

• COLORECTAL CANCER •

Expression of ST13 in colorectal cancer and adjacent normal tissues

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Abstract

AIM: To investigate the *in situ* expression of suppression of tumorigenicity 13 (ST13) mRNA in both colorectal cancer and adjacent normal tissues.

METHODS: Colorectal cancer cell lines SW1116, SW620 and CoLo205 were enrolled to confirm the feasibility of the *in situ* hybridization procedure. Seven colorectal cancer and adjacent normal tissues were included for RNA-RNA *in situ* hybridization.

RESULTS: The expression of ST13 in the seven normal colon tissues was positive and the positive signals appeared in mucosal cells. Only three of the seven colorectal cancer tissues had positive hybridization signals that appeared in adenocarcinoma cells.

CONCLUSION: The expression of ST13 decreases in colorectal cancer tissue compared with that in adjacent normal tissue. ST13 is mostly expressed in colorectal epithelia and adenocarcinoma cells.

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Key words: Colorectal Cancer; Tumorigenicity 13; Gene expression; *In situ* Hybridizations

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INTRODUCTION

Colorectal cancer is a common cancer in China and one of the most rapidly increasing cancers in coastal areas of eastern China^[1-4]. To investigate the factors that are related to colorectal carcinogenesis at the molecular level^[5-7], subtractive hybridization between cDNA of normal mucosal tissues and mRNA of colorectal

carcinoma tissues was performed^[8-12]. Subsequent searching of normal mucosa cDNA libraries and sequence homology analysis with the GenBank database generated two cDNA clones that represented novel genes whose expression was down-regulated in human colorectal carcinoma. One of them is called suppression tumorigenicity 13 (ST13, also called SNC6, HUS17714)^[13,14]. Its cDNA is 3 121 bp and the gene is located on human chromosome 22 as identified by fluorescence *in situ* hybridization combined with fluorescence R-banding technique^[15]. Its protein consists of 271 amino acids^[16,17]. Some studies demonstrated that ST13 expression was significantly lower in colorectal cancer than in adjacent normal colon mucosa^[18-21]. However, to the author's knowledge, which kinds of tissues and cells would mostly express ST13 has not been known. To answer this question, we investigated the expression pattern of ST13 in colorectal cancer and adjacent normal tissue by *in situ* hybridization.

MATERIALS AND METHODS

Cell lines and tissue specimens

Human colorectal carcinoma cell lines SW1116, SW620, and CoLo205 were bestowed from Cancer Institute, Zhejiang University. All cell lines were confirmed expressing ST13 by Northern blot. Approximately, 5×10^6 cell suspension was washed with 0.01 mol/L PBS, and smeared by cytospin-2, the smears were fixed with 4% formaldehyde for 10 min. Seven colorectal cancer and adjacent normal tissue specimens were collected after radical excision in Department of Surgical Oncology, No.2 Hospital, Medical College, Zhejiang University. All patients between 33 and 74 years old had been diagnosed pathologically. Among them, four cases were males. The specimens were minced into 0.5 cm×0.5 cm in size and fixed with 4% polyformaldehyde immediately after resection, then embedded in paraffin, cut into 6 μ m thick sections. And baked at 25 °C overnight, deparaffinized twice with xylene for 30 min, and soaked in 100%, 95%, and 75% alcohol for 5 min respectively, finally washed 3 times with 0.01 mol/L PBS (pH 7.5, DEPC water).

Preparation of primers and probes^[22]

The sequences of ST13 primers were 5'-ACT GCA TTT GAG CTT GTG TG-3' (antisense strand) and 5'-AGAGGAATT TTA CTTTCA CCCACT-3' (sense strand), and the final amplification product was 182 bp. The sequences of β -actin were 5'-TCG ACAACGGCTCCG GCA-3' (AP1) and 5'-CGTACATCGCTG GGG TGT-5' (AP2), and the final amplification product was 370 bp. PCR amplifications of ST13 and β -actin cDNA were performed using above primers (PE GeneAmp PCR System 9 600, perkin elmer). Then the PCR products were cloned to pGEM-Teasy vector (Promega Co, with SP6/T7 promoter). Sequencing analysis showed Sp6 promoter drove transcription of antisense cRNA of ST13 (positive probe) and T7 promoter drove transcription of sense cRNA of ST13 (negative probe) (DNA Sequence Analysis, perkin elmer). Then labeling probes were prepared using the plasmids amplified according to manufacturer's instructions of RNA probe marker kit (Promega Co).

Detection of expression of ST13 in colorectal carcinoma cell lines

CoLo205, SW1116 and SW620 cell smears prepared as described above were fixed with 4% formaldehyde respectively and washed twice with 0.01 mol/L PBS (pH 7.5) for 5 min and 0.2 mol/L HCl for 15 min, respectively. Then the smears were digested with 40 μ L 25 g/mL protease K (without DNase and RNase A, Shanghai Biochemical Technology Company) at 37 °C for 15 min and washed with 0.01 mol/L PBS (pH 7.5) each for 5 min. Before hybridization, the smears were prehybridized at 40 °C for 90 min in 60 μ L prehybridization solution (200 μ L of 20 \times SSC, 500 μ L of 100% formamide, 20 μ L of 50 \times Denhardt, 50 μ L 100 mg/mL ssDNA, and 230 μ L ddH₂O). The hybridization solution consisted of 200 μ L of 20 \times SSC, 100% deionized formamide, 100 μ L of 50 \times dextran sulfate and 130 μ L ddH₂O. A total amount of 150 μ L hybridization solution mixed with 3 μ L probes was denatured at 68 °C for 10 min and laid on ice immediately. Then 50 μ L hybridization solution was added to each smear at 42 °C overnight. After hybridization each smear was washed successively twice with 20 \times SSC for 10 min, 10 \times SSC for 15 min at 37 °C, 10 \times SSC for 10 min and buffer I (1 mol/L Tris-HCl 50 mL+5 mol/L NaCl 15 mL+ddH₂O 435 mL, pH7.5) for 5 min. Then 40-50 μ L of block solution (buffer I 500 μ L+Triton x-100 2.5 μ L+NSS 5 μ L+Dig-Ab 1 μ L) was added on each smear for 30 min. After blocked, each smear was incubated with 40-50 μ L of digoxin antibody alkaline phosphatase complex (buffer I 500 μ L+Triton x-100 2.5 μ L+NSS 5 μ L+Dig-Ab 1 μ L) for 2 h at room temperature was rinsed twice with buffer I each for 5 min and buffer III (1 mol/L Tris-HCl 25 mL+5 mol/L NaCl 5 mL+1 mol/L MgCl 12.5 mL+ddH₂O 260 mL pH9.5) twice for 5 min, then 40-50 μ L of color developing agent (buffer III 1 000 μ L+NBT 4.5 μ L+BCIP 3.5 μ L+levamisole 0.24 mg or buffer III 500 μ L+NBT and BCIP mixture 10 μ L) was added and incubated in a dark room for 72 h. Finally dehydration, clearance and mounting were performed. Northern blot was used to detect expression of ST13 mRNA in above cell lines as positive control. Sense probe and omitted probe were used as negative controls. In our results, hybridization signal appeared as deep-blue granules in cells.

Detection of expression of ST13 in colorectal cancer and adjacent normal tissues

The methods were the same as the procedure described above in cell smears. RNA probe of β -actin was used as positive control. Sense probe and omitted probe were used as negative control.

RESULTS**The Expression of ST13 in colorectal cancer cell lines**

Hybridization signals were seen in all cell line smears, but not in the negative control groups (Table 1).

Table 1 *In situ* expression of ST13 in colon cancer cell lines

Cell lines	Sample number	Antisense probe	Control group	Sense probe
SW1116	6	6 ¹	6 ²	6 ²
SW620	3	3 ¹	3 ²	3 ²
Colo205	2	2 ¹	2 ²	2 ²

¹Positive hybridization signal; ²Negative hybridization signal.

Expression of ST13 in colorectal cancer and adjacent normal tissue sections

Hybridization signals were seen in all specimens of colorectal cancer and normal tissues by using β -actin probe (Table 2), demonstrating that the RNA was well protected in specimens. Hybridization signals could be found in all 7 normal tissues, but only in 3 of 7 colorectal cancer tissues by using ST13 probe (Table 2). These signals appeared mostly in epithelial cells in normal tissues and adenocarcinoma cells in colorectal cancer tissue (Figures 1B, C, 2A, B), but not in negative control groups (Table 2, Figures 1D and 2D), indicating that the results were reliable.

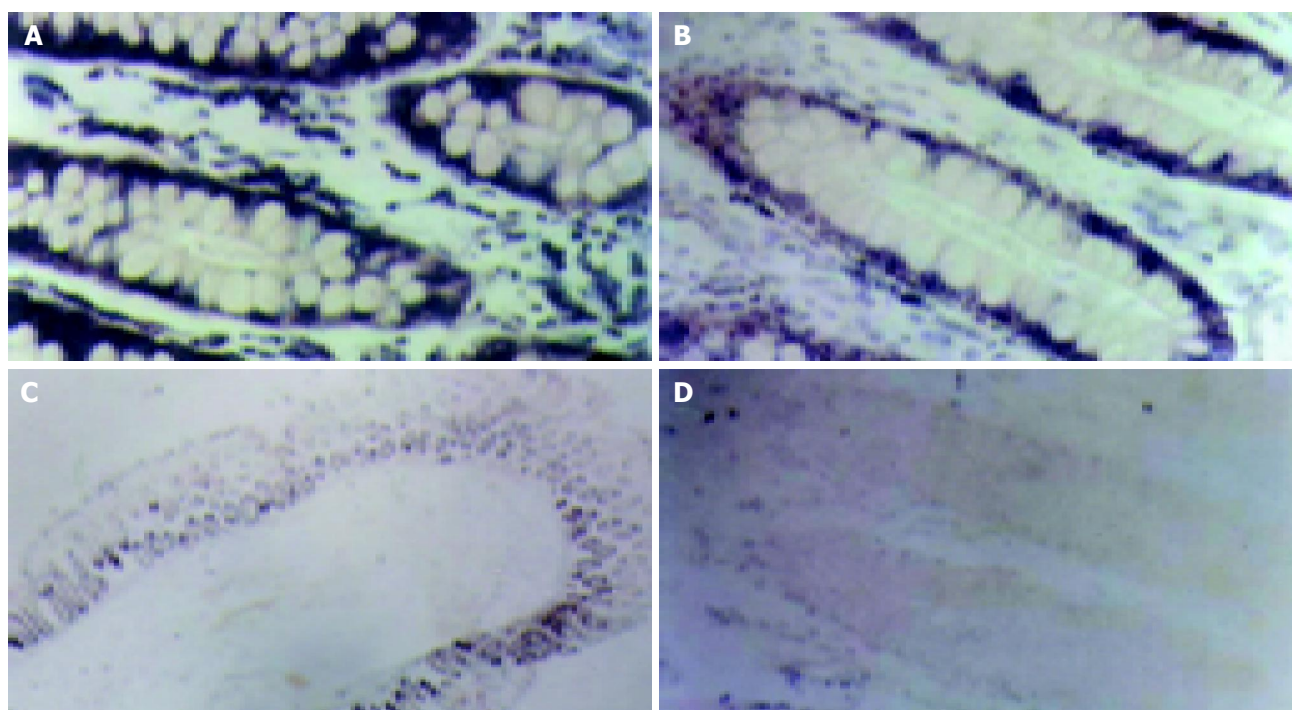


Figure 1 Expression of ST13 in normal mucosa. A: hybridization signal appeared as deep-blue granules in epithelial cells using β -actin probe as positive control; B and C: hybridization signals were seen in normal tissues using ST13 antisense probe, they were located in epithelial cells; and D: hybridization signal was not seen in negative control.

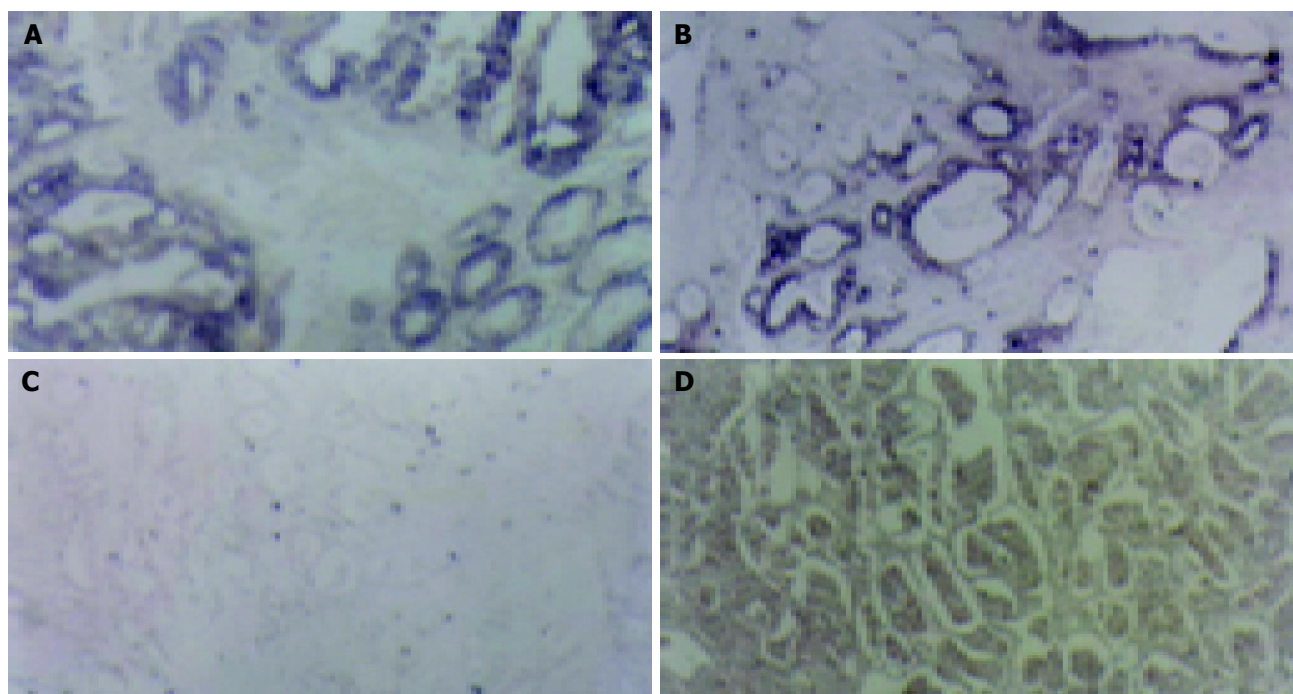


Figure 2 Expression of ST13 in colorectal cancer tissue. A and B: hybridization signals were seen in carcinoma cells using antisense probe of ST13; C: hybridization signals could not be seen using antisense probe of ST13; and D: hybridization signals could not be seen in negative control.

Table 2 *In situ* expression of ST13 in both colon cancer tissue and adjacent normal tissue

Specimens	Sample number	Results			
		β -actin probe	Antisense probe	Control group	Sense probe
Normal tissue	7	7 ¹	7 ¹	7 ²	7 ²
Cancer tissue	7	7 ¹	3 ¹ /4 ²	7 ²	7 ²

¹Positive hybridization signal; ²Negative hybridization signal.

DISCUSSION

ST13 is a novel gene related to colorectal carcinogenesis. Recent studies have demonstrated that ST13 is located on 22q¹³ with a length of 3 121 bp by cDNA sequencing and 271 amino acids in protein coding area^[13-16]. Several studies have indicated that expression of ST13 mRNA is lower in colorectal cancer tissue than in adjacent normal colorectal tissue^[18-21]. However, the precise expression pattern of ST13 in tissues and cells is unclear.

In situ hybridization developed in late 1960, has been confirmed as a stable and reliable technique for novel gene expression research^[23-30]. In this study, the sense and antisense RNA probes of ST13 were designed with 182 bp in length, which was reasonable for RNA-RNA hybridization. In order to develop a stable and effective *in situ* hybridization technique to avoid false positive and false negative results, we designed omitted probe and sense probe for negative controls. Since the negative results could be caused by the low expression of ST13 or by the destruction of RNA in the process of preparation for paraffin sections, we used β -actin RNA probe for positive controls. We applied *in situ* hybridization technique in human colorectal cancer cell lines SW1116, SW620 and COLO205 known to express ST13. Our study revealed that the hybridization signals could be seen in positive controls but not in negative controls

(Table 1) demonstrating that *in situ* hybridization technique in our study was creditable.

Our study showed that all specimens of normal tissue had strong hybridization signals in mucosal epithelia, while only three of seven colorectal cancer specimens had hybridization signals in adenocarcinoma cells (Table 2) suggesting the low expression of ST13 in colorectal carcinoma cells. This is in agreement with the previous studies in which the expression of ST13 was detected in colorectal cancer and adjacent normal tissue by Northern blot^[18-20]. Our study demonstrated that ST13 was expressed mainly in normal colon epithelia and in colorectal adenocarcinoma cells, indicating the different locations of the ST13 expression in different tissues. However, further studies are needed to explore the different intensities of ST13 expression in these different tissues.

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