P.O.Box 2345, Beijing 100023,China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

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• BASIC RESEARCH •

Expression of a novel immunoglobulin gene SNC73 in human cancer and non-cancerous tissues

Jian-Bin Hu, Shu Zheng, Yong-Chuan Deng

Jian-Bin Hu, Department of Radiation Oncology, Sir Run Run Shaw Hospital, Zhejiang University Medical College, Hangzhou 310016, Zhejiang Province, China
Shu Zheng, Cancer Institute, Zhejiang University, Hangzhou 310009, Zhejiang Province, China
Yong-Chuan Deng, Department of Surgical Oncology, The Second Affiliated Hospital of Zhejiang University Medical College, Hangzhou 310009, Zhejiang Province, China
Supported by the National Nature Scientific Foundation of China, No. 30070832
Correspondence to: Shu Zheng, Cancer Institute, Zhejiang University, Hangzhou 310009, Zhejiang Province, China. zhengshu@mail.hz.zj.cn
Telephone: +86-571-87783868 **Fax:** +86-571-87214404
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Abstract

AIM: To investigate the expression of immunoglobulin gene SNC73 in malignant tumors and non-cancerous normal tissues.

METHODS: Expression level of SNC73 in tumors and non-cancerous tissues from the same patient was determined by reverse transcription polymerase chain reaction and enzyme-linked immunosorbent assay (RT-PCR-ELISA) in 90 cases of malignant tumors, including colorectal cancer, gastric cancer, breast cancer, lung cancer and liver cancer. Analysis on the correlation of SNC73 expression with sex, age, site, grade of differentiation, depth of invasion, and metastases in colorectal cancer patients was made.

RESULTS: Expression level of SNC73 in non-cancerous colorectal mucosa and colorectal cancerous tissues was 1.234±0.842 and 0.737±0.731, respectively ($P<0.01$), with the mean ratio of 7.134±14.092 (range, 0.36-59.54). Expression of SNC73 showed no significant difference among gastric cancer, breast cancer, lung cancer and liver cancer when compared with non-cancerous tissues ($P>0.05$). No correlation was found between SNC73 expression level and various clinicopathological factors, including sex, age, site, grade of differentiation, depth of invasion and metastases of CRC patients.

CONCLUSION: Down-regulation of SNC73 expression may be a relatively specific phenomenon in colorectal cancer. SNC73 is a potential genetic marker for the carcinongenesis of colorectal cancer. The relationship of SNC73 expression and carcinogenesis of colorectal cancer merits further study.

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INTRODUCTION

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in developed western countries[1]. A series of molecular changes are involved in colorectal carcinogenesis, including activation of oncogenes, inactivation and/or mutational changes of tumor suppressor genes, microsatellite instability, and so on[2-10]. Fearon *et al* proposed a genetic model of colorectal tumorigenesis[11]. However, despite the tremendous efforts that have been made, there are still many problems unsolved for the model of CRC due to the complexity of carcinogenesis. The early detection and new therapeutic target of CRC have yet to be found. Modern medicine proves that almost all diseases arise from gene function change, which is mainly reflected by the differential gene expression[12]. Hopefully the identification and characterization of genes expressed differently in tumor tissues and normal mucosa will shed light on the mechanisms of CRC and provide useful molecular markers for screening, diagnosis, prognosis and therapeutic monitoring.

To explore new molecular events that are related to carcinogenesis of CRC, Cancer Institute of Zhejiang University constructed CRC negative-associated cDNA libraries by subtractive hybridization[13-17]. Subtractive hybridization between cDNA of normal mucosal tissues and mRNA of CRC tissues was performed and a total of 46 cDNA clones that were expressed in normal mucosal tissues but were either expressed at a significantly reduced level or not expressed at all in cancerous tissues were isolated. SNC73 is one of the 46 CRC negative-associated complement DNA (cDNA) clones. Northern blot, reverse transcription-polymerase chain reaction (RT-PCR), in situ hybridization, and in situ PCR confirmed expression of SNC73 in normal epithelial cells and several non-hematopoietic cancer cell strains[17]. The aim of this study was to confirm the negative association between CRC and SNC73 expression and to examine whether such association also exists in other tumors. In the present study, expression level of SNC73 in 90 cases of malignant tumors (31 cases colorectal cancer, 24 cases gastric cancer, 15 cases breast cancer, 11 cases lung cancer and 9 cases liver cancer) and non-cancerous tissues from the same patient was determined by RT-PCR-ELISA.

MATERIALS AND METHODS

Tissue sample preparation

Fresh samples of surgically resected cancer and its non-cancerous tissues were obtained from the same patient at the Second Affiliated Hospital of Zhejiang University Medical College, and were immediately frozen in liquid nitrogen until used. Several paired specimens were collected for replication. The total RNA was extracted with Trizol reagent (Gibco BRL, USA). RNA integrity was checked on 1 % formaldehyde agarose gel. RNA samples were accepted only when the ratio between absorbance optical density values at 260 nm and at 280 nm was higher than 1.65.

RT-PCR (DIG Labeling)

RNA samples were reverse transcribed with AMV reverse transcriptase (Promega Co.). The primers were labeled with biotin for following immobilization by streptavidin coated microtiter plate modules. The primer for SNC73 was designed

based on its cDNA sequence according to previous study. The sequence is 5' biotin-AAA CAC ATT CCG GCC CGA G 3' and 5' biotin-AGC GGT CGA TGG TCT TCT G 3'. The sequence of primer for β-actin is 5' biotin-TCG ACA ACG GCT CCG GCA 3' and 5' biotin-CGT ACA TGG CTG GGG TGT 3'. RT-PCR was carried out to amplify the mRNA of SNC73 and β-actin. The PCR products were labeled with digoxigenin (dig) by using mixture of dATP, dCTP, dGTP, dTTP and DIG-dUTP in reaction mixture during the amplification process. PCR reaction mixture contained 15.7 μl sterile water, 2.5 μl PCR buffer (10×conc., with $MgCl_2$), 2.5 μl 2 mM PCR DIG labeling mix, 2 μl 10 mM primers mixture, 0.3 μl Taq DNA polymerase and 2 μl template cDNA. The cycling program was denaturation of the template 94 ℃ for 3 min, 22 cycles of amplification: 94 ℃ for 10 s (denaturation), 58 ℃ for 20 s (hybridization), 72 ℃ for 30 s (elongation) and elongation (72 ℃) for 5 min was added after last cycle to ensure the completion of the reaction. PCR products quality was confirmed by electrophoresis in 1 % agarose gel stained with ethidium bromide.

PCR ELISA (DIG Detection)

DIG detection of PCR ELISA was carried out using a modification of the assay protocol according to reagent kit (Boehringer Mannheim Co., German). DIG labelled PCR products were immobilized by incubating appropriate dilutions of amplification reaction at 55 ℃ for 3 h. The solution was discarded and each well was washed 5 times with washing solution. The strips were incubated with anti-digoxigenin peroxidase conjugate at 37 ℃ for 30 min. The solution was discarded and each well was washed 5 times again with washing solution. The colorimetric substrate ABTS was added and incubated at 37 ℃ for 30 min. Absorbance was read in an ELISA-reader at 405 nm. The absorbance optical density values at 405 nm (A_{405}) represented the relative concentrations of PCR products. The results were normalized by the absorbance optical density values of β-actin, which was used as an endogenous standard because of its equal expression in various tissues. This would correct the variation in product abundance due to differences in the efficiencies of individual RT-PCR reaction.

Statistical analysis

The expression level of SNC73 in both cancerous tissues and non-cancerous tissues was interpreted as the ratio of its OD value relative to that of β-actin. SNC73 expression level in various cancerous tissues and non-cancerous tissues and the ratio of non-cancerous tissues to cancerous tissues were calculated. All results were expressed as means ± SD. Statistical differences between means of various cancerous tissues and non-cancerous tissues were determined by Wilcoxon nonparametric test (2-related samples). The differences were considered significant at $P<0.05$.

To get more information about the down-regulation of SNC73, which may be helpful to determine its characteristics, we investigated the relationship between SNC73 expression level and various clinicopathological factors of CRC patients. All CRC patients were grouped according to sex, age, site, grade of differentiation, depth of invasion and metastases (including lymph node metastases and distant metastases). The same functions as those used between various cancerous tissues and non-cancerous tissues were applied among different groups. Statistical differences of means among different groups were determined by analysis of variance. The differences were considered significant at $P<0.05$.

Table 1 SNC73 expression level in human cancerous tissues and non-cancerous tissues (Mean ± SD). The expression level of SNC73 in both cancerous tissues and non-cancerous tissues was expressed as the ratio of its OD value relative to that of β-actin. ($\bar{x}\pm s$)

Tumor	*n*	SNC73 expression level		Non-cancerous tissues / Cancerous tissues
		Non-cancerous tissues	Cancerous tissues	
CRC	31	1.234 ± 0.842	0.737±0.731 [a]	7.134±14.092
Gastric cancer	24	1.098±0.413	1.069±0.606	1.438±1.392
Breast cancer	15	1.279±1.705	0.900±0.690	1.836±2.541
Lung cancer	11	0.834±0.533	1.428±1.904	0.877±0.469
Liver cancer	9	0.793±0.285	0.799±0.322	1.140±0.467

[a] $P<0.01$ *vs* non-cancerous tissues.

Table 2 The relationship between SNC73 expression level and various clinicopathological factors of CRC patients. The expression level of SNC73 was interpreted as the ratio of its OD value relative to that of β-actin. ($\bar{x}\pm s$)

Factor	Group	*n*	SNC73 expression level		Non-cancerous tissues / Cancerous tissues
			Non-cancerous tissues	Cancerous tissues	
Sex	Male	15	1.360±1.011	0.748±0.438	8.822±18.358
	Female	16	1.116±0.657	0.726±0.943	5.551±8.782
Age	<60	14	1.524±0.979	0.737±0.458	9.444±18.843
	⩾60	17	0.995±0.644	0.736±0.911	5.231±8.681
Site	Rectal	17	1.209±0.715	0.616±0.497	10.890±18.260
	Colon	14	1.264±1.002	0.883±0.942	2.572±2.652
Grade of differentiation	Well	14	1.284±1.034	0.756±0.430	2.378±2.488
	Moderately	12	1.188±0.746	0.776±1.086	14.030±21.033
	Poorly	5	1.203±0.536	0.587±0.358	3.898±4.226
Depth of invasion	Mucosa or muscle	8	1.258±0.847	0.973±1.233	9.066±20.511
	Serosa or beyond	23	1.226±0.859	0.654±0.467	6.462±11.624
Metastases	Positive	22	1.225±0.914	0.638±0.479	6.672±11.854
	Negative	9	1.257±0.682	0.977±1.144	8.263±19.334

RESULTS

Expression of SNC73 was significantly down-regulated in CRC compared with non-cancerous colorectal mucosa from the same patient. Expression level of SNC73 in normal colorectal mucosa and colorectal cancerous tissues was 1.234±0.842 and 0.737±0.731, respectively ($P<0.01$), with the mean ratio between them of 7.134±14.092 (range, 0.36-59.54). Among 31cases of CRC, cancerous tissues of 24 cases (77.4 %) expressed lower level SNC73 as compared with non-cancerous colorectal mucosa from the same patient. Study on the expression of SNC73 in other kinds of carcinomas revealed that no differential expression of SNC73 was found in gastric cancer, breast cancer, lung cancer and liver cancer as compared with non-cancerous tissues from the same patient ($P>0.05$, Table1).

Further analysis on the relationship between SNC73 expression level and various clinicopathological factors of CRC patients revealed that no correlation was found between SNC73 expression level and various clinicopathological factors, including sex, age, site, grade of differentiation, depth of invasion and metastases of CRC patients ($P>0.05$, Table 2).

DISCUSSION

RT-PCR-ELISA allows convenient and sensitive detection of PCR products. The sensitivity of the PCR ELISA is in general about one hundredfold higher than conventional analysis of the PCR products in ethidium bromide stained agarose gels[18-20]. The high sensitivity makes detection of low or unknown expression level of genes closer to the real status. The whole process of RT-PCR-ELISA takes no more than ten hours, and a group of samples can be detected at the same time. These are the reasons why this method was used to get a relative estimates about expression levels of SNC73 in different human carcinomas in this study.

SNC73 is one of the 46 cDNA clones of CRC negative-associated cDNA libraries constructed by Cancer Institute of Zhejiang University by subtractive hybridization technique. Sequence analysis revealed that full-length cDNA of SNC73 is 1651bp. Open reading frame analysis showed SNC73 encodes one immunoglobulin (Ig) heavy chain molecule with 384 amino acids, with its constant region identical to that of IgA1. The predicted structure of SNC73 had no apparent difference from other matured IgA molecules that serve in the immune system. Northern Blot, RT-PCR, in situ hybridization and in situ PCR confirmed expression of SNC73 in normal epithelial cells of colorectal mucosa and several non-hematopoietic cancer cell strains[17]. There are several reports about Ig and Ig-like genes expressed in non-hematopoietic cell lines[21-30], and Ig gene rearrangement was confirmed in the epithelial malignant cells[24]. These findings raise the possibility that immunoglobulin genes, whose expression is generally considered to be restricted to lymphocytes-origin cells, can be expressed by non-lymphoid cells. Maybe some factors during de-differentiation of cells activated the rearrangement of Ig genes in malignant cells, but it remains to be explored how Ig genes undergo rearrangement in epithelial cells.

Expression level of SNC73 in CRC and non-cancerous mucosa had been determined by the method of RT-PCR-imaging, Northern-blot and in situ hybridization. The present study confirmed the initial findings that CRC expresses lower level SNC73 than non-cancerous mucosa[17,30]. Expression level of SNC73 in other human cancerous tissuess and non-cancerous tissues was also determined. No significantly different expression was found in gastric cancer, lung cancer, breast cancer and liver cancer compared with non-cancerous tissues from the same patient. This is the first semi-quantitative analysis about SNC73 expression in different human cancers. In this study the number of cases of breast cancer, lung cancer and liver cancer was small, and more cases are needed to draw a conclusion. Interestingly, beyond our expectation, gastric cancer, which also originates from gastrointestinal tract, expressed no significantly different level of SNC73 compared with normal gastric mucosa. Conclusion drawn from the results of this study suggests that down-regulation of SNC73 expression may be a relatively specific phenomenon in CRC, and SNC73 may serve as a potential genetic marker for carcinogenesis of CRC. Further study on expression of SNC73 in tissues of different pathological status, such as adenoma, dysplastic mucosa and para-cancerous tissues, may provide clearer conclusion[31].

The relation between SNC73 and carcinogenesis of CRC remains to be explored. Carcinogenesis of tumor is closely related to immunological surveillance of host. Previous study proved the significance of immune responses to mucosal carcinogens in carcinogenesis[32]. Recently, Ig molecules or Ig-like proteins have been reported to influence the development of tumor directly or through interaction with oncogenes or tumor suppressor genes[24,33,34]. So the relationship between Ig and carcinogenesis of tumor should be further explored besides the conventional hormonal immunity. These clues implicate some role of SNC73 in carcinogenesis of CRC. SNC73 encoded protein can be assumed to be an immune molecule secreted by normal epithelial cells, together with Ig secreted by plasma cells, participating in local anti-tumor activity. The protein affects carcinogenesis of CRC through the similar influence on adhesion or signal transduction to other Ig superfamily components or interaction with some oncogenes or tumor suppressor genes is also possible. Further study on the mechanisms of down-regulation of SNC73 in CRC and the biological function of SNC73 will provide important clues for determining its role in screening, diagnosis, prognosis and therapy of CRC. The relationship of SNC73 expression and carcinogenesis of colorectal cancer merits further study.

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