Expressions of SNCG in Gastric Adenocarcinoma and Its Clinical Significances

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ABSTRACT Objective: The purpose of the study was to detect the expressions of SNCG in gastric adenocarcinoma, to evaluate their roles in carcinogenesis, development, infiltration and metastasis as well as their clinical significances. Methods: The expressions of SNCG was detected by SP immunohistochemical method in 90 cases of gastric adenocarcinoma and 40 cases of normal gastric mucosa; The expressions of SNCGmRNA was detected by RT-PCR method in 29 cases of gastric adenocarcinoma 15 cases of normal gastric mucosa. Results: The expression of SNCG protein and SNCG mRNA in the gastric adenocarcinoma were both remarkably higher than those in the normal gastric mucosa ($P<0.05$); The positive expressions of SNCG protein was correlated with the depth of tumor infiltration and the metastasis of lymph node ($P<0.05$). Conlusion: SNCG may play an important role in carcinogenesis progression and metastasis of gastric adenocarcinoma and serve as target spots in the targeted therapy of gastric adenocarcinoma.

Key Words: SNCG; gastric carcinoma; Immunohistochemistry; RT-PCR

Chinese Library Classification(CLC):R735.2 Document code:A

Article ID:1673-6273(2011)08-1532-04

Troduction

Gastric cancer is one of the most common tumors in China, whose incidence ranks first among various types of tumors, and its mortality takes up 23.2% of the total cancer[1]. Although there are a variety of treatment, such as surgery, chemotherapy and radiotherapy, the invasion and metastasis of cancer is the main cause of death, while the 5-year survival rate is still low [2,3]. Recent studies have found that synuclein family was related to cancer genetics, especially SNCG. Previous research [4] has shown that there was over-expression of SNCG in liver cancer, esophageal cancer, prostate cancer, cervical cancer, colon cancer, breast cancer, lung cancer and other solid tumors, and its expression levels were related to the tumorigenesis and development as well as tumor invasiveness and prognosis even chemotherapy resistance drug of the tumor. This study was to investigate the relation of SNCG expression to clinicopathologic parameters of gastric adenocarcinoma at both mRNA and protein level with specimens of gastric adenocarcinoma and normal gastric mucosa.

1 Materials and Methods

1.1 Materials

130 paraffin-embedded tissue specimen (90 gastric adenocarcinoma specimen and 40 normal gastric mucosa tissue) ,which were 5cm apart from cancer tissues, and 44 fresh specimen (29 gastric adenocarcinoma specimen and 15 normal gastric mucosa tissue) were collected at Qingdao University Medical College affiliated hospital and affiliated hospital of medical college of the Chinese People's Armed Police Force during 2009.3-2009.10. All cases were confirmed no hyperplasia or neoplastic lesions. After the tissues removed from the body, all samples were labled and frozen in liquid nitrogen(-176℃) and stored at -80℃ for later use. The 90 gastric adenocarcinoma specimen consist of 57 males and 33 females among 24-89 years old (the median age were 62), with 31 were well or moderately differentiated adenocarcinoma and 59 were poorly differentiated adenocarcinoma,while 32 cases were node positive . No cases underwent radiotherapy or chemotherapy.

1.2 Reagents and Methods

1.2.1 Reagents Mouse anti-SNCG monoclonal antibody (Santa Cruze,US) , immunohistochemistry kit and DAB solution (Maixin Biotechnology Co. Ltd., P.R. China) were also used.RNAiso Reagent and super RT Kit and PCR Kit were provided by Takara biotechnology (DALIAN) CO., LTD. SNCG was designed by the primer premier 5.0 software.SNCG and primer GAPDH were offered by Sangon Biotech (Shanghai) Co.,Ltd.

1.2.2 Immunohistochemical staining Serial sections of 4um in thickness were cut from paraffin-embedded tissue specimen, and immunohistochemical staining was conducted under the instruction of the kits. Antigen repair was peformed in an autoclave in the presence of citrate for exposing antigen site adequately. Positive control were made by confirmed positive slide,and negative control was made by PBS instead of the primary antibody. A positive reaction was detected as nucleic or plasmatic of oncocyte stain presenting in yellow or brown-yellow color[4]. For each section, 10 high-power fields (400 ×,to avoid large vessels and large areas of

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(Received:2011- 02- 06 Accepted:2011- 02- 31)

mesenchyme) were randomly selected, and in each high-power field 100 cells were scored in terms of staining intensity and percentage of positive cells. Each section got its first score by staining intensity which was delimited as 0(negative), 1(weak), 2(moderate), or 3 (strong). The second score was determined on the basis of the percentage of positively stained cells. The criteria are as following:0(≤ 5 %), 1(6 %~25 %), 2(26 %~50 %), 3(51 %~75 %),4 (≥ 76 %). Each section was then got a multiplied score which was derived from the two scores above, ranging from 0 to 12. And the multiplied score was converted to a ranked value according to the following rules: 0 (-), 1~3 (+), 4~7 (++),8~12 (+++).

1.2.3 RT-PCR Total RNA extraction from 29 cancer tissues and 15 tumor-adjacent tissues and RT-PCR were conducted after the instructions of the kits. Primer SNGG, with a length of 384bp, were as following: forward:5'-ATGGATGTCTTCAAGAAGGG-3', reverse:5'-CTAGTCTCCCCCACTCTGGG-3'. The internal control GAPDH, with a length of 499bp, were as followin: forward:5'- CAAGGTCATCCATGACAACTTTG-3', reverse:5'-CAAGGTCATCCATGACAACTTTG-3'. PCR products were detected by electrophoresis in 2% agarose gels. PCR products of SNGG mRNA and GAPDH have a molecular weight of 384bp and 499bp respectively. Gray levels of band SNGG mRNA and band GAPDH were determined using Quantity One(A software used in gray level analysis).

1.2.4 Statistical analysis SPSS16.0 software was used. Mann-Whitney Test was used in analyzing ranked data.T-test was applied to RT-PCR data analysis. Statistical significance was considered as P-values below 0.05.

2 Results

2.1 Immunohistochemistry

Positive expression rate of SNCG in gastric adenocarcinoma was remarkably higher than that in normal gastric mucosa tissue, and the difference was statistically significant (Uc=7.149, $P<0.05$) (Table 1).

Table 1 Expression of SNCG in gastric adenocarcinoma and normal gastric mucosa tissue

Histotype	n	SNCG				Uc	P
		-	+	++	+++		
gastric adenocarcinoma	90	6	19	22	43	7.149	0.000
normal gastric mucosa	40	24	12	3	1		

2.2 Relation between SNCG expression in gastric adenocarcinoma and clinicopathologic parameters

The expression of SNCG was associated with differentiation and the metastasis of lymph node, the positive expressions of SNCG in invasion to chorion was higher than that invasion to mucosa or muscular layer(Uc=2.742,$P<0.05$); the positive expressions of SNCG in gastric adneocarcinoma with lymphnode metastasis were obviously higher than those without lymphnode metastasis (Uc=3.970,$P<0.05$), (Table 2). There was no apparent correlation between the expression of SNCG and parameters such as age, sex, differentiation($p>0.05$).

Table 2 Relation of SNCG expression in gastric adneocarcinoma to clinicopathologic parameters

clinicopathologic parameters	n	SNCG				Uc	P
		-	+	++	+++		
Sex							
Male	57	3	9	17	28	1.021	P>0.05
Male	33	3	10	5	15		
Age							
<62	44	3	7	12	22	0.663	P>0.05
≥ 62	46	3	12	10	21		
Differentiation							
Well or moderate	31	4	9	6	12	1.874	P>0.05
Low	59	2	10	16	31		
the depth of invasion							
invasion to muscular layer	21	5	8	2	8	2.742	P<0.05
invasion to chorion	69	1	11	20	35		

node involvement							
Node positive	58	2	7	13	36	3.970	P<0.05
Node negative	32	4	12	9	7		

2.3 RT-PCR

The expression of SNCG mRNA in gastric adenocarcinoma was obviously higher than that in normal gastric mucosa tissue, and the difference was statistically significant (t=4.339, P<0.01) (Table 3).

Table 3 Expression of SNCGmRNA in gastric adenocarcinoma and normal gastric mucosa tissue

Histotype	n	expression level ($\bar{x}\pm s$)	t	P
gastric adenocarcinoma	29	0.717±0.175	4.339	0.000
normal gastric mucosa	15	0.470±0.181		

3 Discussions

SNCG, also known as breast cancer specific gene 1 (BCSG 1) or persyn [5], was discovered in 1997 by Ji [6], like α -synuclein and β -synuclein, it belongs to the synuclein gene family. SNCG protein has 127 amino acids and is a natural unfolded protein[7].

SNCG is mainly expressed in the nervous system, which is probably due to the integrity of network structure in the neurofilament. High expression of SNCG was discovered in substantia nigra area and thalamencephal, while low expression of it was seen in testicles, ovary, colon and the heart. SNCG expression has not been found in such tissues [8] as breast, kidney, liver, prostate, lung, small intestine, thymus and placenta.

By immunohistochemistry, Liu [4] et al found that SNCG protein and SNCG mRNA were highly expressed in many solid tumors and that their expression were hardly seen in the adjacent nontumorous tissue, which is consistent with our findings. This suggests SNCG is probably associated with the tumorigenesis of gastric cancer, but its mechanism has not been reported. Our explanation is that demethylation of CPG island[9-11] or the abnormal activation of AP-1 binding site results in an increased transcription level of SNCG, thereby enhancing SNCG expression in tumor tissues. Previous research has suggested blocking of AP-1 or use of AP-1 binding site inhibitors downregulates SNCG expression and inhibits tumor phenotype[12]. In addition, SNCG can activate Extracellular Regulated protein Kinases 1/2 (ERK 1/2) and block the activation of , JNK1, thereby inhibiting apoptosis of tumor cells and promoting tumorigenesis[13].

Researches in the relationship between SNCG and tumor infiltration were rare worldwide. Using immunohistochemistry and RT-PCR, Qing Ye [14] detected SNCG expression in colon cancer at the protein as well as mRNA levels. Its findings suggested they are closely related to clinical stages and lymph node metastasis, which is consistent with our research. This suggest SNCG may be involved inl tumor infiltration and metastasis. The mechanism is reckoned as below. Excessive SNCG has an up-regulate effect on MAPK pathway [13], and following phosphorylation and AP-1 activation, MMPs gene expression is increased[15,16]. MMPs protein degrades extracellular matrix and basement membrane, thereby promotes tumor infiltration and metastasis. Another role of MMPs is to coordinate with MAPs to regulate the structure and assembling of cytoskeleton, so the cellular motility is improved.

Researches on the drug-resistant mechanism of SNCG found a new peptide (ANK) which competitively inhibit the combination of SNCG and BubR1 [17,18] and enhance the sensitivity of high SNCG cells to antineoplastic drug[19,20]. Therefore, inhibitors similar to the aboving peptide may be used for the assistant treatment of tumors.

As a member of synuclein family, SNCG is in close association with tumorigenesis, tumor infiltration and metastasis, and is of great significance in the assistant treatment of tumors. Further studies are needed for its function and clinical application.

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SNCG 在胃癌中的表达及意义

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摘要 目的：探讨 SNCG 在胃癌及正常胃粘膜组织中的蛋白和 mRNA 表达及其与临床病理特征的关系。方法：采用免疫组织化学 SP 法检测 90 例胃癌及 40 例正常胃粘膜组织中 SNCG 蛋白表达情况，同时应用 RT-PCR 检测 29 例胃癌及 15 例正常胃粘膜组织中 SNCG mRNA 的表达情况。结果：SNCG 蛋白在胃癌组中的表达高于正常胃粘膜组（Uc=7.149，P <0.05），胃癌组中 SNCG 蛋白阳性表达与癌组织的浸润深度以及有无淋巴结转移有关（Uc= 2.742，Uc,3.970，P 均 <0.05），而与患者的性别、年龄、及癌组织的分化程度无关。SNCG mRNA 在胃癌组织中的表达量明显高于正常胃粘膜组织（t=4.399，P <0.01）。结论：SNCG 在胃癌组织中的高表达与胃癌发生发展及浸润转移密切相关，可能会成为胃癌早期发现、早期诊断以及判断转移、预后的重要参考指标。

关键词：胃癌；SNCG；免疫组织化学；RT-PCR

中图分类号：R735.2 **文献标识码**：A **文章编号**：1673-6273（2011）08-1532-04

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（收稿日期：2011-02-06 接受日期：2011-02-31）