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GRK6 expression in patients with hepatocellular carcinoma

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ABSTRACT

Objective: To investigate the expression and potential roles of G protein-coupled receptor kinase 6 (GRK6) in hepatocellular carcinoma (HCC) patients. **Methods:** Immunohistochemistry and Western blot was performed to determine GRK6 expression in 73 HCC samples. And the correlation with clinicopathological features was also analyzed. **Results:** GRK6 expression was significantly higher in HCC than that in normal hepatic tissue. GRK6 was positively correlated with proliferation marker Ki-67, clinical stage, metastasis and survival time. **Conclusions:** Our results suggested that GRK6 overexpression plays an important role in HCC. Monitoring the expression of GRK6 maybe helpful in early diagnosis and prognosis of HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is one of commonest malignant tumors, its pathogenesis is still not completely clear, and its morbidity is rising year by year in China. Because of its high morbidity and poor prognosis, it is hot spot research to discover the molecular mechanism for HCC metastasis. G protein-coupled receptor kinase 6 (GRK6) is an important protein kinase in human, which participates in the pathological process of many diseases[1-5]. GRK6 can regulates the activity of chemokine receptor by phosphorylation[6,7] , and is related to the adhesion and movement of many cancer cell lines. It is reported that the lack of GRK6 can inhibit cancer cell migration[8]. The function and expression of GRK6 in HCC has not been reported. This study is to determine GRK6 expression in HCC by immunohistochemical assay and Western blot, and to analyze the relationship between the expression level and clinicopathological features of HCC.

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2. Materials and methods

2.1. Material

A total of 73 HCC samples were collected from patients admitted from May 2010 to August 2011, and all samples were confirmed by pathological histological analysis. The age was 34 to 75 years old, averaged 53 years old; it included male 50, female 2; Ⅰ grade 24, Ⅱ grade 36, Ⅲ grade 13 (Table 1). All patients had informed consent. The material from the operation was equally divided into two copies, one was fixed with formaldehyde, embedded with paraffin, and used for immuohistochemical stainin, and another one was preserved in cryogenic refrigerator under -80 ℃ after liquid nitrogen frozen for Western blot.

GRK6 antibody from rabbits and β-actin antibody from rats were from Santa cruz Company, chemiluminescent solution was supplied by CST company, SP kit was from Dako, and the other chemical reagent was analytical reagent made in china.

2.2. Method

2.2.1. Immuohistochemical assay

SP method was performed. The paraffin section was under conventional dewaxing, incubated in room temperature

in 3% methanol solution for 15 min, washed 3 times with PBS, closed with normal goat serum working solution for 1 h, and then incubated in GRK6 antibody at 4 ℃ for a night. Then the sections was rinsed in PBS, incubated in room temperature for 30 min, developed by DAB, stained by hematoxylin, dehydrated by ethanol, transparented and fixed by neutral xylene balata. GRK6 expression was observed and analyzed under light microscope, and was presented by positive cell percentage. Antibody dilution ratio of GRK6 was 1:200.

2.2.2. Western blot assay

Histiocyte lysate was prepared in 100 mg/mL, including 1 M Tris-HCl pH 7.5, 1% TritonX-100, 1% NP-40, 10% SDS, 0.5% sodium deoxycholate, 0.5 M EDTA, 10 μg/mL leupeptin, 10 μg/mL aprotinin, 1 mm PMSF, 40 mmol/L DTT and deionized water. And then histiocyte lysate was kept in ice for 1h, centrifuged in 4 ℃ at 12 000 r/min for 20 min. Upper homogenate was tabken for Bradford test, and kept in -80 ℃ for reserve. The total protein was degenerated for 10 min under boiling and analyzed in SDS PAGE gel. After then the protein was diverted with electro transfer from SDS PAGE gel to PVDF membrane. After washed with TBST, PVDF membrane was temperature closed in TBST with 5% defatted milk powder, and added once with antibody (rabbit anti human GRK6 1:1 000), incubated in 4 ℃ for a night, washed with TBST 3 times and then added twice with antibody (horse radish peroxidase marked goat anti-rabbit IgG, 1:1 000), incubated in room temperature for 2 h, fondly washed with TBST 3 times (10 min), developed in ECL color. GRK6 expression was presented by ratio of banding gray value of GRK6 and β-actin.

2.3. Statistical analysis

All statistical analyses were carried out with SPSS 13.0 statistical software. χ^2 test was used for GRK6 expression, and Spearman correlation analysis was used for clinical index correlation. The survival rate acquired from follow-up visit was analyzed with Kaplan Meier survival curve and Single variable Log rank test. Statistical significance was set at $P<0.05$.

3. Result

3.1. GRK6 expression on HCC tissue and pericarcinomatous tissue

Immunohistochemical result shows that the expression of GRK6 and Ki67 in HCC tissue was significantly higher than that in pericarcinomatous tissue ($Z = -2.422$, $P < 0.05$) (Figure 1). Western blot test was performed for the GRK6 expression in fresh tissue, and the outcome shows there was a specific banding in 66 kD, whose molecular weight was the same with GRK6, indicating the GRK6 expression. Figure 2 shows the GRK6 expression in pericarcinomatous tissue was significantly lower (0.14± 0.02) than in HCC tissue (0.79± 0.21) ($P < 0.05$).

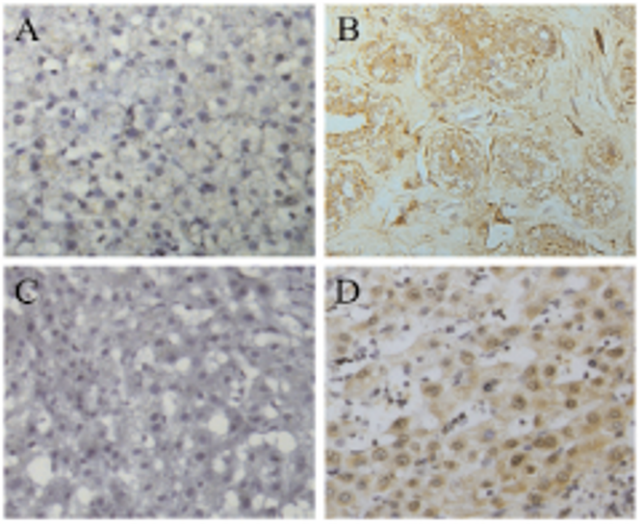

Figure 1. Expression of GRK6 and Ki-67 (SP × 200).
A: GRK6 expression in pericarcinomatous tissue; B: GRK6 expression in HCC tissue; C: Ki-67 expression in pericarcinomatous tissue; D: Ki-67 expression in HCC tissue.

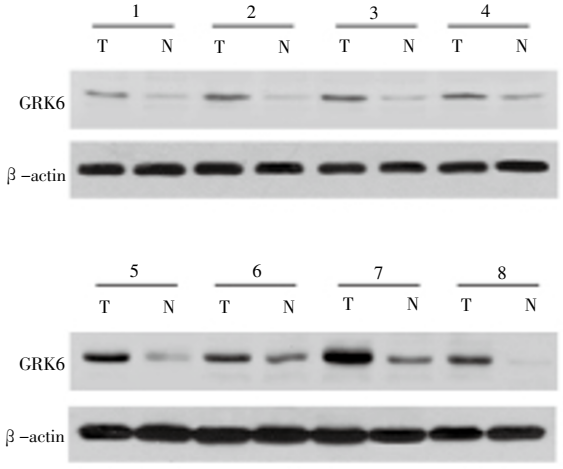

Figure 2. GRK6 expression in carcinoma tissue (T) and in pericarcinomatous tissue (N).

3.2. Relationship between GRK6 expression and clinicopathological features

GRK6 expression was related to age, sex, the size of tumor, hard state, AFP and HBeAb. GRK6 expression in poor-differentiated carcinoma tissue was significantly higher than that in high-differentiated ($P = 0.005$). And that in tumor metastasis carcinoma tissue was significantly higher than that in not metastasis ($P = 0.045$). Ki-67 was a index for tumor cell proliferation, whose expression was linear related to GRK6 expression (Figure 3).

Table 1
Relationship between GRK6 expression and clinic opathological features.

Parameters	Total	GRK6		*P*
		Low≤ 0.46	High>0.46	
Age (years)				
≤ 45	30	14	16	0.235
>45	43	14	29	
Gender				
Male	50	21	29	0.440
Female	23	7	16	
Histological grade				
Well	24	13	11	0.005
Mod	36	15	21	
Poor	13	0	13	
Metastasis				
Positive	46	22	24	0.045
Negative	27	6	21	
Tumor size (cm)				
≤ 5	57	21	36	0.772
>5	16	7	9	
Cirrhosis				
Positive	23	10	13	0.841
Negative	50	23	27	
AFP (ng/mL)				
≤ 50	57	26	31	0.895
>50	16	7	9	
HbSAg				
(+)	57	46	11	0.056
(–)	16	9	7	

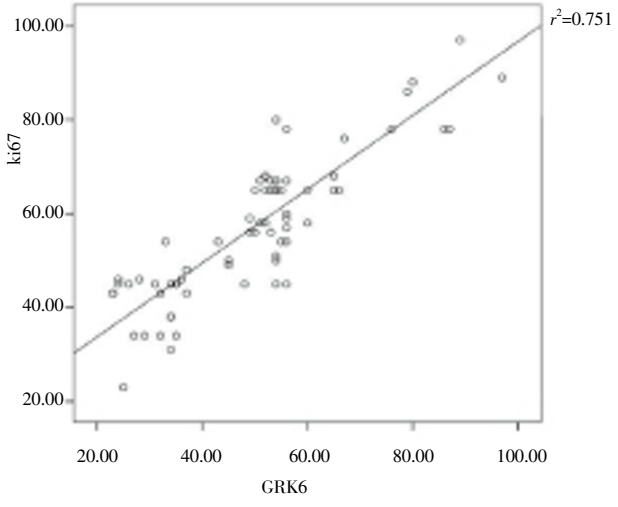

Figure 3. Linear relationship between the expression in Ki-67 and GRK6.

3.3. Influence of GRK6 expression on survival after operation

Survival data of 52 cases was collected from 73 cases, including 33 patients with high GRK6 expression and 19 patients with low GRK6 expression. Survival rate of high GRK6 expression group was 39.4%, and low group was 78.9%. The result shows that GRK6 expression (P = 0.009), Ki-67 expression (P = 0.009) and histilogical grade were correlated with survival (Table 2). Survival analysis and single variable log rank test showed that the higher was, the lower survival rates was (χ^2 = 4.821, P<0.05); and the shorter survival time was, the worse prognosis was (Figure 4).

Table 2
Survival analysis of patients.

	Total	Survival status		*P*
		Dead	Alive	
Age (years)				
≤ 45	19	9	14	0.412
>45	33	15	14	
Gender				
Male	19	13	6	0.021
Female	33	11	22	
Histological grade				
Well	13	6	7	0.045
Mod	17	4	13	
Poor	22	14	8	
Metastasis				
Positive	31	9	22	0.004
Negative	21	15	6	
Tumor size (cm)				
≤ 5	39	19	20	0.749
>5	13	5	8	
Cirrhosis				
Positive	19	14	5	0.868
Negative	33	25	8	
AFP (ng/mL)				
≤ 50	39	20	19	0.528
>50	13	5	8	
HbSAg				
(+)	33	18	15	0.219
(–)	19	7	12	
Ki-67				
Low expression	24	4	20	0.000
High expression	28	20	8	
GRK6				
Low expression	19	4	15	0.009
High expression	33	20	13	

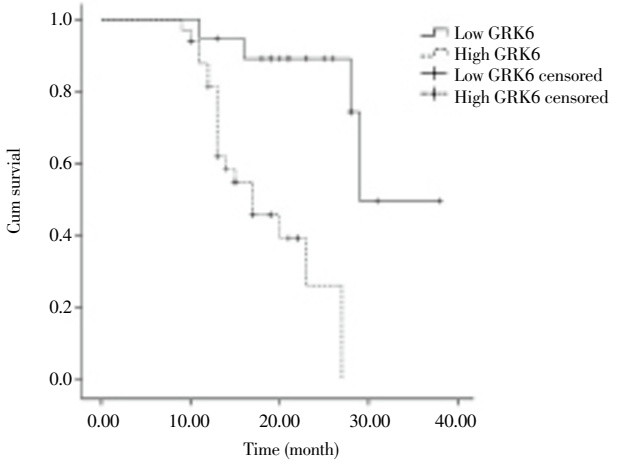

Figure 4. Relationship between GRK6 expression and survival rate after surgery.

4. Discussion

HCC has high malignant degree and morbidity, and postoperative recurrence and metastasis are the important factors of prognosis. The metastasis of the HCC is a multiple stage process. The first display of HCC cell is abating adhesivity and enhancing athletic ability, and secretes substances such as matrix metalloproteinase to degrade extracellular matrix and then the HCC cell can cross through the basement membrane and extracellular matrix[9-12]. The metastasis of the HCC cell to a great extent depends on the signal and the function of the chemokine[13,14].

GRK6 belongs to GRKs family. The member of GRKs contains a high conserved regulator of G protein signaling protein domain, a Ser-Thr kinase domain which is similar to AGC protein kinase domain, and a carboxyl terminal which is affected with cell membrane[15]. In addition to mediate phosphorylation dependent desensitization effect of G protein coupled receptor, GRKs still can phosphorylation regulate other substrate. The reaserch shows that GRKs is closly related to the movement and migration of the cell, participates in cell movement and cell migration of the integin[16], and participate in the regulation of chemotaxis T/B cell and neutrophil[17]. GRK6 is related to the adhere and movement of many kinds cancer cell, and it can disturb and significantly inhibit the migration of PC3, MB231 and Hela cell. GRK6 interacted with GRK-interacting protein (GIT1) adjust the cell adhere and cytoskeleton reconstruction[8,18]. In addition, GRK6 can affect the migration and invasion of cancer cell through second messenger such as cAMP, calmodulin and EGFR[19, 20].

This study found that GRK6 is high expressed in HCC, and related to the migration and prognosis of HCC. Determining GRK6 expression can help early diagnosis of HCC and to evaluate the prognosis of the patients. GRK6 may participate in the regulation for migration and invasion of HCC cell, but the molecular mechanism is still needed further research.

Conflict of interest statement

We declare that we have no conflict of interest.

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