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Expression and Clinical Significance of SNCG in the Cholangiocarcinoma Tissues*

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ABSTRACT Objective: To investigate the expression of the γ-synuclein (SNCG) in the cholangiocarcinoma and normal bile ducts tissues, and explore the role and its clinical significance of SNCG in the occurrence, development, invasion and metastasis of the cholangiocarcinoma. **Methods:** Immunohistochemical staining was used to detect the expression of SNCG protein among the 72 cases of cholangiocarcinoma and 41 cases of normal bile duct tissues. Its relationship with the clinicopathological features of the cholangiocarcinoma was analyzed. **Results:** The expression positive rate of SNCG in the carcinoma of bile duct tissues was 73.61% (53/72), which was higher than that in normal bile duct tissues (4.88%, 2/41), and the difference was statistically significant (P<0.01). The expression of SNCG protein was correlated with the depth of tumor invasion and lymph node metastasis (P<0.01), but it had not the relationship with the age, sex of patients and the degree of tumor differentiation (P>0.05). **Conclusions:** The expression of SNCG protein was correlated with the occurrence, development, invasion and metastasis of the cholangiocarcinoma, and may play an important role in the invasion and metastasis of the carcinoma of bile duct. SNCG is expected to become a new tumor marker of the cholangiocarcinoma and provides the basis for its prognosis and developing appropriate treatment programs.

Key words: γ-Synuclein protein; Cholangiocarcinoma; Immunhistochem

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Introduction

Cholangiocarcinoma is the carcinoma of bile duct which is primarily occurred in the biliary epithelial cells, in recent years, the incidence and mortality of cholangiocarcinoma have been increased year by year, because its onset is insidious, the early clinical diagnosis is difficult, the prognosis is poor, and the surgical resection rate is low (15% to 20%)[1-3]. Even when patients are eligible for surgical intervention, overall survival is poor, with 5-year survival rates of 20%-43%[4]. The synuclein protein of neurons (SNCG) is called the breast cancer special gene 1 (BCSG1), and Ji discovered the specific expression of SNCG in breast cancer for the first time[5], and confirmed that SNCG could promote the invasion and metastasis of the breast cancer[6]. In recent years, the study of many scholars at home and abroad has found that SNCG had specific expression in many tumors, but the study in cholangiocarcinoma at home and abroad was rarely reported[7-13]. In this study, immunohistochemical staining was used to detect the expression of SNCG in the cholangiocarcinoma and normal bile duct tissues, and to analyze and discuss its clinical significance.

1 Materials and Methods

1.1 Clinical data

113 cases of paraffin-embedded tissue specimens were collected from the Affiliated Hospital of Qingdao University during November 2011 to October 2013, including 72 cases of carcinoma of bile duct and 41 cases of normal bile duct tissues (all taken from autopsy tissues and liver transplanted organ donors without biliary disease, and confirmed by pathological examination). Among the 72 cases of carcinoma of bile duct tissues, there were 50 males and 22 females aged 32 to 88, with the median age of 58 years old; There were 59 cases of highly differentiated adenocarcinoma (33 cases of papillary adenocarcinoma and 26 cases of highly differentiated adenocarcinoma), 13 cases of poorly differentiated adenocarcinoma (10 cases of poorly differentiated adenocarcinoma and 3 cases of mucinous adenocarcinoma); 14 cases of patients without lymph node metastasis, and 58 cases of patients with lymph node metastasis; There were 26 cases of patients at T1+T2 stage and 46 cases of patients at T3+T4 stage according to the TNM staging system of the Union for International Cancer Control (UICC). All patients with cholangiocarcinoma had no preoperative radiotherapy and chemotherapy. All specimens were fixed in 10% neutral formalin, embedded in paraffin, sliced, and performed with HE staining and immunohistochemical staining.

1.2 Methods

Immunohistochemical EnVision two-step method was used, and the ready-to-use immunohistochemistry MaxVision detection kit (KIT-5020) was purchased from Fuzhou Maixin Biotechnology

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Co., Ltd.; DAB coloration was performed according to the DAB chromogenic kit (purchased from Fuzhou Maixin Biotechnology Co., Ltd.); The reagents SNCG mouse anti-human monoclonal antibodies were purchased from the U.S. Santa Cruze Biotechnology. Specific operations were referred to the kit instructions.

1.3 Results determination

The cell count method of SNCG protein positive was: randomly selected 10 fields with 400 times in the tumor region of each slice. 100 cells were counted in each field according to the white blood cell count method. They were scored according to the tinting strength and coloring cells counts and their mean values were calculated, which are: ① scoring by the tinting strength, the counted cells without coloration was 0 point; cells colored with pale yellow was 1 point; cells colored with brown or golden yellow was 2 points; cells colored with tan was 3 points. ② Scoring according to the percentage of positive cells: 0 point, the percentage of positive cells was below 5%; 1 point, the percentage of positive cells was 5% to 25%; 2 points, the percentage of positive cells was 26% to 50%; 3 points, the percentage of positive cells was 51% ~ 75%; 4 points, the percentage of positive cells was above 75%. After multiplied by two kinds of points, 0 point represented that SNCG protein expression was negative (-), 1 point and higher than 1 point represented positive, of which 1 to 3 points represented weakly positive (+), 4 to 7 points represented positive (++), 8 to 12 points represented strongly positive (+++)[14].

1.4 Statistical Methods

SPSS 11.0 statistical software was used for statistical analysis, and non-parametric statistic rank sum test was used for the ranked data (Mann-Whitney test); Spearman rank correlation analysis was used for correlation analysis. The significance level was $\alpha = 0.05$.

2 Results

2.1 The test results about the expression of SNCG in cholangiocarcinoma and normal bile duct tissue

Of 72 cholangiocarcinoma tissues, there was 53 cases which had SNCG positive expression, and the positive expression rate was 73.61%, which was higher than that in normal bile duct tissues(4.88%, 2/41), P <0.01. (Fig 1 and Table 1).

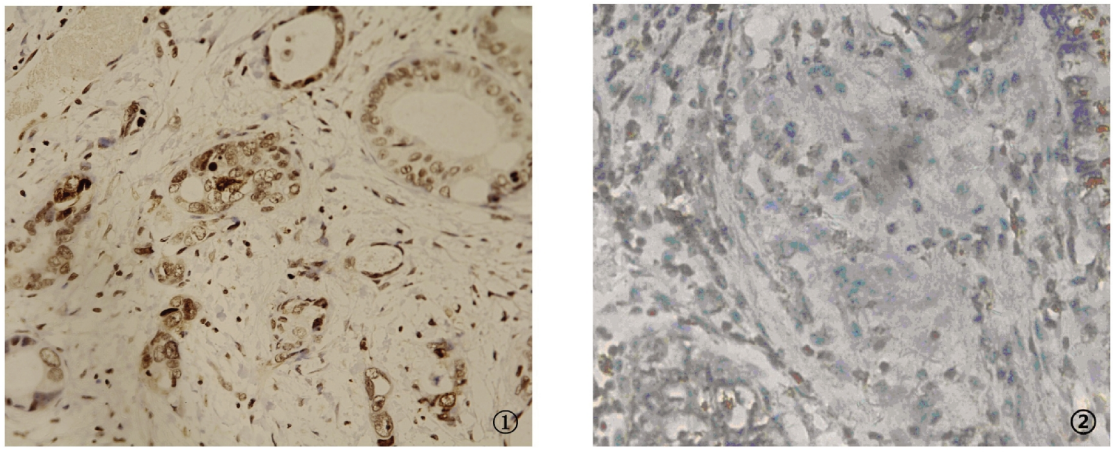

Fig. 1 Expressions of SNCG in carcinoma of bile duct and normal bile duct tissues(SP × 200).① : Carcinoma of bile duct tissue; ② : Normal bile duct tissue

Table 1 Results of expressions of SNCG in carcinoma of bile duct and normal bile duct tissues

Tissue	n	Expression of SNCG				Mean rank	x^2 value	P value
		-	+	++	+++			
Carcinoma of bile duct tissue	72	19	8	17	28	36.5	12.72	P<0.01
Normal bile duct tissue	41	39	1	1	0	93.0		

2.2 The relationship between the expression of SNCG and its clinical pathological features in cholangiocarcinoma tissues

The results were shown in Table 2. From which it could be seen that the expression of SNCG was related with the clinical TNM stage of cholangiocarcinoma (the depth of tumor invasion) and lymph node metastasis (P<0.05), but it had not relationship with age, gender and degree of tumor differentiation of patients (P>0.05). The positive expression rate of SNCG protein in T3+T4 stage was higher than that in T1+T2 stage (P<0.005), which suggested that the tumor infiltrating was deeper, the positive expression of SNCG protein was higher. The positive expression rate of SNCG protein in lymph node metastasis was higher than that without lymph node metastasis (P<0.005).

Table 2 The relationship between the expression of SNCG and clinicopathologic features of bile duct carcinoma

Clinicopathologic feature		n	Expression of SNCG (case)				Positive rate (%)	Mean rank	P value
			-	+	++	+++			
Gender	Male	50	13	6	11	20	74.00	25.5	0.945
	Female	22	6	3	6	7	72.73	62.0	
Age(year, M)	<58	35	8	4	12	11	77.14	18.0	0.525
	≥ 58	37	11	5	5	16	70.27	54.0	
Differentiation grade of tumor	Highly	59	16	7	14	22	72.88	30.0	0.753
	Poorly	13	4	1	2	6	69.23	66.0	
Lymph node metastasis	Yes	58	7	7	16	28	87.93	29.5	P<0.005
	No	14	12	1	1	0	14.29	65.5	
TNM staging	T1+T2	26	12	6	3	1	38.46	13.5	P<0.005
	T3+T4	46	5	1	13	27	89.13	49.5	

3 Discussion

Cholangiocarcinoma is a kind of gastrointestinal cancer which is a serious threat to human health, and the incidence and mortality rates are more rapid upward; The incidence of cholangiocarcinoma is occult, and it is lack of an ideal marker for early diagnosis, and has poor sensitivity to radiotherapy and chem-otherapy, so the prognosis of patients with cholangiocarcinoma is poor [15-17]. SNCG was discovered and its corresponding DNA was sequenced in 1997. This protein, consisting of 127 amino acids, is an intracellular protein. It is widely present in brain tissue and is homologous with neural nuclear protein. Studies have shown that SNCG is mainly expressed in neurons, particularly rich in presynaptic terminals, and its unusual expression has a certain relevance with neurodegenerative lesions and malignant cells [18]. SNCG protein positive was mainly localized in the nucleus and cytoplasm of tumor cells, showing as brownish yellow granular; few lymphocytes, vascular endothelial cells and smooth muscle cells also have a small amount of SNCG protein positive expression, The expression of SNCG may be a specific protein occurred in the process of the transition of the body's normal cells into cancer cells. It has low or no expression in precancerous lesions (pericarcinomatous tissue). Once the tumor is formed, SNCG will be expressed because of certain incentives, which has a certain relationship with the progression of tumor. Reports has preliminarily confirmed it in the research of gastric cancer and ovarian cancer [20]. Results further suggest that SNCG can be applied as a more specific tumor markers to be used in clinical because SNCG is not or rarely expressed in the pericarcinomatous tissues of a variety of tumors. Even if it can not be used for early diagnosis, it still can be used as adjuvant means to help clinicians make the final diagnosis of the tumor.

Iwaki found that the expression of SNCG protein was found in the urine of patients with bladder cancer [21]. In addition, the expression of SNCG was extremely increased in the study of prostate cancer, lung cancer, colorectal cancer, cervical cancer and many other tumors [22-24]. And Zhou found that SNCG expression was low in the research of esophageal squamous cells [25]. Therefore, it remains to be further studied on the relationship of SNCG with a variety of tumors. This study found that SNCG almost had no expression in normal bile duct tissues, whereas showed high expression in cholangiocarcinoma tissues. This was related with TNM stage and with or without lymph node metastasis, suggesting SNCG expression may be associated with the occurrence, development and prognosis of cholangiocar cinoma.

In summary, the unusual expression of SNCG possibly plays an important role in the development and invasion and metastasis process of tumor, so it may serve as a potential target for cancer therapy by inhibiting the activity of SNCG, blocking its action with other molecules, and thus inhibiting the proliferation and metastasis of tumor cells. SNCG can be used as a new tumor marker and a new target for cancer treatment, and it will have broad prospect of clinical application in the assessment of invasion and metastasis, prognosis and treatment of tumor.

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胆管癌组织中 SNCG 的表达及其临床意义 *

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摘要 目的:观察 γ-synuclein(SNCG)在胆管癌和正常胆管组织中的表达,并探讨其在胆管癌发生、发展中的临床意义。**方法:**采用免疫组化方法检测 SNCG 蛋白在 72 例胆管癌组织及 41 例胆管正常组织中的表达水平, 并分析其与胆管癌临床病理特征的关系。**结果:**SNCG 蛋白在胆管癌组织中的阳性表达率为 73.61%(53/72),高于其在胆管正常组织中的阳性表达率 (4.88%, 2/41),其差异有统计学意义 (P<0.01)。SNCG 蛋白的表达与肿瘤的淋巴结转移相关(P<0.01),但与患者的年龄、性别及肿瘤分化程度无关(P>0.05)。**结论:**SNCG 蛋白的表达与胆管癌的发生、发展正相关,并对胆管癌的浸润转移发挥重要的促进作用。对 SNCG 蛋白的研究将可能为胆管癌的早期诊断提供新的肿瘤标志物,并为胆管癌的预后判断和诊治提供重要理论依据。

关键词:γ- 突触核蛋白;胆管癌;免疫组化
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