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Original Article

Guanine nucleotide binding protein-like 3 is a potential prognosis indicator of gastric cancer

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Abstract: Guanine nucleotide binding protein-like 3 (GNL3) is a GIP-binding nuclear protein that has been reported to be involved in various biological processes, including cell proliferation, cellular senescence and tumorigenesis. This study aimed to investigate the expression level of GNL3 in gastric cancer and to evaluate the relationship between its expression and clinical variables and overall survival of gastric cancer patients. The expression level of GNL3 was examined in 89 human gastric cancer samples using immunohistochemistry (IHC) staining. GNL3 in gastric cancer tissues was significantly upregulated compared with paracancerous tissues. GNL3 expression in adjacent non-cancerous tissues was associated with sex and tumor size. Survival analyses showed that GNL3 expression in both gastric cancer and adjacent non-cancerous tissues were not related to overall survival. However, in the subgroup of patients with larger tumor size (≥ 6 cm), a close association was found between GNL3 expression in gastric cancer tissues and overall survival. GNL3-positive patients had a shorter survival than GNL3-negative patients. Our study suggests that GNL3 might play an important role in the progression of gastric cancer and serve as a biomarker for poor prognosis in gastric cancer patients.

Keywords: Guanine nucleotide binding protein-like 3, gastric cancer, prognosis, survival

Introduction

Cancer is one of the four major non-communicable diseases, accounts for ~14.6% of all human deaths. At present, there are more than 100 different kinds of cancer and more than 500 genes involved in cancer. Gastric cancer is the second most common cause of cancer-related death in the world [1]. China is one of the countries with high incidence of gastric cancer [2, 3], accounting for more than 40% of annual new cases of gastric cancer in the world. Most gastric tumors are malignant and gastric adenocarcinoma accounts for 95% of the overall number of malignancies [4]. The mortality of gastric cancer is still quite high in spite of adjuvant therapy and technical progress in surgery.

GTPases are a large family of hydrolase enzymes that bind and hydrolyze guanosine triphosphate (GTP) [5]. Guanine nucleotide binding protein-like 3 (GNL3) is a protein identified as a nuclear GTPase that is overexpressed in stem cells, cancer cells and other proliferating cells [6-8]. GNL3 belongs to YlqF/YawG GTPases subfamily and regulate the shuttle of protein between nucleolus and nucleoplasm [9, 10]. With the latest advance in research on tumorigenesis, the importance of GNL3 in cancer is gradually recognized. Increasing evidence has shown that GNL3 involved in various malignant tumors, such as esophageal, breast, bladder, and lung cancer [11-16]. However, there are few reports regarding the role of GNL3 in gastric cancer [17, 18]. In the present study, we examined the expression levels of GNL3 in gastric cancer and paracancerous tissues, as well as their relationship to clinical features and prognosis of gastric cancer patients.

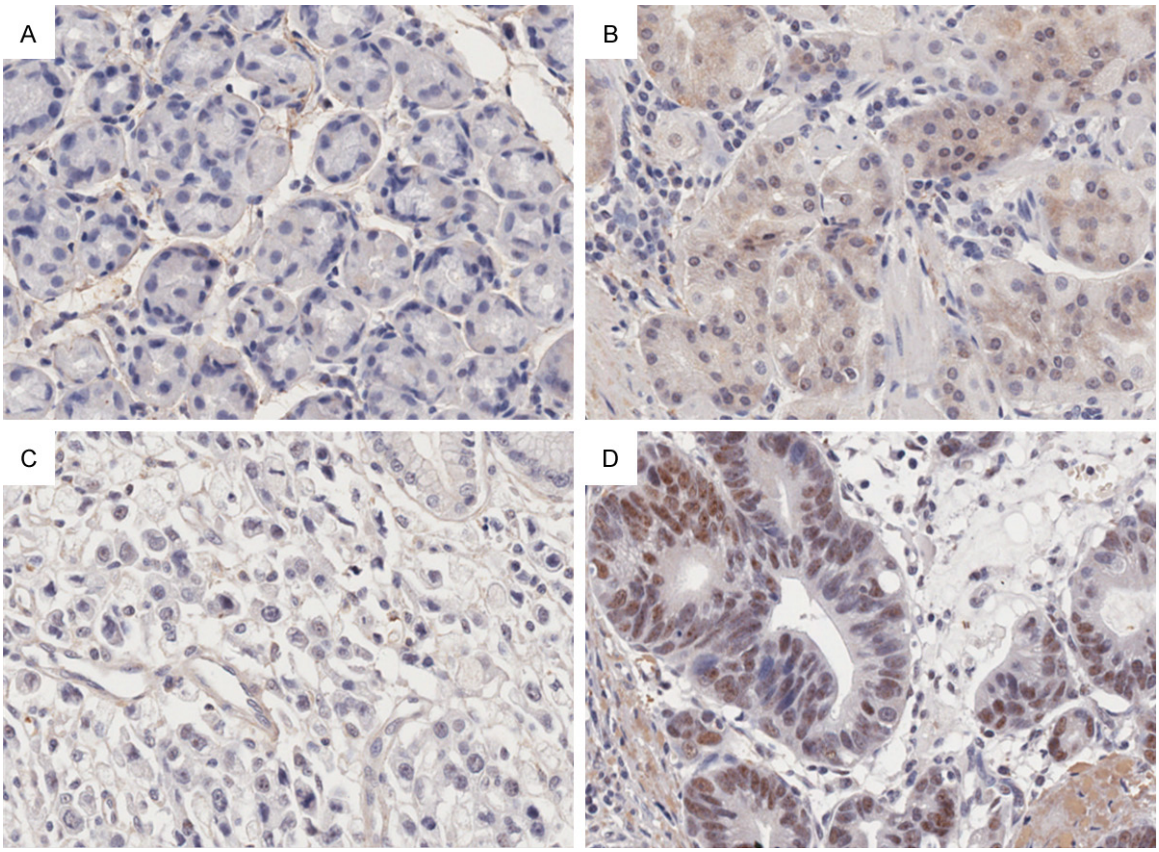

Figure 1. IHC staining of GNL3 in gastric cancer and paired and paracancerous tissues. A. GNL3-negative paracancerous tissues. B. GNL3-positive paracancerous tissues. C. GNL3-negative gastric cancer tissues. D. GNL3-positive gastric cancer tissues.

Methods and materials

Gastric cancer samples

Gastric cancer and their adjacent tissues were obtained from 89 patients with histologically confirmed gastric adenocarcinoma randomly chosen from the Biobank of National Engineering Center for Biochip at Shanghai. This study was approved by the Ethics Committees of National Engineering Center for Biochip at Shanghai. Each patient gave written informed consent. The follow-up data was mainly obtained by telephone and out-patient review. Patients who had inadequate follow-up were excluded from this study. Tissues were fixed in 10% formaldehyde and embedded in paraffin.

Tissue microarray construction

Tissue microarrays were constructed as described previously [19]. Briefly, we dig holes on the paraffin block by using tissue microarray instrument. The diameter is about 0.6 mm and the depth of holes was about 2 mm. Pathologists assisted us to select representative gastric cancer and paracancerous tissues. The fine hollow needles were used to punch donor block, and the sample was pressed into a recipient block. Samples were arrayed to the duplicated blocks to minimize the loss of tissue. Finally, the tissue microarray was confirmed by two pathologists using HE staining.

Immunohistochemistry (IHC) assay

The streptavidin peroxidase (SP) of IHC was used to detect the expression of GNL3 in gastric cancer and paracancerous tissues. Tissue microarray was taken out from the refrigerator and placed in a rack for rewarming. Tissue microarray was heated at 60°C for one hour to melt the seal wax away from the surface. Then, we soaked the slide twice in xylene for 13 minutes and subsequently in absolute alcohol for 10 minutes. The slide was soaked the slide in 95% ethanol for another 6 minutes. At last, the

Table 1. Association of GNL3 expression with clinical features of gastric cancer patients

Clinical features	GNL3 expression in paracancerous tissues			GNL3 expression in GA tissues		
	Positive	Negative	*P* value	Positive	Negative	*P* value
Sex						
Male	14	47	0.031	31	30	0.144
Female	1	27		9	19	
Age (years)						
> 60	12	45	0.239	24	33	0.511
≤ 60	3	29		16	16	
Histologic grade						
1+2	2	13	1.000	8	7	0.474
3	13	61		32	42	
Tumor size						
≥ 6 cm	13	35	0.009	19	29	0.271
< 6 cm	2	39		21	20	
LNM						
Positive	11	53	1.000	28	12	0.717
Negative	4	21		36	13	
TNM stage						
I+II	9	46	0.875	24	31	0.828
III+IV	6	28		16	18	

slide was placed in 70% ethanol for 5 minutes to the paraffin wax. Antigen retrieval was carried out in citrate buffer for 10 minutes in a steam oven. GNL3 antibody (ABCAM, CA, USA) was added at dilutions of 1:1000. Subsequently, a secondary antibody was applied and was incubated for 30 minutes. Then, the slide was added with horseradish peroxidase-conjugated streptomycin working solution. Finally, the slide was stained with DAB/H_2O_2 reaction. The results were blindly determined by two pathologists.

Statistical analyses

X^2 test and Fisher's exact test were used to analyze the relationship between GNL3 expression and clinical features. The Kaplan-Meier and log-rank test were used to analyze the survival rates. Cox proportional hazard model was used to determine factors related to patient survival. All statistical analyses were performed using SPSS 20.0.

Results

Clinical features

There were 89 gastric cancer cases enrolled in our study, including 61 males (68.5%) and 28 females (31.5%). The mean age was 64, ranged from 42 to 83. Tumor size ranged from 2 cm to 27 cm, with the mean of 6.49 cm. Sixty-four cases (71.9%) had regional lymph node metastasis (LNM), whereas 25 cases (28.1%) had no regional lymph node. One case (1.1%) was well differentiated, 14 (15.7%) were moderately differentiated, and 74 (83.1%) were poor differentiated. According to the AJCC staging system, there were 6 (6.7%) stage I cases, 28 (31.5%) stage II cases, 54 (60.7%) stage III cases, and 1 (1.1%) stage IV case.

Expression levels of GNL3 in gastric cancer tissues and paracancerous tissues

IHC results showed that GNL3 was significantly overexpressed in cancer tissues compared with that in paracancerous tissues ($P < 0.001$). Positive GNL3 staining in paracancerous tissues was 16.85% (15/89), whereas 74 (83.1%) gastric cancer cases showed positive GNL3 staining (**Figure 1**).

Association of GNL3 expression with clinical features of patients with gastric cancer

We further examined the association between GNL3 expression and clinical features of patients with gastric cancer. GNL3 expression in noncancerous tissues was associated with sex and tumor size ($P = 0.031$ and $= 0.009$, respectively). There was no significant difference between GNL3 expression in noncancerous tissues and other clinical features. However, GNL3 expression in gastric cancer tissues showed no association with clinical features, including sex, age, histological grades, tumor size, LNM, and TNM stage (**Table 1**).

Relationship between GNL3 expression and overall survival of GA patients

We further analyzed the effect of GNL3 expression on overall survival. No significant association was found between GNL3 expression in both gastric cancer and paracancerous tissues and overall survival of gastric cancer patients.

Table 2. Stratification analysis of GNL3 expression associated with survival of gastric cancer patients

Clinical features	GNL3 expression in cancer tissues		GNL3 expression in paracancerous tissues	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age, years				
≤ 60	1.248 (0.505-3.083)	0.631	2.282 (0.299-17.432)	0.426
> 60	1.033 (0.539-1.981)	0.923	1.462 (0.609-3.508)	0.395
Sex				
Male	1.195 (0.626-2.283)	0.589	1.723 (0.717-4.143)	0.224
Female	0.910 (0.345-2.400)	0.849	0.348 (0.043-2.788)	0.320
Histologic grade				
1+2	1.229 (0.274-5.518)	0.788	0.706 (0.084-5.910)	0.748
3	1.248 (0.505-3.083)	0.631	1.686 (0.717-3.967)	0.231
Tumor size				
< 6 cm	0.529 (0.216-1.295)	0.163	22.533 (0.050-54.834)	0.429
≥ 6 cm	2.166 (1.115-4.207)	0.022	1.872 (0.816-4.292)	0.139
LNM				
Positive	1.282 90.738-2.228)	0.377	1.316 (0.592-2.927)	0.500
Negative	0.699 (0.117-4.189)	0.695	27.048 (0.081-40.132	0.517
TNM*				
III+IV	1.255 (0.698-2.257)	0.449	1.766 90.693-4.496)	0.233

*Since there was only 13 patients with TNM stages I and II, those were excluded from further stratification analysis.

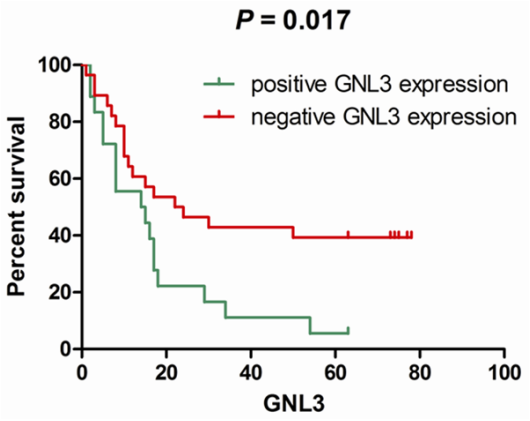

Figure 2. Kaplan-Meier curves estimates of the probability of survival in gastric cancer patients with larger tumor size according to the GNL3 expression status.

In addition, we performed stratification analysis based on clinical features to evaluate the effect of GNL3 expression on overall survival. As shown in **Table 2**, the subgroup of patients with larger tumor size (≥ 6 cm), patients whose tumor tissue contained positive GNL3 staining had a tendency to have shorter survival time compared with those with negative GNL3 staining (HR = 2.166, 95% CI: 1.115-4.207, *P* = 0.022) (**Figure 2**).

Discussion

In the present study, we evaluated the expression levels of GNL3 in gastric cancer and analyzed its impact on the prognosis of gastric cancer patients. The most important observation in this study indicates a major influence of GNL3 expression in gastric cancer tissues on patient survival only in those with larger tumor size.

GNL3 has been shown to affect cell proliferation and growth by regulating ribosome biosynthesis and inhibiting tumor suppressor P53 protein. GNL3 can bind directly to p53 and knockdown of GNL3 leads to p53 stabilization [7, 20, 21]. Suppression of GNL3 resulted in G1-S phase arrest and inhibited cell proliferation [22, 23]. Recent studies have revealed that besides G1-S phase arrest, knockdown of GNL3 could cause G2-M phase arrest [21, 22, 24]. Recent studies have showed that GNL3 played an important role in the development and progression of human cancer [12, 17, 25, 26]. In squamous cell carcinoma of the head and neck, GNL3 was connected with malignant transformation [12]. In breast cancer, GNL3 expression had an independent impact as a prognostic indicator [26]. A study by Yoshida et al. [25] showed that overexpression of GNL3 resulted in an advanced malignant phenotype and a poor prognosis in oral squamous cell carcinoma. Asadi et al. [17] found that the expression level of GNL3 in high-grade gastric adenocarcinoma was significantly higher than that in low-grade gastric adenocarcinoma. Moreover, a significant change in the morphology and cell cycle distribution of the cells was noticed following inhibition of GNL3. The round shape cells changed into a hummingbird-like appearance. Knockdown of GNL3 resulted in an increased number of distributed

cells of G1 phase and a decreased number of distributed cells within S phase [17]. In agreement with results of previous study [12, 17, 25, 26], we found that GNL3 was upregulated in gastric cancer and affected the prognosis of patients with gastric cancer. Taken together, GNL3 may contribute to the development and progression of gastric cancer.

In conclusion, our results demonstrated that GNL3 was significantly upregulated in gastric cancer, and positive GNL3 expression was associated with poor prognosis in patients with larger tumor size. Since our sample size is limited, further studies with larger sample are needed to validate our results. Moreover, relevant studies should be conducted to reveal the mechanism by which GNL3 affect patient prognosis.

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Disclosure of conflict of interest

None.

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