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Original Article

Expression of SNCG, MAP2, SDF-1 and CXCR4 in gastric adenocarcinoma and their clinical significance

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Abstract: Objectives: The purpose of the study was to detect the expression of SNCG, MAP2, SDF-1 and CXCR4 in gastric adenocarcinoma, and to evaluate their roles in the carcinogenesis of gastric adenocarcinoma, development, invasion and metastasis as well as their clinical significance. Methods: The expression of SNCG, MAP2, SDF-1 and CXCR4 was detected by SP immunohistochemical method in 225 cases of gastric adenocarcinoma and 105 cases of nonneoplastic adjacent gastric tissue. The expression of SNCG, MAP2, SDF-1 and CXCR4 mRNA was also detected by RT-PCR method in 50 cases of gastric adenocarcinoma and 30 cases of nonneoplastic adjacent gastric tissue. Results: The expression of SNCG, MAP2, SDF-1 and CXCR4 in the gastric adenocarcinoma was remarkably higher than those in the nonneoplastic adjacent gastric tissue ($P < 0.01$); The positive expression of SNCG and MAP2 was correlated with the depth of tumor invasion and the metastasis of lymph nodes ($P < 0.05$), and that of SDF-1 and CXCR4 was correlated with the metastasis of lymph nodes ($P < 0.05$). Conclusions: SNCG, MAP2, SDF-1 and CXCR4 may play an important role in the carcinogenesis, progression, invasion and metastasis of gastric adenocarcinoma. However, it still needs more exploration whether they can serve as promising therapeutic targets of gastric adenocarcinoma.

Keywords: SNCG, MAP2, SDF-1, CXCR4, gastric adenocarcinoma, immunohistochemistry, RT-PCR

Introduction

Gastric cancer is one of the most frequent tumors in the world, whose mortality ranks third among various types of tumors. Each year, about 723,000 people died of gastric cancer, and its mortality takes up 8.82% of the total cancer [1, 2]. Although there are a variety of treaments, such as surgery, chemotherapy and radiotherapy, the invasion and metastasis of gastric cancer are the main causes of death and the 5-year survival rate is still low [3, 4].

SNCG (γ-synuclein), also known as breast cancer specific gene 1 (BCSG 1), was discovered in 1997 by Ji *et al.* [5]. Like α-synuclein and β-synuclein, it belongs to the synuclein gene family [6]. SNCG protein contains 127 amino acids and is a natural unfolded protein. Microtubule-associated protein 2 (MAP2), as a member of structural microtubule-associated protein family, is an important regulator of microtubule dynamics. SDF-1, also known as CXCL12, as an important member of the chemokine family, is a specific ligand of CXCR4. CXCR4 is a highly conserved seven-transmembrane G protein-coupled receptor consisting of 352 amino acids. SDF-1 can combine with CXCR4 to form SDF-1/CXCR4 axis, which can start cell signal transduction and possess a variety of biological functions such as the extracellular transmission of information and cell migration.

This study was mainly to detect the expression of SNCG, MAP2, SDF-1 and CXCR4 of gastric adenocarcinoma at both protein and mRNA levels and to discuss the relation of them with clini-

copathological characteristics of occurence, invasion and metastasis in gastric adenocarcinoma to search for potential therapeutic targets of gastric cancer on the basis of experiments.

Materials and methods

Tissue specimens

With the Institutional Review Board approval, 225 cases of gastric adenocarcinoma tissues were derived from the surgical pathology files at the Affiliated Hospital of Logistics College of CAPF (Tianjin, China) during January 2009 to March 2014. The tissue specimens were fixed in 10% formalin, and then embedded in parafin. Among them, 105 eligible paraffin-embedded blocks of nonneoplastic adjacent gastric tissue (more than 5 cm distance from cancerous tissue and no proliferation or tumor lesions) were cut into serial 7 sections of 4 μm thickness in 1 week, one of which was H&E counterstained and the pathological diagnosis rechecked by two expert pathologists in double-blind method. The remaining six were adhered to APES rubber processing section for immunohistochemical staining. 80 fresh tissue specimens (50 gastric adenocarcinoma specimens and 30 nonneoplastic adjacent tissues) were also collected at the Affiliated Hospital of Logistics College of CAPF (Tianjin, China) during July 2013-March 2014. After the tissues removed from the body, all samples were labeled and frozen in liquid nitrogen (-196°C). No cases underwent radiotherapy or chemotherapy.

Immunohistochemistry

Sections of immunohistochemical staining were deparaffinized with xylene. Following rehydration in distilled water, antigen retrieval was accomplished by heating with Target Retrieval Solution High pH (Dako, Carpinteria, CA). Endogenous peroxidase activity was blocked by incubating in the peroxidase-blocking reagent (Dako, Carpinteria, CA) at room temperature for 10 minutes. Nonspecific antibody binding was blocked with 5% goat serum for 10 minutes at room temperature. Slides were then incubated with mouse SNCG monoclonal antibody (Santa Cruz Biotech, CA) at 1:100 dilution at 4°C overnight. MAP2 (rabbit polyclonal antibody) was bought from Abcam Biotech and incubated at 1:150 dilution at 4°C overnight. SDF-1 (rabbit polyclonal antibody) was bought from Santa Cruz Biotech, CA, and incubated at 1:100 dilutions at 4°C overnight. CXCR4 (mouse monoclonal antibody) was bought from ABGENT Biotech and incubated at 1:50 dilution at 4°C overnight. Following washed three times with phosphate-buffered saline (PBS), slides were incubated with biotin-labeled rabbit anti-mouse IgG (DAKO, Carpinteria, CA) for 30 minutes at 37°C. After washing three times with PBS, the staining was accomplished by using 3, 3V-diaminobenzidine + substrate chromogen systems (DAKO, Carpinteria, CA). Sections were counterstained with hematoxylin, dehydrated, cleared and mounted. In this experiment, PBS solution replaced primary antibodies as negative control and positive blank sections provided by the antibody company were used as positive control, which was stained in the same lot.

Judgment of the results: positive cases were defined by the presence of intracellular staining with brown color, as seen in positive controls. SNCG positive substance is located in the nucleus and cytoplasm. MAP2 positive substance is located in the nucleus and cytoplasm. SDF-1 positive substance is located in the cell membrane or cytoplasm. CXCR4 positive sustance is located in the cytoplasm. All of them appeared tan fine granular. Negative cases were defined by the absence of specific intracellular staining, as seen in negative cotrols. A semiquantitative scoring system based on the average number of SNCG, MAP2, SDF-1 and CXCR4-positive cells from ten randomly chosen fields of 400× was used to grade the expression levels and the staining intensity. Samples were evaluated under light microscope independently by two pathologists without prior knowledge of the patients' clinical data. The slides for each section, 10 highpower fields (400×, to avoid large vessels and large areas of mesenchyma) 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 follows: 0 (≤5%), 1 (6%~25%), 2 (26%~50%), 3 (51%~75%), and 4 (≥76%). Each section was then got a multiplied score which was derived from the two scores above, ranging

Table 1. Expression of SNCG, MAP2, SDF-1 and CXCR4 in gastric adenocarcinoma and nonneoplastic adjacent gastric tissue

	Histological type	n	-	+	X^2	*P*
SNCG	Gastric adenocarcinoma	225	86	139	37.245	0.000**
	Nonneoplastic adjacent gastric tissue	105	78	27		
MAP2	Gastric adenocarcinoma	225	74	151	66.24	0.000**
	Nonneoplastic adjacent gastric tissue	105	85	20		
SDF-1	Gastric adenocarcinoma	225	51	174	55.02	0.000**
	Nonneoplastic adjacent gastric tissue	105	68	37		
CXCR4	Gastric adenocarcinoma	225	71	154	39.947	0.000**
	Nonneoplastic adjacent gastric tissue	105	72	33		

$^{**}P < 0.01$.

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 (+++). Eventually, we think that (-) was negative, (+) and above were positive and use those for statistical analysis.

RT-PCR

RNAiso Reagent, Super RT Kit and PCR kit were bought from Takara Biotech (Dalian) CO., LTD. Primer SNCG, MAP2, SDF-1, CXCR4 and GAPDH were offered by Sangon Biotech (Shanghai) Co., Ltd. Total RNA extraction from 50 cancer tissues and 30 tumor-adjacent tissues and RT-PCR were conducted according to the instructions of the kits. Reverse transcription reaction mixtures were then incubated at 65°C (5 min), 42°C (20 min) and 95°C (5 min), respectively. cDNA samples were stored at -20°C prior to use. Primer SNGG, with a length of 384 bp, was as follows: forward: 5'-ATG GAT GTC TTC AAG AAG GG-3', reverse: 5'-CTA GTC TCC CCC ACT CTG GG-3'. Primer MAP2, with a length of 320 bp, was as follows: forward: 5'-TCA GAG GCA ATG ACC TTA CC-3', reverse: 5'-GTG GTA GGC TCT TGG TCT TT-3'. The internal control GAPDH (1), with a length of 492 bp, was as follows: forward: 5'-CAA GGT CAT CCA TGA CAA CTT TG-3', reverse: 5'-CAA GGT CAT CCA TGA CAA CTT TG-3'. Primer SDF-1, with a length of 103 bp, was as follows: forward: 5'-GAG CCA ACG TCA AGC ATC TCA-3', reverse: 5'-TTC GGG TCA ATG CAC ACT TGT-3'. Primer CXCR4, with a length of 173 bp, was as follows: forward: 5'-TGG CCT TAT CCT GCC TGG TAT-3', reverse: 5'-GGA GTC GAT GCT GAT CCC AAT-3'. The internal control GAPDH (2), with a length of 299 bp, was as follows: forward: 5'-CGG GAA ACT GTG GCG TGA T-3', reverse: 5'-AGT GGG TGT CGC TGT TGA AGT-3'. Reaction mixtures contained 1× PCR master mix (Takara Biotech, Dalian, LTD); forward and reverse primers (Sangon Biotech, Shanghai, Ltd) at a concentration of 10 μM; for SNCG, MAP2, SDF-1, CXCR4 and GAPDH amplification 50 ng cDNA templates; made to a total volume of 20 μl with sterile H_2O. Thermal cycling parameters included activation at 94°C (1 min) followed by 40 cycles each of denaturation at 94°C (30 s), annealing at 57°C (30 s) and extending at 72°C (1 min), then extending at 72°C (10 min). PCR products were detected by electrophoresis in 2% agarose gels. PCR products of SNGG mRNA, MAP2 mRNA and GAPDH (1) mRNA have a molecular weight of 384 bp, 320 bp and 492 bp respectively. PCR products of SDF-1 mRNA, CXCR4 mRNA and GAPDH (2) mRNA have a molecular weight of 103 bp, 173 bp and 299 bp, respectively. Gray levels of band SNGG, MAP2, SDF-1, CXCR4 mRNA and band GAPDH were determined by using Quantity One (software used in gray level analysis).

Statistical analysis

SPSS16.0 software was used. Chi-square Test was used in the positive rate of immunohistochemistry. T-test was applied to RT-PCR data analysis. Statistical significance was considered as *P*-values below 0.05.

Results

Immunohistochemical findings

Positive expression rates of SNCG, MAP2, SDF-1 and CXCR4 in gastric adenocarcinoma were remarkably higher than those in nonneoplastic adjacent gastric tissue, and the differences were statistically significant ($P < 0.01$) (**Table 1**; **Figure 1**).

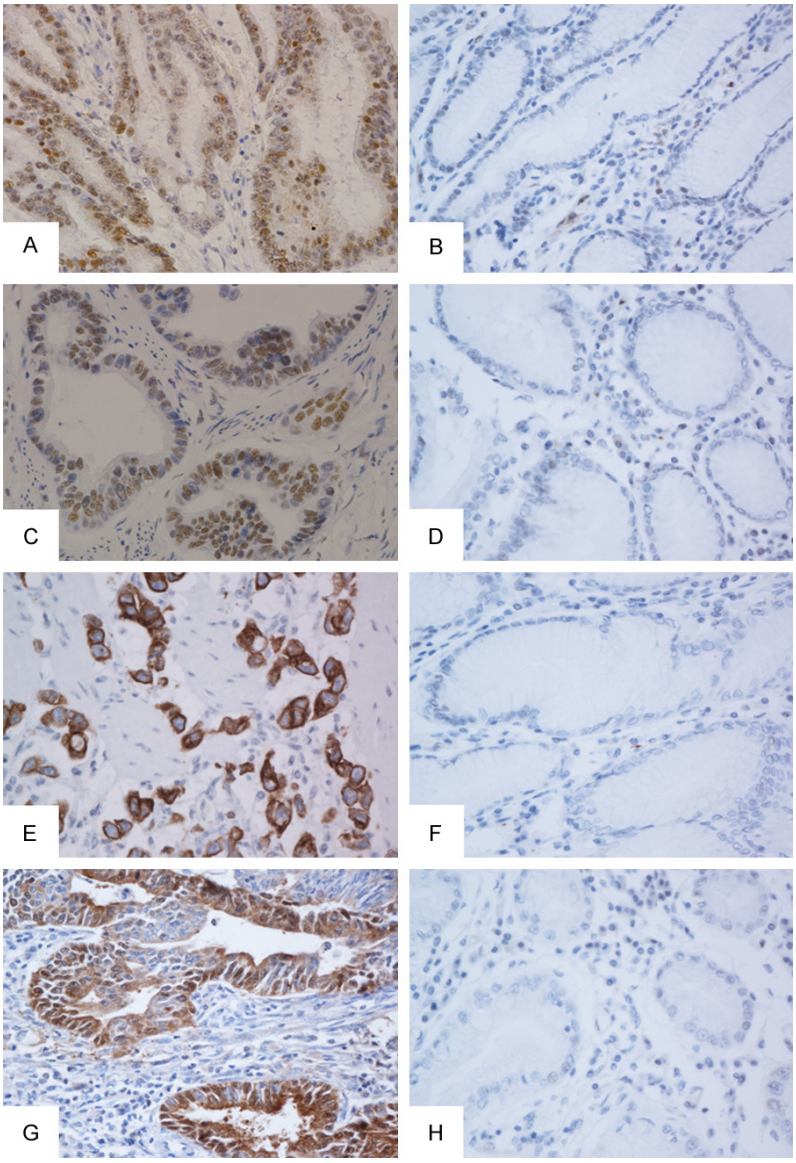

Figure 1. Expression of SNCG, MAP2, SDF-1 and CXCR4 in gastric adenocarcinoma and nonneoplastic adjacent gastric tissue. A. The positive expression of SNCG in gastric adenocarcinoma (400×). B. The negative expression of SNCG in nonneoplastic adjacent gastric tissue (400×). C. The positive expression of MAP2 in gastric adenocarcinoma (400×). D. The negative expression of MAP2 in nonneoplastic adjacent gastric tissue (400×). E. The positive expression of SDF-1 in gastric adenocarcinoma (400×). F. The negative expression of SDF-1 in nonneoplastic adjacent gastric tissue (400×). G. The positive expression of CXCR4 in gastric adenocarcinoma (400×). H. The negative expression of CXCR4 in nonneoplastic adjacent gastric tissue (400×).

Relations between SNCG, MAP2, SDF-1 and CXCR4 protein expression in gastric adenocarcinoma and clinicopathologic parameters

The expression of SNCG and MAP2 proteins was associated with the depth of invasion and the metastasis of lymph nodes (**Table 2**): the positive expression of SNCG and MAP2 proteins in invasion to serosa was higher than that invasion to submucosa or muscular layer ($P < 0.01$, $P < 0.05$); the positive expression of SNCG and MAP2 proteins in gastric adenocarcinoma with lymph node metastasis was obviously higher than those without lymph node metastasis ($P < 0.05$). The expression of SDF-1 and CXCR4 proteins in gastric adenocarcinoma with lymph node metastasis was obviously higher than those without lymph node metastasis ($P < 0.01$, $P < 0.05$). There was no apparent correlation between the expression of SNCG, MAP2, SDF-1, CXCR4 proteins and parameters such as age, sex, differentiation ($P > 0.05$). SDF-1 and CXCR4 proteins have no distinct correlation with the depth of invasion ($P > 0.05$).

RT-PCR findings in gastric adenocarcinoma tissues

The expression of SNCG, MAP2, SDF-1 and CXCR4 mRNA in gastric adenocarcinoma was obviously higher than those in nonneoplastic adjacent gastric tissue, and the differences were statistically significant ($t = 2.861$, $t = 2.860$, $t = 7.808$, $t = 8.073$, $P < 0.01$) (**Table 3**; **Figures 2**-**4**).

Relations between SNCG, MAP2, SDF-1 and CXCR4 mRNA expression in gastric adenocarcinoma and clinicopathologic parameters

The expression of SNCG and MAP2 mRNA were associated with the depth of invasion and the metastasis of lymph nodes (**Table 4**): the positive expression of SNCG and MAP2 mRNA in gastric adenocarcinoma with invasion to the serosa was higher than those with invasion to sub-

Table 2. Relations between SNCG, MAP2, SDF-1 and CXCR4 protein expression in gastric adenocarcinoma and clinicopathologic parameters

Clinicopathologic parameters	n	SNCG protein		MAP2 protein		SDF-1 protein		CXCR4 protein	
		-	+	-	+	-	+	-	+
Sex		$P = 0.074$		$P = 0.546$		$P = 0.621$		$P = 0.364$	
Male	152	52	100	48	104	33	119	45	107
Female	73	34	39	26	47	18	55	26	47
Age		$P = 0.296$		$P = 0.276$		$P = 0.447$		$P = 0.338$	
< 60	112	39	73	33	79	23	89	32	80
≥ 60	113	47	66	41	72	28	85	39	74
Differentiation		$P = 0.951$		$P = 0.577$		$P = 0.897$		$P = 0.504$	
Well or moderate	91	35	56	28	63	20	70	31	60
Low	134	51	83	46	88	31	104	40	94
Depth of invasion		$P = 0.001$**		$P = 0.044$*		$P = 0.793$		$P = 0.615$	
Invasion to submucosa or muscular layer	74	40	34	31	43	16	58	25	49
Invasion to serosa	151	46	105	43	108	35	116	46	105
Node involvement		$P = 0.016$*		$P = 0.015$*		$P = 0.002$**		$P = 0.026$*	
Positive	135	43	92	36	99	21	114	35	100
Negative	90	43	47	38	52	30	60	36	54

**$P < 0.01$, *$P < 0.05$.

Table 3. Expression of SNCG, MAP2, SDF-1 and CXCR4 mRNA in gastric adenocarcinoma and nonneoplastic adjacent gastric tissue

	Histological type	n	Expression level ($\bar{x}$±s)	*t*	P
SNCG	Gastric adenocarcinoma	50	0.7094±0.1523	2.861	0.005**
	Nonneoplastic adjacent gastric tissue	30	0.6133±0.1326		
MAP2	Gastric adenocarcinoma	50	0.6737±0.1944	2.860	0.005**
	Nonneoplastic adjacent gastric tissue	30	0.5585±0.1339		
SDF-1	Gastric adenocarcinoma	50	0.7092±0.0818	7.808	0.000**
	Nonneoplastic adjacent gastric tissue	30	0.5995±0.0850		
CXCR4	Gastric adenocarcinoma	50	0.814±0.0802	8.073	0.000**
	Nonneoplastic adjacent gastric tissue	30	0.6634±0.0817		

**$P < 0.01$.

mucosa or muscular layer ($P < 0.05$, $P < 0.01$); the positive expression of SNCG and MAP2 mRNA with lymph node metastasis was obviously higher than those without lymph node metastasis ($P < 0.05$). There was no apparent correlation between the expression of SNCG and MAP2 mRNA and parameters such as age, sex, differentiation ($P > 0.05$). The expression of SDF-1 and CXCR4 mRNA was associated with the metastasis of lymph node: the positive expression of SDF-1 and CXCR4 mRNA in gastric adenocarcinoma with lymph node metastasis was obviously higher than those without lymph node metastasis ($P < 0.05$). SDF-1 and CXCR4 mRNA have no apparent correlation with age, sex, differentiation and the depth of invasion ($P > 0.05$).

Discussions

This experiment results have shown that the expression of SNCG protein and mRNA in gastric adenocarcinoma was higher than in the nonneoplastic adjacent gastric tissue and correlated with the depth of tumor invasion and the metastasis of lymph nodes, which suggested that SNCG may have relationship with the pathogenesis, invasion and metastasis of gastric adenocarcinoma.

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 thalamencephalon, while low

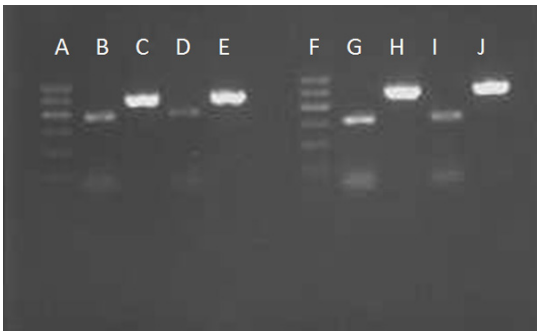

Figure 2. Expression of SNCG mRNA and MAP2 mRNA in gastric adenocarcinoma and nonneoplastic adjacent gastric tissue. A. DNA Ladder Marker (2000 bp). B. The expression of SNCG mRNA in gastric adenocarcinoma (384 bp). C. The expression of GAPDH mRNA in gastric adenocarcinoma (492 bp). D. The expression of SNCG mRNA in nonneoplastic adjacent gastric tissue (384 bp). E. The expression of GAPDH mRNA in nonneoplastic adjacent gastric tissue (492 bp). F. DNA Ladder Marker (2000 bp). G. The expression of MAP2 mRNA in gastric adenocarcinoma (320 bp). H. The expression of GAPDH mRNA in gastric adenocarcinoma (492 bp). I. The expression of MAP2 mRNA in nonneoplastic adjacent gastric tissue (320 bp). J. The expression of GAPDH mRNA in nonneoplastic adjacent gastric tissue (492 bp).

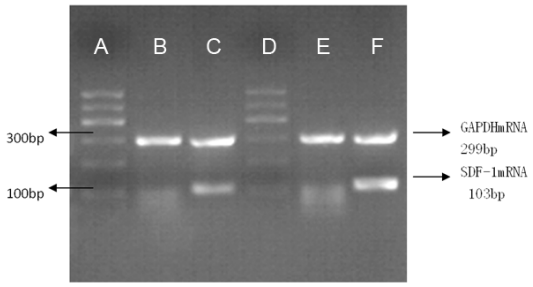

Figure 3. Expression of SDF-1 mRNA and GAPDH mRNA in gastric adenocarcinoma and nonneoplastic adjacent gastric tissue. A. DNA Ladder Marker (1000 bp). B. The expression of GAPDH mRNA and SDF-1 mRNA in nonneoplastic adjacent gastric tissue (299 bp and 103 bp). C. The expression of GAPDH mRNA and SDF-1 mRNA in gastric adenocarcinoma without lymph node metastasis (299 bp and 103 bp). D. DNA Ladder Marker (1000 bp). E. The expression of GAPDH mRNA and SDF-1 mRNA in nonneoplastic adjacent gastric tissue (299 bp and 103 bp). F. The expression of GAPDH mRNA and SDF-1 mRNA in gastric adenocarcinoma with lymph node metastasis (299 bp and 103 bp).

expression of it was seen in testicles, ovary, colon and heart. Recent studies have found that synuclein family is related to cancer genetics, especially SNCG. Previous research [7] had shown that there is 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 level is related to the tumor genesis, development, invasiveness and prognosis, even the resistance of the chemotherapy drug can affect its expression, especially the sensitive of Taxol anti-microtubule drugs [8], which is similar with our experiment results. The high expression of SNCG in gastric cancer may be related with existence of a special CPG island in exon 1. The increasing degree of CPG island methylation or the abnormal activation of AP-1 binding sites can lead to increase SNCG transcription level, which causes SNCG overexpression in tumor tissues [9-11]. The high SNCG expression can activate extracellular regulated protein kinases 1/2(ERK 1/2) and block the activation of c-Jun amino terminal kinase (JNK1) to inhibit apoptosis of tumor cells and promote tumorigenesis [12]; SNCG can combine with MAP2 to adjust the structure of cytoskeleton system and dynamic assembly such as promoting tubulin polymerization, microtubule bundle [13] and improve the sports ability of tumor cells, which is conducive to the migration of tumor cells and metastasis. SNCG also has an unregulated effect on MAPK pathway following by phosphorylation and AP-1 activation, which led to the increased expression of MMPs gene. Then MMPs protein degraded extracellular matrix and basement membrane to promote tumor metastasis [14]. So the proportion of cells was changed at different phase in the cell cycle to increase G0/G1 phase cells, decrease the G2/M phase cells and reduce anti microtubules drugs sensitivity of tumor cells such as taxol and vincristine. Previous research has proved that blocking of AP-1 or using AP-1 binding site inhibitors could downregulate SNCG expression and inhibit tumor phenotype [15]. To date, studies on the drug-resistant mechanism of SNCG found a new peptide (ANK, ankyrin-based peptide), which competitively inhibited the combination of SNCG and BubR1 and enhanced the sensitivity of high SNCG expression cells to antineoplastic drug [16, 17]. Therefore, whether this pathway and peptide inhibitors like ANK could be used for the assistant treatment of tumors needs more exploration.

This experiment results have also shown that the expression of MAP2 protein and mRNA in

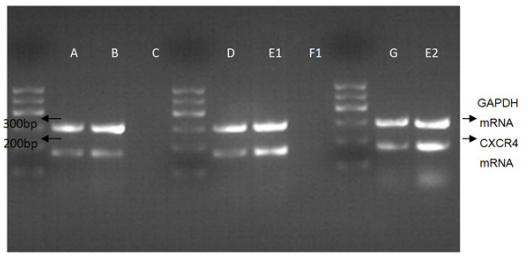

Figure 4. Expression of CXCR4 mRNA and GAPDH mRNA in gastric adenocarcinoma and nonneoplastic adjacent gastric tissue. A. DNA Ladder Marker (1000 bp). B. The expression of GAPDH mRNA and CXCR4 mRNA in nonneoplastic adjacent gastric tissue (299 bp and 173 bp). C. The expression of GAPDH mRNA and CXCR4 mRNA in gastric adenocarcinoma without lymph node metastasis (299 bp and 173 bp). D. DNA Ladder Marker (1000bp). E1. The expression of GAPDH mRNA and CXCR4 mRNA in nonneoplastic adjacent gastric tissue (299 bp and 173 bp). F1. The expression of GAPDH mRNA and CXCR4 mRNA in gastric adenocarcinoma with lymph node metastasis (299 bp and 173 bp). G. DNA Ladder Marker (1000 bp). E2. The expression of GAPDH mRNA and CXCR4 mRNA in nonneoplastic adjacent gastric tissue (299 bp and 173 bp). F2. The expression of GAPDH mRNA and CXCR4 mRNA in gastric adenocarcinoma with lymph node metastasis (299 bp and 173 bp).

gastric adenocarcinoma was obviously higher than in the nonneoplastic adjacent gastric tissue and correlated with the depth of tumor infiltration and the metastasis of lymph nodes.

Chen *et al*. [18] have found that the expression rate of MAP2 in oral carcinoma was higher than in normal mucosa. Liu *et al.* [19] have found that the migratory ability of cancer cells transfected MAP2 was significantly higher than control group, and then conjectured that MAP2 was closely associated with tumor occurrence, invasion and metastasis, which was consistent with our result. The possible reasons are as follows: (1) ERKs, PKA, PKC, calmodulin dependent protein kinaseII (CAMKII) and glycogen synthesis kinase (GSK23β) were significantly increased in the tumor tissue leading to the increase of MAP2 phosphorylation and cell cycle protein dependent kinase (cdc2) multiple sites phosphorylation. The phosphorylation made the M-phase promoting factor (MPF) activity decreased and hindered polymerization of lamin monomers, so the cells arrested in M phase resulting in the formation of polyploid cells and tumor genesis [20-22]. (2) Increasing MAP2 phosphorylation levels could decline the combination ability between MAP2 and microtubules and changed the dynamic behavior inside microtubules. So that the microtubule cytoskeleton reorganized and structural became abnormal, this led to the enhanced capacity of tumor motion and migration [19].

Our experiment results suggested that SDF-1 and CXCR4 proteins expressed higher in gastric cancer than in non-neoplastic mucosa and both were closely associated with lymph node metastasis.

Under normal circumstances, the biological axis binded by SDF-1 and its specific receptor CXCR4 could take part in various pathophysiological processes, such as participating in embryonic development, regulating migration and homing of hematopoietic stem cell, mediating immune and inflammatory responses, promoting angiogenesis and mediating HIV infection [23, 24], etc. Studies have shown that CXCR4 exhibited abnormal expression and excessive activation in human malignant tumors and played an important role in tumor invasion and metastasis [24-26], which have become a research hotspot in this field. Our experiment results have suggested that SDF-1/CXCR4 biological axis was related with tumorgenesis and metastasis of gastric cancer. The possible mechanism could be as follows: (1) The overexpression of SDF-1 can inhibit the increase of mitochondrial membrane electric potential and the release of cytochrome from mitochondria to cytoplasm, and decrease the activity of caspase and aspartic protease-3, which could in hibit the cell apoptosis and promote tumorgenesis; (2) SDF-1/CXCR4 axis can activate extracellular signal-regulated kinase (ERK-1/2), increase the secretion of matrix metalloproteinases (such as MMP-2 or MMP-9), and promote the degradation of type IV collagen fibers and destruction of the basement membrane leading to the invasion and metastasis of tumor cell [27]; (3) Another study showed that MMP-2 could do positive feedback regulation of the signaling pathways of SDF-1/CXCR4 to generate more SDF-1, which could up-regulate the expression of CXCR4 and PI3K, increase the phosphorylation of AKt (Ser473), enhance the interactions between MMP-2 and vascular endothelial cell prime αVβ3 integration, and promote the vascular remodeling in tumor [28]. (4) Meanwhile, combining SDF-1 with CXCR4 could activate MAPK p42/44 and AKT signal transduction pathway and promote tumor cell proliferation [29].

Table 4. Relation of SNCG, MAP2, SDF-1 and CXCR4 mRNA expression in gastric adenocarcinoma to clinicopathologic parameters

Clinicopathologic parameters	n	SNCG mRNA	MAP2 mRNA	SDF-1 mRNA	CXCR4 mRNA
Sex		$P = 0.167$	$P = 0.76$	$P = 0.401$	$P = 0.341$
Male	28	0.6829±0.1672	0.6662±0.1677	0.718±0.0827	0.8237±0.0809
Female	22	0.7431±0.1267	0.6834±0.2278	0.6981±0.0813	0.8017±0.0795
Age		$P = 0.142$	$P = 0.631$	$P = 0.305$	$P = 0.233$
≤ 61	25	0.7411±0.1358	0.6871±0.2135	0.7212±0.0675	0.8004±0.0911
> 61	25	0.6777±0.1638	0.6603±0.1768	0.6972±0.0939	0.8277±0.0668
Differentiation		$P = 0.904$	$P = 0.592$	$P = 0.319$	$P = 0.301$
Well or moderate	30	0.7072±0.1483	0.6615±0.2064	0.6997±0.0855	0.8044±0.0828
Low	20	0.7126±0.1620	0.6920±0.1787	0.7235±0.0758	0.8286±0.0759
Depth of invasion		$P = 0.027$*	$P = 0.003$**	$P = 0.582$	$P = 0.458$
Nvasion to submucosa or muscular layer	26	0.6640±0.1413	0.5965±0.1325	0.703±0.0715	0.8058±0.0811
Invasion to serosa	24	0.7585±0.1513	0.7575±0.2178	0.716±0.0928	0.8229±0.0799
Node involvement		$P = 0.048$*	$P = 0.027$*	$P = 0.029$*	$P = 0.035$*
Positive	27	0.7485±0.1553	0.7292±0.1884	0.7324±0.0729	0.8358±0.0702
Negative	23	0.6634±0.1381	0.6087±0.1846	0.682±0.0849	0.7883±0.0851

**$P < 0.01$, *$P < 0.05$.

The experiments showed that the expressions of SNCG, MAP2, SDF-1 and CXCR4 proteins and mRNA were positively correlated. All of them could promote tumor cell invasion and metastasis through improving the activity of ERK1/2, increasing matrix metalloproteinases (MMPs) expression and accelerating the degradation of the extracellular matrix. The enhanced activity ERK1/2 could contribute to the phosphorylation of MAP2 to adjust the recombinant tubulin. The increased expression of SDF-1/CXCR4 could regulate actin assembly, which caused aggregation and redistribution of cytoskeletal proteins in tumor cells. The colocalization of SNCG with microtubules could promote polymerization of tubulin to form microtubule bundles and change morphological of microtubule.

In conclusion, SNCG, MAP2, SDF-1 and CXCR4 may have a synergistic effect on the structural changes of cytoskeletal protein, which could increase the chance of tumor occurrence, invasion and metastasis. However, further understanding the underlying mechanisms of the four proteins in the pathogenesis of gastric adenocarcinoma needs more exploration.

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Disclosure of conflict of interest

None.

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