growing body of evidence had reported that the aberrant expression of *Rab25* was confirmed in a variety types of tumors [11, 12]. In addition, a study suggested that knockdown of *Rab25* could suppress cell growth, cell cycle progression and induce cell apoptosis of ovarian cancer [13]. Moreover, *Rab25* had

been proven to be up-regulated in HCC, and its over-expression contributed to tumor cell proliferation as well as invasion [14]. However, little is known about the prognostic value of *Rab25* in HCC.

In this study, we detected the relative mRNA expression levels of *Rab25* in HCC tissues and corresponding adjacent normal tissues. The relationship between *Rab25* expression and clinical characteristics of patients was analyzed. The prognostic value of *Rab25* was also assessed.

Methods

Patients and samples

We recruited 132 HCC patients who underwent curative resection at Cancer Hospital ,Chinese Academy of Medical Sciences & Peking Union Medical College. None of the patients had received any treatments before surgery. Histopathological diagnosis was confirmed by the pathologists with extensive experience. This study was approved by the Ethical Committee of the hospital and the written informed consents were obtained from all patients in advance.

HCC tissues and corresponding adjacent normal tissues were collected from HCC patients and immediately frozen in liquid nitrogen. The tissue samples were stored at − 80℃ before RNA extraction. The detail clinicopathological characteristics of HCC patients were shown in Table 1. Patients' follow-up was conducted with 5 years via a telephone or questionnaires. Patients whose deaths were not directly associated with HCC should be excluded from this study.

Table 1
Relationship between *Rab25* expression and clinical features of HCC patients

Clinical Features	Cases (n = 132)	*Rab25* expression		χ^2	*P*
		High (n = 64)	Low (n = 68)		
Age (years)				0.732	0.392
≤ 53	69	31	38		
> 53	63	33	30		
Gender				0.500	0.480
Male	91	46	45		
Female	41	18	23		
Liver cirrhosis				1.582	0.208
No	42	17	25		
Yes	90	47	43		
AFP level (ng/mL)				1.377	0.241
≤ 20	79	35	44		
> 20	53	29	24		
Tumor size (cm)				1.853	0.173
≤ 5	74	32	42		
> 5	58	32	26		
Differentiation				2.999	0.083
Well + Moderate	68	28	40		
Poor	64	36	28		
TNM stage				5.103	0.024
□–□	67	26	41		
□–□	65	38	27		
Metastasis				5.226	0.022
Negative	89	37	52		
Positive	43	27	16		
Invasion classification				4.273	0.039
T1 + T2	82	34	48		

Clinical Features	Cases (n = 132)	*Rab25* expression		χ^2	*P*
		High (n = 64)	Low (n = 68)		
T3 + T4	50	30	20		

RNA extraction and qRT-PCR

The TRIzol reagent (Invitrogen, USA) was used to isolate total RNA from tissue samples. The reverse transcription was performed with PrimeScript® 1st strand cDNA synthesis kit (Takara, China). SYBR Green PCR kit (Takara, China) was applied to detect the relative mRNA expression of *Rab25* in tissues. *GAPDH* was used as internal control and the the relative mRNA quantification of *Rab25* expression was calculated by the $2^{-\Delta\Delta Ct}$ method. Each samples was examined in triplicate.

Statistical analysis

SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5.0 (GraphPad Software Inc., USA) were utilized to perform all the statistical analyses. The data were presented as mean ± standard deviation (SD). The difference between two groups was evaluated via Student's t-test. And chi-square test was applied to analyze the relationship between *Rab25* expression and clinical characteristics of patients. The overall survival of patients with different *Rab25* expression was compared by Kaplan-Meier analysis. Univariate and multivariate cox regression analyses were used to estimate the prognostic value of *Rab25* in HCC. When *P* less than 0.05, the difference was considered statistically significant.

Results

Demographic data of the study subjects

The clinical characteristics of HCC patients were shown in Table 1. 41 female and 91 male patients were enrolled in this study (mean age, 53.37 ± 14.95 years; range, 36–81 years). Among these patients, 90 patients had liver cirrhosis and 79 patients had AFP levels less than 20 ng/mL. 74 tumors were less than 5 cm in size and 58 tumors were more than 5 cm in size. There were 68 cases with well or moderate differentiation and 64 cases with poor differentiation. Of 132 patients, 67 patients were at stage □-□ and 65 patients were at stage □-□. There were 43 patients with positive metastasis and 50 patients of T3 + T4 invasion classification.

Rab25 expression was up-regulated in HCC patients

The relative mRNA expression levels of *Rab25* in HCC tissues and adjacent normal tissues were detected by qRT-PCR. Compared with normal tissues, *Rab25* mRNA expression was significantly increased in HCC tissues ($P < 0.001$, Fig. 1). According to the median *Rab25* expression, 132 HCC patients were divided into high *Rab25* expression group and low *Rab25* expression group.

Relationship between Rab25 expression and clinical features of HCC patients

To explore whether *Rab25* was involved in the development of HCC, we analyzed the correlation between its expression and clinical characteristics of patients. As shown in Table 1, a notable correlation was found between *Rab25* expression and TNM stage ($P = 0.024$), metastasis ($P = 0.022$) as well as invasion classification ($P = 0.039$). However, *Rab25* expression was not obviously associated with age, gender, liver cirrhosis, AFP level, tumor size or differentiation ($P > 0.05$, Table 1).

Increased expression of Rab25 was associated with poor prognosis of HCC patients

In Kaplan-Meier analysis, the overall survival time of HCC patients with low *Rab25* expression was markedly longer than those with high expression of *Rab25* (log rank test, $P < 0.001$, Fig. 2). To estimate whether *Rab25* expression was associated with the prognosis of HCC, cox regression analysis was conducted. Univariate and multivariate analyses using cox regression analysis revealed that *Rab25* expression (HR = 2.149, 95%CI = 1.276–3.618, $P = 0.004$), TNM stage (HR = 2.041, 95%CI = 1.215–3.429, $P = 0.007$) and metastasis (HR = 1.860, 95%CI = 1.125–3.078, $P = 0.016$) were all correlated with the overall survival and they might be independent prognostic factors of HCC patients (Table 2).

Table 2
Univariate and multivariate cox regression analyses of prognostic factors in patients with HCC patients

Factors	Univariate analysis			Multivariate analysis		
	HR	(95%CI)	*P*	HR	(95%CI)	*P*
Rab25 (High vs Low)	2.512	1.503-4.200	< 0.001	2.149	1.276–3.618	0.004
Age (years) (> 53 vs ≤ 53)	1.023	0.624–1.677	0.929	-	-	-
Gender (Male vs Female)	1.401	0.794–2.471	0.245	-	-	-
Liver cirrhosis (Yes vs No)	1.353	0.776–2.360	0.287	-	-	-
AFP level (ng/mL) (> 20 vs ≤ 20)	1.644	1.003–2.696	0.049	-	-	-
Tumor size (cm) (> 5 vs ≤ 5)	1.659	1.010–2.723	0.045	-	-	-
Differentiation (Poor vs Well + Moderate)	1.789	1.080–2.966	0.024	-	-	-
TNM stage (□-□ vs □-□)	2.211	1.322–3.698	0.002	2.041	1.215–3.429	0.007
Metastasis (Positive vs Negative)	2.037	1.238–3.353	0.005	1.860	1.125–3.078	0.016
Invasion classification (T3 + T4 vs T1 + T2)	1.749	1.066–2.871	0.027	-	-	-
Note: - indicated no data.						

Discussion

Rab GTPases family members, belonging to the rat sarcoma (Ras) oncoprotein small GTPases, are key regulators of intracellular vesicle transport that plays an important role in carcinogenesis and tumor progression [7, 15]. It reported that Rab GTPases was associated with several tumor-related processes [8].

Similar to other members of Rab GTPases family, *Rab25* had been proven to be specific in epithelial cells and function in various cancers [16]. Previous study had showed that *Rab25* could enhance the aggressiveness of tumors through directly interacting with integrin and thereby promoting tumor cell invasion [17]. It also revealed that the aberrant expression of *Rab25* might be involved in tumorigenesis, progression and prognosis of neoplasia [18]. However, the clinical significance of *Rab25* in HCC is still unclear.

In the present study, we evaluated the relative mRNA expression levels of *Rab25* in HCC tissues and corresponding adjacent normal tissues. The outcome suggested that *Rab25* gene expression was obviously increased in HCC tissue samples compared to that in normal tissues. It revealed that *Rab25* might exhibit tissue-specific function as an oncogene in HCC. This result was accorded with the previous studies. A study of Mitra et al. demonstrated that the mRNA and protein expression levels of *Rab25* were significantly elevated in breast cancer cell lines [19]. In the study of Sheach et al., tissue microarray section showed the protein expression of *Rab25* was higher in ovarian cancer [20].

To explore the effect of *Rab25* on the progression of HCC, we analyzed the relationship between *Rab25* expression and clinical characteristics of patients. Interestingly, patients with high *Rab25* expression tended to have advanced TNM stage, positive metastasis and high invasion classification which were aggressive clinical parameters representing advanced progression, invasion and metastasis. Similar findings were occurred in other cancers. Li et al. mentioned that the increased expression level of *Rab25* was closely associated with invasion classification, lymph node metastasis and pathological stage of renal cell carcinoma patients [21]. In gastric cancer, elevated *Rab25* expression was more often observed in patients with late pathological stage, positive lymph node metastasis and distant metastasis [22]. According to these studies, we hypothesized that *Rab25* expression was involved in the development of human tumors, including HCC.

Recently, some clinical factors, such as serum AFP level, TNM stage and metastasis, are shown to be associated with the prognosis of HCC [23]. However, they may not accurately assess the prognosis due to heterogeneity in HCC patients. In this study, *Rab25* expression was closely correlated with TNM stage and metastasis, suggesting *Rab25* might be linked with the prognosis of HCC. Furthermore, the result of Kaplan-Meier analysis showed that patients with high expression of *Rab25* had markedly shorter overall survival than those with low *Rab25* expression. Univariate and multivariate cox regression analyses indicated that *Rab25* might be an independent prognostic factor of HCC. Similar to our finding, a large number of researches had demonstrated that *Rab25* had potency to predict the overall survival and disease-free survival of tumors [24, 25]. Moreover, metastasis and TNM stage both were independent factors in this study.

Conclusions

In conclusion, we prove that *Rab25* expression is up-regulated in HCC and correlated with tumor progression. Besides, *Rab25* may be an independent prognostic factor of HCC. However, considering the

constraints of samples size and its source, further studies are needed.

List Of Abbreviations

quantitative real-time polymerase chain reaction (qRT-PCR)

hepatocellular carcinoma (HCC)

standard deviation (SD)

rat sarcoma (Ras)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Cancer Hospital ,Chinese Academy of Medical Sciences & Peking Union Medical College and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

Z.D., H.Y. design of the work; X.H., Y.L. the acquisition, analysis, Y.L., S.L. interpretation of data; S.L., X.J. the creation of new software used in the work; X.H. have drafted the work or substantively revised it. All

authors read and approved the final manuscript.

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Figures

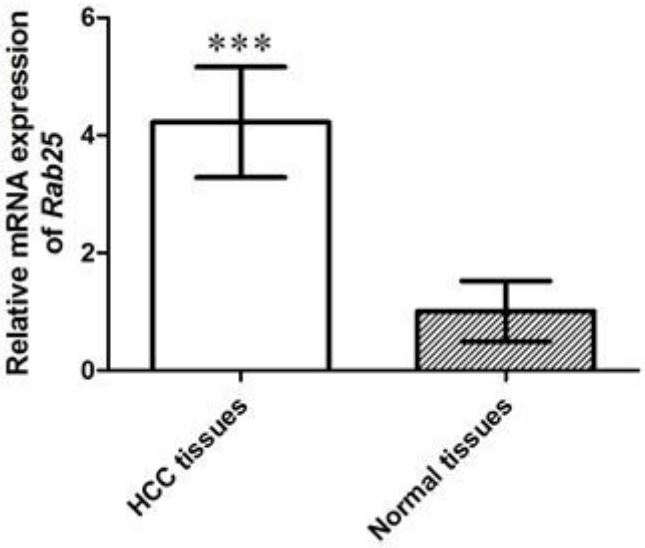

Figure 1

The relative mRNA expression levels of Rab25 in HCC tissues and paired adjacent normal tissues. Compared with normal tissues, the expression of Rab25 was obviously up-regulated in HCC tissue samples at mRNA level (P<0.001). *** indicated P less than 0.001.

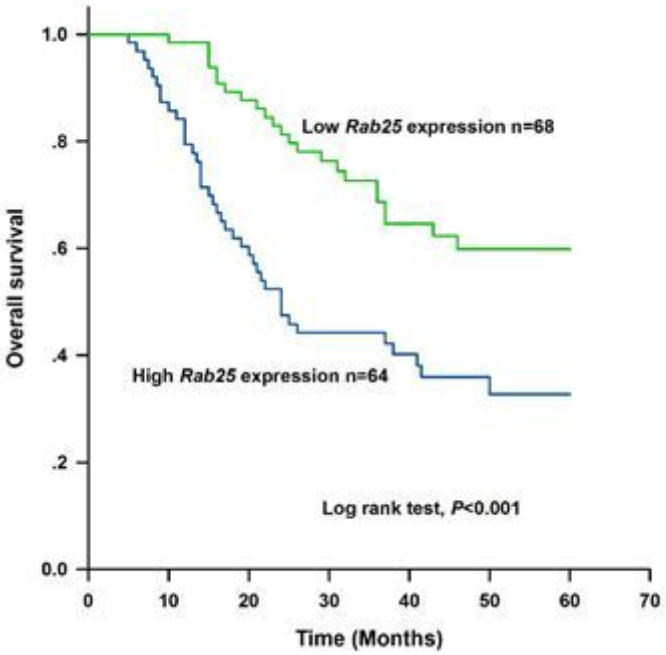

Figure 2

Kaplan-Meier analysis for the overall survival of HCC patients. The overall survival of patients with low expression of Rab25 was significantly longer than those with high Rab25 expression (log rank test, P<0.001).