ISSN 1009 - 3095 *Journal of Zhejiang University SCIENCE* V.3, No.1, P.100 - 106, Jan. - Mar., 2002
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Genomic structure analysis of SNC6, a progesterone-receptor associated protein gene, and cloning and characterization of its 5'-flanking region*

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Received July 18, 2001; revision accepted Sept.18, 2001

Abstract: Objective: To analyze the genomic structure of SNC6, a progesterone-receptor associated protein gene and its regulatory elements in its 5'-flanking region. Methods: Genomic sequence from GenBank database (accession number: Z98048) covering the whole SNC6 gene was used to analyze the genomic structure of SNC6 and design primers for PCR amplification of its 5'-flanking region. A 1894 bp fragment of the 5'-flanking region (−1814 to +75) was cloned by PCR using genomic DNA from a healthy donor peripheral blood lymphocyte as template. This fragment, as well as 3 shorter derivative fragments (1423 bp, 632 bp and 416 bp, which correspond to −1344 to +75, −552 to +75 and −337 to +75 respectively), were subcloned into pCL2 series luciferase reporter vectors. These constructs were introduced into colorectal cancer cell line SW620 for transient expression of reporter gene and luciferase activities were measured. Results: The genomic structure analysis showed there are 12 exons for SNC6 gene, which spans 32017 bp (nt71529 to nt39513 in Z98048 sequence). All transfected SW620 cells with the above 5-flanking region-containing constructs showed luciferase activities. The highest luciferase activities were measured in transfected cells with vectors containing 1894 bp fragments, and the lowest luciferase activities were measured in transfected cells with vectors containing 416 bp fragments. Luciferase activities were higher in transfected cells with vectors containing 632 bp fragments than that in transfected cells with vectors containing 1423 bp fragments. Conclusion: The basic transcription-promoting element (promoter) for SNC6 expression resides between 0 to −337, and two transcription-enhancing elements (enhancer) resides between −337 to −552 and −1344 to −1814, whereas one transcription-inhibiting element (silencer) exists between −552 to −1344.

Key words: SNC6 gene, genomic structure, 5'-flanking region, promoter, luciferase assay
Document code: A　　**CLC number:** Q751; Q812

INTRODUCTION

The development of cancer always exhibits up-regulated and down-regulated expression of certain genes associated with cell growth, division and differentiation. Whether these changes are the causes or results of malignant transformation, the more we identify them, the better we understand the mechanism. They also provide us more diagnostic or prognostic markers of cancer. There are many ways to identify genes differentially expressed in normal tissues and their malignant counterparts, such as subtractive hybridization and its modifications (Sagerstrom et al., 1997), mRNA differential display (DD-RT-PCR) (Liang et al., 1997), serial analysis of gene expression (SAGE) (Velculescu et al., 1995), as well as the more recently widely-applied microarray (DeRisi et al., 1996) and DNA chip (Kurian et al., 1999) technologies.

SNC6 is one of the novel human genes we cloned when performing subtractive hybridization

* Project supported by grants from National 863 High-Technology Project of China (No.102-10-01-07), National 9-5 Key Project (No.969090121) and National Natural Science Foundation of China (Nos.39570785, 39770818).

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between normal human mucosal cDNA and colorectal cancer mRNA(Zheng et al., 1997). Full-length cDNA of SNC6 was obtained(Geng et al., 1999) and the sequence data has been determined and deposited in GenBank (accession number U17714)(Cao et al., 1997). The 3145 bp-long cDNA encodes a progesterone receptor-associated molecular chaperon consisting of 369 amino acid residues (Prapapanich et al., 1996). In our previous work, we mapped this gene to human chromosome 22q13(Cai et al., 1997). Though not well defined, there was a tendency of down-regulated expression of this gene in some colorectal cancer specimens(Mo et al., 1996). While the expression profile of this gene needs further elucidation, we set to study its regulation elements. Due to the rapid progress of the human genome project (HGP) and public release of genomic sequence data on human chromosome 22, we were able to analyze the genomic structure of SNC6. A PAC clone sequence, deposited in the GenBank under accession number Z98048, covers the whole SNC6 gene. Here we report our recent work on analysis of the SNC6 genomic structure and characterization of its 5'-flanking regulatory region.

MATERIALS AND METHODS

1. Materials

E. coli strain XL1-Blue (Stratagene, USA) was routinely maintained on tetracycline-containing LB plate. All culture mediums, restriction enzymes and other enzymes were purchased from Life Technologies Inc., USA.

2. Analysis of genomic structure of SNC6 and cloning of its 5'-flanking region

The full-length SNC6 cDNA sequence was used to search the homologue sequence in a PAC clone (408N23) on human chromosome 22q13 (GenBank accession number Z98048) using NCBI online Blast program (http://www.ncbi.nlm.nih.gov/BLAST/). Genomic context of SNC6 was analyzed, and primers were designed for PCR amplification of the 5'-flanking region: 5'-GGT ACC TTT AGT CAG GCT GGG GAG-3' (upstream), 5'-CCT CGA GGT AGG GAG GTG GTG GGC GA-3' (downstream). Genomic DNA from a healthy donor's peripheral blood lymphocytes was extracted and used as template in PCR reaction. The 1894 bp PCR product was cloned into pGEM-T-Easy vector (Promega, USA) and cut with restriction endonucleases Kpn I, Xho I, EcoR I, Mlu I and Sac I to generate different-sized fragments (see Results for details). All these fragments were subcloned into luciferase reporter vectors pGL2-Basic, pGL2-Promoter and pGL2-Enhancer (Promega, USA).

3. Transfection

Colorectal cancer cell line SW620 (from ATCC) was maintained in RPMI1640 (100 U/ml penicillin, 100 μg/ml streptomycin) supplemented with 10% fetal calf serum. Cells were seeded (at density of 2×10^5 cells/well) into a 24-well plate the day before transfection. On the day of transfection, 2 μg of each plasmid DNA was diluted with RPMI1640 (serum-free, antibiotics-free) to a final volume of 60 μl in a 1.5 ml eppendorf tube; then 5 μl of SuperFect Transfection Reagent (QIAGEN Inc, USA) was added to and mixed thoroughly with the solution. After 7 minutes incubation at room temperature, 350 μl RPMI1640 (with serum and antibiotics) was added to and mixed well with the solution before it was transferred to each well of PBS-pre-washed SW620 cells in a 24-well plate (each transfection was done in a triplicate). After 3 hours incubation at 37 ℃ and 5% CO_2, 500 μl RPMI1640 with serum and antibiotics was added to each well and was further incubated till harvest.

4. Luciferase assay

Transfected cells were harvested for luciferase assay 48 hours after transfection using Luciferase Assay System (Promega, USA) according to the manufacturer's instructions. Briefly, cells were washed twice with ice-cold PBS, then 50 μl 1X Cell Culture Lysis Reagent was added to each well. The cell lysate was transferred to a 1.5 ml Eppendorf tube and centrifuged at top speed for 30 seconds. The supernatant was collected and stored at −70 ℃. Upon luciferase activity assay, 20 μl supernatant was rapidly mixed with 100 μl Luciferase Assay Reagent (both room temperature pre-equilibrated) in a 96 well plate and read with a VICTOR™ multilabel counter (Wallac Oy, Finland).

RESULTS

1. Analysis of the genomic structure of SNC6 and cloning of its 5'-flanking region

Sequence analysis of SNC6 full-length cDNA against PAC clone 408N23 (GenBank accession number Z98048) revealed 12 exons for SNC6 gene which cover 32017 bp in Z98048 sequence (nt71529 to nt39513), as shown in Table 1 and Fig.1.

Table 1 Distribution of exons and introns of ST13 Gene

	Length(bp)	Position in Z98048
exon	184	71529 – 71345
intron	5550	71344 – 65795
exon	58	65794 – 65737
intron	2453	65736 – 63284
exon	76	63283 – 63208
intron	3384	63207 – 59824
exon	71	59823 – 59753
intron	4146	59752 – 55607
exon	67	55606 – 55540
intron	4738	55539 – 50802
exon	85	50801 – 50717
intron	131	50716 – 50585
exon	111	50584 – 50474
intron	2888	50473 – 47586
exon	103	47585 – 47483
intron	1611	47482 – 45871
exon	117	45870 – 45754
intron	1164	45753 – 44589
exon	49	44588 – 44540
intron	2396	44539 – 42144
exon	134	42143 – 42010
intron	429	42009 – 41581
exon	2072	41580 – 39513

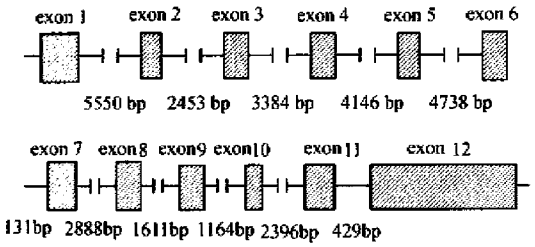

Fig.1 Genomic context of SNC6

The 5'-flanking region (+75 to −1814) was obtained by PCR. This 1894 bp DNA fragment was cut with Kpn I, Xho I, EcoR I, Mlu I and Sac I to generate a series of fragments as shown in Fig.2. These different-sized fragments were subcloned into pGL2 luciferase reporter vectors(Fig.3), and used for transfection in the next step analysis.

2. Luciferase assay

All transfected SW620 cells with the above 5-flanking region-containing constructs showed luciferase activities. As shown in Table 2 and Fig. 4, the highest luciferase activities were measured in transfected cells with all three pGL2 vectors containing 1894 bp fragments, and the lowest luciferase activities were measured in transfected cells with all pGL2 vectors containing 416 bp fragments. Luciferase activities are higher in transfected cells with vectors containing 632 bp fragments than those in transfected cells with vectors containing 1423 bp fragments. These data suggest that, the basic transcription-promoting element (promoter) for SNC6 expression resides between 0 to −337, and that two transcription-enhancing elements (enhancer) resides between −337 to −552 and −1344 to −1814, whereas one transcription-inhibiting element (silencer) exists between −552 to −1344.

Table 2 Luciferase activity of SW620 cell extracts in different transfections

	Luciferase activity (counts per second)
Blank	215.3 ± 2.8
pGL2-Control	1304.1 ± 283.9
pGL2-Basic(1.9)	24971.1 ± 2353.3
pGL2-Basic(1.4)	8167.4 ± 415.6
pGL2-Basic(0.6)	18685.3 ± 936.9
pGL2-Basic(0.4)	2324.8 ± 717.0
pGL2-Promoter(1.9)	9515.9 ± 2936.0
pGL2-Promoter(1.4)	5784.4 ± 714.2
pGL2-Promoter(0.6)	8041.6 ± 1082.4
pGL2-Promoter(0.4)	1473.1 ± 231.0
pGL2-Enhancer(1.9)	9925.3 ± 2729.0
pGL2-Enhancer(1.4)	4968.3 ± 528.1
pGL2-Enhancer(0.6)	5736.9 ± 1421.6
pGL2-Enhancer(0.4)	2122.9 ± 282.2

-1814

GGCTCCTTTTA GTCAGGCTGG GGAGAGCCTT TCAAATAAAA GCAAATTCCA GGCCTGGGGC
GGTGGCTCAG AGAGGAGGGT GGATCGCTTG AGGCCAGGAG TTCGAGATCA GCTTGGCCAA
CGTGGCAAAA CCTGGTTTCT ACTACAATTA CAAAAATTAG CCAGGCGTGG TGGCACACGC
CTATAATCCC AGCTACCTCG GGAGGCTGAG GCACGAGAAT TGCTTGAACT CAGGAGGCAG
AGGTTGCAAA TGAGCAGAGA TTGCCACTGC ACTCCAGTCT GAGCAACAGA ATGAGAGCCT
GTCTCAAAAA AAGACTGAAA AATTGCACAT CTTTCTTTTC ACCTAATCCC ACGGAGAGTT
GTCTTTCTGC AGCTTTACTA TGAGAACTGA AGAACAAGTT CTGTGCACTG GACAATAACT

EcoRI

TTTTATTTTT TATAGTAAGA AGTCGATGTA ATTAACATTC CATTTTAAAT GAATTCAAGT
ATATCAACAT AAACATATGC CAAGACATAG CTTTGATGTC AAATACCATA CGTTCTGGTT
CACACATTGT TCATATATTG ACCCAAAAAA CTGAAATACG ATCCAATGGC AGATCCGCCT
GCAAGGAAGG TGATTCAGTT TTCACTGATT AAGCTTTATG TTCATCAGAT TAATTGCTCG
CAATAGAGGA CACCCTACAA AAAATTATTC TCCTTATCCT TCGTCTTGTC TTTGCAAACA
GGACTTTCAG ACACAGAACG CCCCAGAAGT ATTTCGAAAC AGTTACCTCT CCTAAGAGGA
GCTGCTGAAA GAATTTGTTA CTAAACATGC TTCGTGTATA TTCTGGAGCA AGCTCTCACG
CCTAACAGAA ACAAGAGCTA CTTGATGTGA GACACTTTGT CACTTAAGTG TGATAATTGC
CACAACTTCG GAGTCCGAGT AACTTGGAAG ACAGCCCCTC TGGGGGATGG CACACTTTTC
TCTCTGCAGT CATTTCGGCT TCACAAAATA CCTCAACGGT AGAGAACACA CTGGGCCCAC
CCCTTTCCAG GAGCAGCCGG TATAGAAGAA ATCCGCGGAG CACAGAGGGT TGGGGAGCGG
AGGCCCCACC AGGAGAAGGG TCAGCCGAAC CAGGGATCGC CTGAGACCAA CTTGGGTCCC
TCCAGGGGAC ACCATGGGAG GAGACCGTGG GAGAAGAGTC TAACCTGAGA GGCCGCGGAC
GTTTGCTACA GCGGGAACCA GCTTGGGGGC TGAGAGCAGC CAAGGCATTA CGGCCTAACT

MluI

CACGCGTCGG GAAGAGAAAG AGGGTAACGA CGGGGAACGC AACCGCCTGC AGGGCCGCGT
CGCCAGGAAA CCGGGCCAGC CGCACCTCCC CTTCCGGCGG AGGCGGCTCG CGGGCCTTCC
TTGCGCGGCG GCGCAGGTTG TGACGTCATG CCCCGAGCTG GGACCGACCC TCCGGCTCCA

SacI

CGCCCTTTTT TTTTTTTTTT TTTAGGTCCC GGGCGTTGAG CTCTACTCAG TGCACGCAGC
CACACAATAT GCTCTTGGAC GTCGCCCCCT GGAGGGGATG TTATCTCCCG CCTCCTTGAA
GTCGCCCATT TCTGGGGTTG CCCCATCCAG GGAGCTGGTT CCTAGTTAAG AATATGAGAA
TGTATGCGCA GGGAGGCAGG CAACAGCACG AACAGCCACG CTTCTAGAAG ATTCTAGGGA
GCGCGCAGGA GCAGCGCAGA GGGAGTAGGA ATGAGCAGGC GGAGGACCCG AGGTCACGAG
ACCGTTGGGT GGGAGGAGCC AGCGGCCGGG GAGGTTCTAG TCTGTTCTGT CTTGCGGCAG

-1

CCGCCCCCTT CTGCGCCGGTC ACGCCGAGCC AGCGCCTGGG CCTGGAACCG GGCCGTAGCC

-75

CCCCCAGTTT CGCCCACCAC CTCCCTACCA TGGA

Fig.2A Nucleotide sequence of 5'-flanking region of SNC6

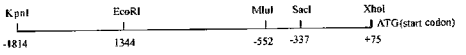

Fig.2B Illustration of cloning of 5'-flanking region of SNC6

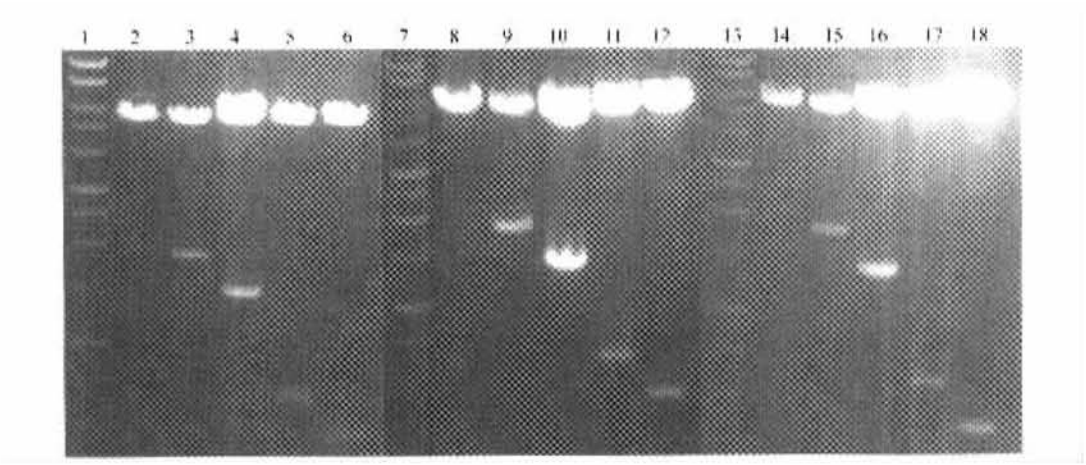

Fig.3 Construction of luciferase reporter vectors with SNC6 5'-flanking region

1, 7, 13: ϕX174/HaeIII marker; 2: pGL2-Basic vector; 3: pGL2-Basic-1894/KpnI + XhoI; 4: pGL2-Basic-1423/KpnI + XhoI (via subclone); 5: pGL2-Basic-632/MluI + XhoI; 6: pGL2-Basic-416/SacI + XhoI; 8: pGL2-Enhancer vector; 9: pGL2-Enhancer-1894/KpnI + XhoI; 10: pGL2-Enhancer-1423/ KpnI + XhoI; 11: pGL2-Enhancer-632/MluI + XhoI; 12: pGL2-Enhancer-416/SacI + XhoI; 14: pGL2-Promoter vector; 15: pGL2-Promoter-1894/KpnI + XhoI; 16: pGL2-Promoter-1423/KpnI + XhoI; 17: pGL2-Promoter-632/MluI + XhoI; 18: pGL2-Promoter-416/SacI + XhoI.

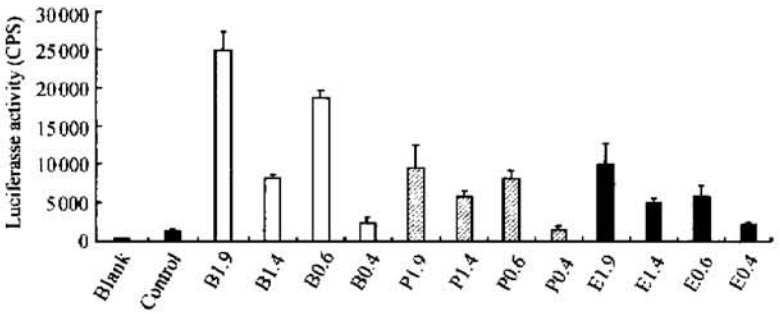

Fig.4 Luciferase assay for different 5'-flanking region of SNC6 gene

Blank: vector-free transfection; Control: pGL2-Control vector transfection; B 1.9: pGL2-Basic-1894/KpnI + XhoI; B 1.4: pGL2-Basic-1423; B 0.6: pGL2-Basic-632; B 0.4: pGL2-Basic-416; P 1.9: pGL2-Promoter-1894; P 1.4: pGL2-Promoter-1423; P 0.6: pGL2-Promoter-632; P 0.4: pGL2-Promoter-416; E 1.9: pGL2-Enhancer-1894; E 1.4: pGL2-Enhancer-1423; E 0.6: pGL2-Enhancer-632; E 0.4: pGL2-Enhancer-416

DISCUSSION

SNC6 encodes an hsp70-interacting molecular chaperon, involved in progesterone-receptor complex assembly. Northern blot showed that this gene is expressed in a wide variety of normal human tissues including heart, skeletal muscle, pancreas, ovary, small intestine, colon, brain, placenta, lung, liver, kidney, spleen, thymus, prostate, testis and peripheral blood lymphocytes, and that there are 4 distinct alternately spliced transcripts (Zheng et al., 1997). In situ hybridization and immunohistochemical staining showed that SNC6 is expressed abundantly in colorectal epithelial cells (paper in preparation). Results from current study showed that the 5'-flanking region of SNC6 gene can strongly promote downstream gene expression; and the efficiency is even higher than that of the control

SV40 promoter (which is widely used in universal mammalian expression vectors) in the specific cell line SW620. Preliminary work which transfected pGL2-basic/1.9 vector into another 2 cell lines (breast cancer cell Bcap37 and colorectal cancer cell SW480) also showed similar high promoting efficiency (data not shown). This may suggest the potential use of this 5'-flanking region or part of it as a strong promoter in some tissue-specific expression vector constructions.

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