Int J Clin Exp Med 2015;8(11):21482-21486
www.ijcem.com /ISSN:1940-5901/IJCEM0015465

Original Article

Low expression of *GNAI3* predicts poor prognosis in patients with HCC

Guodong Chen[1,2], Xiaoyan Li[3], Gengsheng He[2], Zijian Yu[2], Jiaxing Luo[2], Jun He[2], Zonghai Huang[1]

[1]Department of General Surgery, Zhujiang Hospital, Southern Medical University, Guangzhou, China; Departments of [2]General Surgery, [3]Endocrinology, First Affiliated Hospital, University of South China, Hengyang, China

Received September 1, 2015; Accepted November 1, 2015; Epub November 15, 2015; Published November 30, 2015

Abstract: Purposes: This study was performed with an aim to explain the underlying role of GNAI3 on the prognosis of patients with HCC. Methods: The expression of GNAI3 at protein level was detected with the utilization of Immunohistochemistry (IHC). Chi-square test was conducted to assay the relationship between GNAI3 expression and clinical parameters of HCC patients. The correlation between expression level of GNAI3 and survival time after surgeries of HCC patients was evaluated by Kaplan-Meier method. Finally, the Cox regression was established to evaluate the relationship between GNAI3 expression and the prognosis of patients with HCC. Results: In this study, the negative rate of GNAI3 expression in HCC samples was about 76.6%, which was significantly higher than that in paired normal specimens (12.5%). Result showed that there was no correlation between GNAI3 expression and age, gender, liver cirrhosis and vein invasion (P>0.05), but tight relationship between GNAI3 expression and TNM stage and tumor size was found (P<0.05). The following Kaplan-Meier analysis result illustrated that negative expression of GNAI3 induced high mortality of HCC patients. Cox regression result revealed that GNAI3 might be a biomarker for prognosis of patients with HCC (HR: 0.218, P=0.016, 95% CI 0.063-0.750). Conclusion: Generally, results of this study demonstrated that expression of GNAI3 shared a tight relationship with the prognosis of patients with HCC. Therefore, *GNAI3* could be considered as a novel index for prognosis of patients with HCC.

Keywords: HCC, *GNAI3,* prognosis

Introduction

Liver cancer consists of primary hepatic carcinoma and metastatic hepatic carcinoma. He patocellular carcinoma (HCC) accounts for 80% of primary hepatic carcinoma in adults [1-3], which is one of the most common malignancies in Africa and Asia, especially in China and Japan [4-6]. Studies have shown that HCC has an increasing incidence and a poor 5-year survival rate of about 7% despite treatment [7-9]. Currently, therapy of HCC was mainly surgical resection, and sometimes liver transplantation, radiotherapy and some other therapies are also adopted. However, due to advanced disease at the time of diagnosis, lack of suitable organ donors and the influence of radiosensitivity and other factors, the effects of therapies are not obvious [10-14]. Therefore, it is crucial to develop beneficial markers and therapeutic targets for HCC [15].

Guanine nucleotide binding proteins (G-proteins) are a family of signal mediators that are essential for a variety of cellular functions [16]. They widely exist in cells and can function as switches to transduce and regulate signals from outside to inside in the cells [17-19]. Heterotrimeric G protein complexes are typically made up of α, β and γ subunits [20]. The Guanine nucleotide binding protein, alpha inhibiting activity polypeptide 3 (*GNAI3*) belongs to the α subunit [21], which locates at chromosome hand 17q22-24. *GNAI3* has been shown to affect cytokinesis. Recent years, accumulating evidences have demonstrated that *GNAI3* is involved in regulating lots of cellular functions such as proliferation, migration, invasion and apoptosis [22-26]. Besides, *GNAI3* can produce seemingly paradoxical promotion and inhibition on invasion of different cell lines.

Recently, with the urgent demand to find novel and promising markers for HCC, *GNAI3* has

Table 1. Different expression level of *GNAI3* in HCC tissues and paired normal tissues

Tissue	Case NO.	Expression		Negative rate	*P* Value
		Positive	Negative		
HCC	64	15	49	76.6%	*P*<0.0001
Normal	64	8	56	12.5%	

Table 2. Association between clinical parameters and *GNAI3* expression

Characteristics	Case number	Protein expression		χ^2	*P* Value
		Positive	Negative		
Gender				0.145	0.703
Male	40	10	30		
Female	24	5	19		
Age (years)				0.040	0.841
≤55	50	12	38		
>55	14	3	11		
Liver cirrhosis				0.731	0.393
Yes	36	7	29		
No	28	8	20		
TNM stage				5.066	0.024
I, II	35	12	23		
III	29	3	26		
Tumor size (cm)				4.348	0.037
≤4.5	41	13	28		
>4.5	23	2	21		

attracted more and more attentions from scholars in this field.

Method and materials

Patients and specimens

HCC samples and paired normal specimens used in this study were obtained from 64 preoperatively untreated patients with histologically confirmed HCC, including 40 males and 24 females, aged from 21 to 65, with a mean age of 37 years, in Zhujiang Hospital. None of the selected patients experienced preoperative cancer treatment. The surgical specimens were cut into small pieces and stored in liquid nitrogen under aseptic condition immediately. This investigation lasted for about 4 years, from April 2010 to May 2014. This retrospective study was approved by our institution's research ethics board. All patients involved in this study were asked to write an informed consent.

Immunohistochemistry

The expression level of *GNAI3* in 64 cases of HCC tissues and paired normal tissues were tested with the utilization of immunohistochemistry (IHC) method. Concretely, the samples were fixed in 3% formaldehyde solution, embedded in paraffin and then cut into 4 μm-thick sections. Then the prepared sections were deparaffinized and rehydrated in a graded series of alcohols after baking at 65°C for 30 min. Following, 0.01 M citrate buffer (pH 6.0) was used to incubate with the sections at 100°C for 15 min, and cooled at room temperature for another 20 min. After that, the primary antibody rabbit anti-*GNAI3* was added to the sections and the mixture was incubated at 4°C overnight. Later, we added the Biotin-labeled second antibody to each section, incubating 15 min at room temperature, followed by washing with PBS twice, each for 3 min. Finally, staining signaling was developed using DAB by the avidin-biotin- proxidase method. The sections were airdried and reserved to use. Positive staining of *GNAI3* protein showed mainly in cytoplasm.

Statistical analysis

Data collected in this study was analyzed by SPSS18.0 software (SPSS Inc, USA). The correlation between *GNAI3* expression and clinical parameters of patients with HCC was evaluated by Chi-squared test. Kaplan-Meier survival method was adopted to determine survival rates of patients with HCC after operation. Then Cox regression analysis was conducted to evaluate the factors that could influence the prognosis of patients with HCC. We considered statistical significance existed when *P* value was less than 0.05.

Results

Low GNAI3 protein level in HCC tissues

We next explored the expression of *GNAI3* in HCC patients with IHC. The result showed that, among the 64 HCC samples, only 15 (23.4%) specimens were of positive expression, but among those paired normal samples, 56 (87.5%) specimens featured with positive expression of

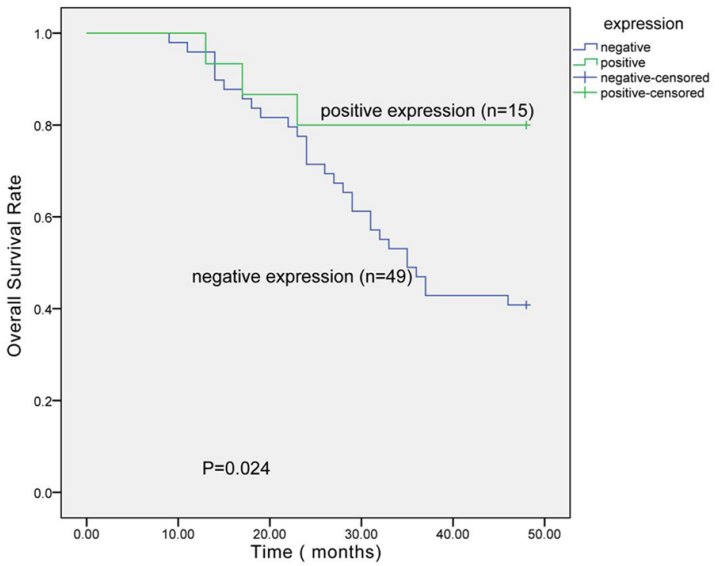

Figure 1. We use Kaplan-Meier survival analysis to evaluate the correlation between *GNAI3* expression and survival time of HCC patients. The consequence declared that the survival time of patients with HCC was tightly associated with the expression level of *GNAI3*. Patients with low expression of *GNAI3* had lower survival rate than those with high expression of *GNAI3*.

Low expression of GNAI3 associated with poor prognosis of HCC patients

Kaplan-Meier survival analysis was utilized to analyze the correlation between the survival time of patients with HCC and the expression of *GNAI3* at protein level. A follow-up of patients with HCC was executed after surgeries, ranging from 1 to 48 months with an average value of 34 months. During the follow-up, 29 (59.2%) of the 49 patients with negative *GNAI3* expression died, but of the 15 patients with positive *GNAI3* expression, only 3 (20%) died. According to the Kaplan-Meier survival curve, we could conclude that the overall survival rate of patients with negative *GNAI3* expression was significantly lower than those with positive *GNAI3* expression (**Figure 1**).

Table 3. Multivariate analysis of clinical parameters

Clinical parameters	HR	*P* value	95% CI
Liver cirrhosis	2.274	0.037	1.050-4.925
Tumor size	7.898	0.001	2.359-26.442
GNAI3 expression	0.218	0.016	0.063-0.750

GNAI3 (**Table 1**). The result illustrated that the expression of *GNAI3* protein in HCC tissues was significantly lower than that in paired normal tissues ($P<0.05$).

Correlation between GNAI3 protein expression and clinical parameters of HCC patients

Further investigation of relationship between the expression of *GNAI3* and the clinical parameters of HCC patients was performed. Chi-square result showed that there was a significant relationship between the expression level of *GNAI3* and certain clinical parameters, such as TNM stage, tumor size ($P<0.05$), but no tightly correlation was found between expression level of *GNAI3* and other clinical parameters, likely age, gender, liver cirrhosis and vein invasion ($P>0.05$) (**Table 2**).

Then we further researched the correlation between expression of *GNAI3* and prognosis of patients with HCC by multivariate analysis with the utilization of Cox regression. Data showed in **Table 3** demonstrated that negative expression of *GNAI3* predicted poor prognosis of patients with HCC, indicating that *GNAI3* could be an potential biomarker of prognosis of patients with HCC (HR: 0.218, $P=0.016$, 95% CI 0.063-0.750) (**Table 3**).

Discussion

HCC is one of the most common malignancies all over the world, which carries a heavy socioeconomic burden [27]. Due to the time of diagnose, lack of organ donors, high recurrence and some other reasons, a novel gene therapy was urgently needed. *GNAI3* involves in a series of biological processes. Nevertheless, the function of *GNAI3* on HCC remains unclear. Therefore, this study was conducted with the aim to evaluate the possibility of *GNAI3* as a predictor of HCC.

In this research, we first determined the expression of *GNAI3* in patients with HCC at protein level by IHC. According to the result, we found

that *GNAI3* was always downregulated in HCC tissues compared to paired normal tissues. Besides, Yu Zhang et al. reported that *GNAI3* had lower expression in HCC tissues than that in normal tissues, which was consistent with our results.

Based our previous consequence, further investigations were conducted to explore the underlying relationship between *GNAI3* expression and clinical parameters of patients with HCC. Statistical significance was found between *GNAI3* expression and clinical parameters, such as TNM stage and tumor size ($P<0.05$), indicating that *GNAI3* might be an independent predictor for prognosis of HCC patients.

After that, Kaplan-Meier survival analysis and Cox regression analysis were established to validate our hypothesis. It could be concluded that there was a high mortality in HCC patients with low expression of *GNAI3*. It was also verified that *GNAI3* expression was significantly associated with the prognosis of HCC patients, indicating that it can be regarded as a predictor for prognosis of HCC patients. Low expression of *GNAI3* stated poor prognosis.

Because *GNAI3* was a newly discovered gene, reports on it were minor. Our research was the first time to explore the prognostic function of *GNAI3* on HCC, which could provide theory evidence for further investigations on prognosis of diseases.

Taken together, our findings revealed that *GNAI3* expression was significantly lower in HCC tissues compared to paired normal tissues. The result showed that *GNAI3* could act as an independent indicator for prognosis of HCC patients. Low expression of *GNAI3* could induce poor prognosis.

Acknowledgements

Colleges and Universities Scientific Research Projects of Hunan Province (15C1199).

Disclosure of conflict of interest

None.

Address correspondence to: Zonghai Huang, Department of General Surgery, Zhujiang Hospital, Southern Medical University, No. 253 Gongye Road M, Guangzhou 510282, Guangdong, China. E-mail: dahai112015@sina.com

References

[1] Ng KK, Lam CM, Poon RT, Ai V, Tso WK and Fan ST. Thermal ablative therapy for malignant liver tumors: a critical appraisal. J Gastroenterol Hepatol 2003; 18: 616-629.

[2] Yaghi C, Sharara AI, Rassam P, Moucari R, Honein K, BouJaoude J, Slim R, Noun R, Abdul-Baki H, Khalifeh M, Ramia S and Sayegh R. Hepatocellular carcinoma in Lebanon: Etiology and prognostic factors associated with short-term survival. World J Gastroenterol 2006; 12: 3575-3580.

[3] Bosch FX, Ribes J, Diaz M and Cleries R. Primary liver cancer: worldwide incidence and trends. Gastroenterology 2004; 127: S5-S16.

[4] Zhang JW, Feng XY, Liu HQ, Yao ZW, Yang YM, Liu B and Yu YQ. CT volume measurement for prognostic evaluation of unresectable hepatocellular carcinoma after TACE. World J Gastroenterol 2010; 16: 2038-2045.

[5] Okuda K, Ohtsuki T, Obata H, Tomimatsu M, Okazaki N, Hasegawa H, Nakajima Y and Ohnishi K. Natural history of hepatocellular carcinoma and prognosis in relation to treatment. Study of 850 patients. Cancer 1985; 56: 918-928.

[6] Wu KT, Wang CC, Lu LG, Zhang WD, Zhang FJ, Shi F and Li CX. Hepatocellular carcinoma: clinical study of long-term survival and choice of treatment modalities. World J Gastroenterol 2013; 19: 3649-3657.

[7] Cheung TT, Ng KK, Chok KS, Chan SC, Poon RT, Lo CM and Fan ST. Combined resection and radiofrequency ablation for multifocal hepatocellular carcinoma: prognosis and outcomes. World J Gastroenterol 2010; 16: 3056-3062.

[8] Carr BI. Hepatocellular carcinoma: current management and future trends. Gastroenterology 2004; 127: S218-224.

[9] Merion RM. Current status and future of liver transplantation. Semin Liver Dis 2010; 30: 411-421.

[10] Cao DX, Li ZJ, Jiang XO, Lum YL, Khin E, Lee NP, Wu GH and Luk JM. Osteopontin as potential biomarker and therapeutic target in gastric and liver cancers. World J Gastroenterol 2012; 18: 3923-3930.

[11] Lee JI, Lee JW, Kim JM, Kim JK, Chung HJ and Kim YS. Prognosis of hepatocellular carcinoma expressing cytokeratin 19: comparison with other liver cancers. World J Gastroenterol 2012; 18: 4751-4757.

[12] Ni JY, Sun HL, Chen YT, Luo JH, Chen D, Jiang XY and Xu LF. Prognostic factors for survival after transarterial chemoembolization combined with microwave ablation for hepatocellular carcinoma. World J Gastroenterol 2014; 20: 17483-17490.

[13] Hiraoka A, Kawamura T, Aibiki T, Okudaira T, Toshimori A, Yamago H, Nakahara H, Suga Y, Azemoto N, Miyata H, Miyamoto Y, Ninomiya T, Murakami T, Ishimaru Y, Kawasaki H, Hirooka M, Abe M, Matsuura B, Hiasa Y and Michitaka K. Prognosis and therapy for ruptured hepatocellular carcinoma: problems with staging and treatment strategy. Eur J Radiol 2015; 84: 366-371.

[14] Ikeda K, Osaki Y, Nakanishi H, Nasu A, Kawamura Y, Jyoko K, Sano T, Sunagozaka H, Uchino K, Minami Y, Saito Y, Nagai K, Inokuchi R, Kokubu S and Kudo M. Recent progress in radiofrequency ablation therapy for hepatocellular carcinoma. Oncology 2014; 87 Suppl 1: 73-77.

[15] Alsina AE, Nakshabandi A, Makris AM and Torres EA. Liver transplantation for hepatocellular carcinoma in Puerto Ricans: underutilization of a curative therapy. P R Health Sci J 2014; 33: 170-176.

[16] Zhang Y, Yao J, Huan L, Lian J, Bao C, Li Y, Ge C, Li J, Yao M, Liang L and He X. GNAI3 inhibits tumor cell migration and invasion and is post-transcriptionally regulated by miR-222 in hepatocellular carcinoma. Cancer Lett 2015; 356: 978-984.

[17] Sarwar M, Samuel CS, Bathgate RA, Stewart DR and Summers RJ. Serelaxin-mediated signal transduction in human vascular cells: bell-shaped concentration-response curves reflect differential coupling to G proteins. Br J Pharmacol 2015; 172: 1005-1019.

[18] Bourne HR, Sanders DA and McCormick F. The GTPase superfamily: a conserved switch for diverse cell functions. Nature 1990; 348: 125-132.

[19] Gilman AG. G proteins: transducers of receptor-generated signals. Annu Rev Biochem 1987; 56: 615-649.

[20] Wu T, Li Y, Huang D, Han F, Zhang YY, Zhang DW and Han J. Regulator of G-protein signaling 19 (RGS19) and its partner Galpha-inhibiting activity polypeptide 3 (GNAI3) are required for zVAD-induced autophagy and cell death in L929 cells. PLoS One 2014; 9: e94634.

[21] Engelhardt S and Rochais F. G proteins: more than transducers of receptor-generated signals? Circ Res 2007; 100: 1109-1111.

[22] Kelly P, Moeller BJ, Juneja J, Booden MA, Der CJ, Daaka Y, Dewhirst MW, Fields TA and Casey PJ. The G12 family of heterotrimeric G proteins promotes breast cancer invasion and metastasis. Proc Natl Acad Sci U S A 2006; 103: 8173-8178.

[23] Fukuhara S, Chikumi H and Gutkind JS. RGS-containing RhoGEFs: the missing link between transforming G proteins and Rho? Oncogene 2001; 20: 1661-1668.

[24] Rasheed SA, Teo CR, Beillard EJ, Voorhoeve PM and Casey PJ. MicroRNA-182 and microRNA-200a control G-protein subunit alpha-13 (GNA13) expression and cell invasion synergistically in prostate cancer cells. J Biol Chem 2013; 288: 7986-7995.

[25] Yao J, Liang L, Huang S, Ding J, Tan N, Zhao Y, Yan M, Ge C, Zhang Z, Chen T, Wan D, Yao M, Li J, Gu J and He X. MicroRNA-30d promotes tumor invasion and metastasis by targeting Galphai2 in hepatocellular carcinoma. Hepatology 2010; 51: 846-856.

[26] Jiang L, Dai Y, Liu X, Wang C, Wang A, Chen Z, Heidbreder CE, Kolokythas A and Zhou X. Identification and experimental validation of G protein alpha inhibiting activity polypeptide 2 (GNAI2) as a microRNA-138 target in tongue squamous cell carcinoma. Hum Genet 2011; 129: 189-197.

[27] Mendes LS, Nita ME, Ono-Nita SK, Mello ES, da Silva LC, Alves VA and Carrilho FJ. Prognostic factors for progression of liver structural lesions in chronic hepatitis C patients. World J Gastroenterol 2008; 14: 2522-2528.