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SQUAMOUS CELL CARCINOMA ANTIGEN IS A NEW MEMBER OF THE SERINE PROTEASE INHIBITORS

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SUMMARY: We have cloned cDNA of squamous cell carcinoma antigen. Sequence analysis of the complete 1711 basepairs SCC antigen cDNA revealed that it coded 390 amino acids and no typical signal sequence in the NH_2-terminus. Northern blot analysis of human squamous cell poly $(A)^+$ RNA using this cDNA insert as the probe showed a single RNA species of about 1.7 kilobases. The cDNA was expressed in *Escherichia coli* and the product was detected by immunological methods using antibodies against SCC antigen, indicating that this cDNA encodes the entire SCC antigen sequence. The amino acid homology search revealed that SCC antigen was a member of the serine protease inhibitors family.

Squamous cell carcinoma antigen (SCC antigen) is a tumor associated protein which was first isolated from squamous cell carcinoma tissue of the uterine cervix and reported as TA-4 in 1977 (1), and is widely used for the diagnosis and management of squamous cell carcinoma of various tissues (2-4). Although SCC antigen is not tumor specific, the release of SCC antigen from the cell is apparently increased in squamous cell carcinoma and reflects the infiltrative growth and the degree of histologic differentiation of the tumor (5), suggesting that it may have important roles in the malignant behavior of the tumor cells. To investigate its function, it is essential to clarify the molecular nature of this protein.

In this report, we describe the cloning and characterization of cDNA of SCC antigen and its expression in *E.coli*. The amino acid homology search revealed that SCC antigen had a high

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Abbreviations: SCC antigen, squamous cell carcinoma antigen; *E.coli*, Escherichia coli; serpins, serine protease inhibitors; PCR, polymerase chain reaction; bp, basepairs; SDS, sodium dodecil sulfate; PBS, phosphate buffer-saline; CTL, cytotoxic T lymphocyte.

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homology with each member of the serine protease inhibitors (serpins) family (6). We will also discuss the implications of its homology with serpins.

MATERIALS AND METHODS

RNA Isolation Total RNA was isolated from SKGIIIa cell (7) and each tissue using RNA extraction kit (Amersham, UK). Poly $(A)^+$RNA was prepared with Oligotex-dT30 (Nippon Roche, Japan).

Construction of cDNA Library The SKGIIIa cell cDNA library in λgt10 was constructed using a cDNA synthesis and cloning system (Amersham, UK).

Determination of Partial Amino acid Sequence Starting with liver metastatic tissue of squamous cell carcinoma of the uterine cervix, purification of the SCC antigen was performed as described by Kato (1) with some modifications; use of Sephadex G-100 instead of G-200 and final addition of reverse phase chromatography. The protein, 95.4% pure, was digested with lysil endopeptidase and amino acid sequence was determined.

cDNA Cloning Two pairs of oligonucleotide primer pools were synthesized on the basis of two amino acid sequences. The first pair was derived from amino acid 248-253 (Fig. 3, sense primer, primer 1; 5'-CCNAAYCARATHGAYGG-3' and antisense primer, primer 2; 5'-CCRTCDATYTGRTTNGG-3') and the second pair was deduced from amino acid 307-312 (Fig. 3, sense primer, primer 3; 5'-ATHTTYAACGGNGAYGC-3' and antisense primer, primer 4; 5'-GCRTCNCCGTTRAADAT-3'). Polymerase chain reaction (PCR) was performed with primer 1 and 4, or primer 2 and 3, and double stranded cDNA of SKGIIIa cells using a Perkin Elmer Cetus thermal cycler and Taq polymerase (Gene Amp). After 30 cycles of PCR with 1 min of denaturation at 94°C, 2 min of annealing at 37°C and 3 min of extension at 72°C, the resulting fragments were purified, subcloned into M13mp18 and 19, and sequenced using T7 DNA polymerase (Sequenase, Stratagene, USA). The DNA fragment, which could encode the amino acid sequence identical with that of the lysil endopeptidase fragments, was labelled with [α-^{32}P]dCTP by the method of Feinberg and Vogelstein (8) to screen a SKGIIIa cell cDNA library. The cDNA obtained from a positive clone was used to synthesize primers corresponding to nucleotide 442-462 (primer 5, see Fig. 3) and 350-370 (primer 6, see Fig. 3) to which a *Xba*I site was added at the 5'-end. One additional primer was synthesized which corresponded to the sense strand 54 bp upstream (primer 7; 5'-GGGAGCTCGCTGGGTAGTCCCCACCTTT-3') from an *Eco*RI site of λgt10 vector containing a *Sac*I site for subcloning into M13 sequence vector. Using primer 7, cDNA from the library was amplified with primer 5 and re-amplified with primer 6. The resulting fragment was subcloned and sequenced.

Southern and Northern Blot Analyses Human lymphocytes DNA (10 μg) was digested and the fragments were subjected to electrophoresis and blotted on Hybond-N+ membrane (Amersham, UK). The RNA was resolved on to a 1% agarose gel containing 20% formaldehyde and blotted on Hybond-N+ membrane. Hybridization was performed as described (9). Washing was done at 65°C in 0.1 x SSC (Southern blot) or 0.2 x SSC (Northern blot) containing 0.1% SDS for 20 min. Filters were exposed for 1day at -80°C.

Construction of Expression Plasmid Starting with λSCC-1, an expression plasmid pKK-SCC-1 was constructed, in which the coding sequence for SCC antigen was placed after the *trc* promoter (10) on an expression vector pKK233-2 (Pharmacia, Sweden). pKK233-2 was digested with *Nco*I and λSCC-1 was partially digested with *Eco*RI. The resulting 1.6 kb DNA fragment from λSCC-1 was purified on an agarose gel. After repairing the cohesive ends with Klenow Fragment, both DNAs were ligated with T4 DNA ligase and the sequence was confirmed.

Immunoblot Analysis *E.coli* JMI09 containing pKK-SCC-1 was grown at 37°C in 5 ml of L-broth containing 50 mg/ml of ampicillin in the presence of 1mM isopropyl-β-D-thiogalactopyranoside for 9hrs and precipitated by centrifugation and resuspended in 0.2 ml of sample buffer (11) for SDS-polyacrylamide gel electrophoresis. SKGIIIa cells were washed and resuspended in PBS. After freezing and thawing 3 times, supernate was recovered by

centrifugation. For cervical cancer tissue and normal squamous epithelium, each tissue was homogenized in PBS using a Potter homogenizer and centrifuged. Supernates were precipitated with 10% trichloroacetic acid and the precipitates were rinsed in water saturated diethylether and dissolved in sample buffer. After boiling for 2 min and centrifugation, the supernates were subjected to electrophoresis. Proteins were transferred electrophoretically to Clearblot-P membrane (Atto, Japan) and subjected to immunoblot analysis with a monoclonal antibody for SCC antigen (Mab-13; ref. 12) by PAP method.

RESULTS AND DISCUSSION

Isolation and characterization of SCC antigen cDNA

To isolate SCC antigen cDNA, PCR was performed with primers designed from lysil endopeptidase fragments of SCC antigen (Fig. 1) and cDNA from SKGIIIa, a SCC antigen-productive cell line. Using primer 1 and primer 4, nine DNA fragments (about 190 bp -540 bp) were amplified, and these fragments were purified and sequenced. One of these DNA fragments, 194 bp, could encode the amino acid sequence identical with that of lysil endopeptidase fragments and was used to probe a cDNA library of SKGIIIa. Of 5 x 10^5 plaques screened, one clone (λSCC-1, Fig. 2) was isolated. As λSCC-1 lacked 5'-extreme end, the remainder portion of cDNA (pcSCC-2, Fig. 2) was isolated by one-side PCR amplification of cDNA library DNA, using the primers in the SCC antigen sequence (primer 5 or 6) and the primer in the vector sequence (primer 7). Sequence analysis revealed that λSCC-1 and pcSCC-2 overlapped completely and the complete 1711-bp SCC antigen cDNA consisted of a 61 nucleotide 5'-untranslated sequence region, 1170 nucleotides coding for 390 amino acids, a 3'-untranslated region of 462 nucleotides and a poly(A) sequence of 18 nucleotides (Fig. 3). All of the amino sequence of cDNA except fragment 13, although N-terminal five amino acids of fragment 13 corresponded to amino acid 113-117, and Gln in the fragment 21-1 which was replaced by Glu in the deduced amino acid sequence of cDNA.

Southern and Northern blot analyses

To estimate the number of the SCC antigen gene in human genome, genomic human DNA was analyzed by Southern blot using SCC antigen cDNA as the probe. A few fragments were identified in digestions of the restriction enzymes examined (Fig. 4*a*). Thus, we tentatively assumed that SCC antigen gene exists in a single copy in the human genome.

Fragment No:

10 Val, Leu, Glu, Ile, Pro, Tyr, Lys

13: Tyr, Leu, Phe, Leu, Gln or Phe, Tyr, Glu, Arg, Phe, Ser, Val, Pro

19: Gly, Met, Val, X , Ile, Phe, Asn, Gly, Asp, Ala, Asp, Leu, Ser, Gly, Met, Thr, Gly, Ser, Arg, Gly, Leu, Val, Leu

21-1: X ,Leu, Ser, Met, Ile, Val, Leu, Leu, Pro, Asn, Gln, Ile, Asp, Gly, Leu, Gln, Lys

21-2: X ,Met, Phe, X , Leu, Phe, Gln, Gln, Phe, Arg, Lys

FIG. 1. Amino acid sequence of lysil endopeptidase fragments. The underlines indicate the position of primers used for first round PCR.

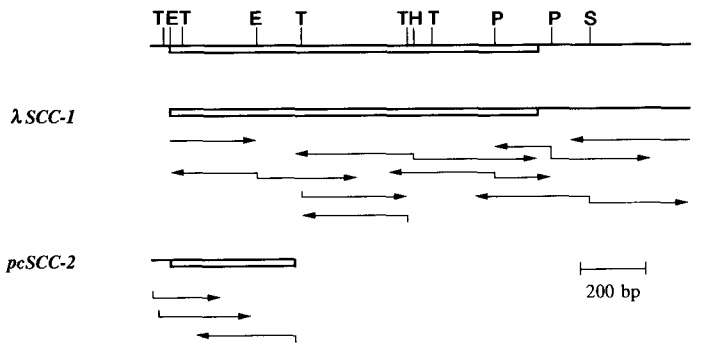

FIG. 2. Restriction map and sequence strategy. T: *Taq*I, E: *Eco*RI, H: *Hin*dIII, P: *Pst*I, S: *Sph*I. Arrows show the extent and direction of sequence determination. Box indicates the coding region.

In order to examine the length of SCC antigen mRNA, poly $(A)^+$ RNAs extracted from SKGIIIa cell and human tissues were subjected to Northern blot analysis using SCC antigen cDNA as the probe. As shown in Fig. 4*b*, a single band corresponding to about 1.7 kilobases, almost the same size as the isolated cDNA, was observed in SKGIIIa cell, cervical squamous cancer tissue and normal squamous epithelium, suggesting that the cDNA sequence described above was a full length cDNA. It is notable that the mRNA level in normal squamous epithelium was higher than in cervical squamous cancer tissue. This result is not consistent with that of the concentration and immunostaining analyses of SCC antigen in both tissues (13, 14), although there is one report that the concentration of SCC antigen correlate with our result (5). Thus, the possibility arises that the expression of SCC antigen in malignant cells is regulated at least in part at the post-transcriptional level.

Expression of SCC antigen cDNA in E.coli

The complete cDNA encoding SCC antigen was cloned into the expression vector pKK233-2 under the control of the *E.coli* promoter *trc* (pKK-SCC-1) and expressed in *E.coli*. The protein produced by transformed *E.coli* was detected by sandwich enzyme immuno assay system (IMx, DaiNabot, Japan; ref. 15) with monoclonal antibodies recognizing SCC peptide epitopes. Approximately 3 μg of recombinant SCC antigen was produced from 1 ml of *E.coli* culture (data not shown). The produced protein was also analyzed by Western blot analysis using a monoclonal antibody (Mab-13) for SCC antigen. The size of immunoblotted protein was 45KDa which was equivalent with the calculated molecular weight of the deduced amino acid sequence (M_r, 44532) (Fig. 5). Furthermore, the size of the recombinant protein was identical to either purified SCC antigen or SCC antigen found in extracts from SKGIIIa cells, cancer tissue and normal squamous epithelium. These findings indicate that there is no cleavable signal sequence as is expected from the deduced amino acid sequence and that the cDNA encodes the entire SCC antigen sequence. Although SCC antigen is largely secreted when squamous cells have malignant potential (1, 5), the mechanism of secretion is yet unknown. After the treatment of SCC antigen purified from the tissue with sialidase, it showed no change in its mobility on SDS-polyacrylamide gel electrophoresis (data not shown). These results also suggest that the content

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                                                                                CTCTCTGCCCACCTCTGCTTCCTCTAGGAACACAGGAGTTCCAGATCACATCGAGTTCACC   61

  1                                                 10                                                  20
Met Asn Ser Leu Ser Glu Ala Asn Thr Lys Phe Met Phe Asp Leu Phe Gln Gln Phe Arg
ATG AAT TCA CTC AGT GAA GCC AAC ACC AAG TTC ATG TTC GAC CTG TTC CAA CAG TTC AGA  121
                                    30                                          40
Lys Ser Lys Glu Asn Asn Ile Phe Tyr Ser Pro Ile Ser Ile Thr Ser Ala Leu Gly Met
AAA TCA AAA GAG AAC AAC ATC TTC TAT TCC CCT ATC AGC ATC ACA TCA GCA TTA GGG ATG  181
                                    50                                          60
Val Leu Leu Gly Ala Lys Asp Asn Thr Ala Gln Gln Ile Lys Lys Val Leu His Phe Asp
GTC CTC TTA GGA GCC AAA GAC AAC ACT GCA CAA CAG ATT AAG AAG GTT CTT CAC TTT GAT  241
                                    70                                          80
Gln Val Thr Glu Asn Thr Thr Gly Lys Ala Ala Thr Tyr His Val Asp Arg Ser Gly Asn
CAA GTC ACA GAG AAC ACC ACA GGA AAA GCT GCA ACA TAT CAT GTT GAT AGG TCA GGA AAT  301
                                    90                                         100
Val His His Gln Phe Gln Lys Leu Leu Thr Glu Phe Asn Lys Ser Thr Asp Ala Tyr Glu
GTT CAT CAC CAG TTT CAA AAG CTT CTG ACT GAA TTC AAC AAA TCC ACT GAT GCA TAT GAG  361
                                   110                                         120
Leu Lys Ile Ala Asn Lys Leu Phe Gly Glu Lys Thr Tyr Leu Phe Leu Gln Glu Tyr Leu
CTG AAG ATC GCC AAC AAG CTC TTC GGA GAA AAA ACG TAT CTA TTT TTA CAG GAA TAT TTA  421
                                   130                                         140
Asp Ala Ile Lys Lys Phe Tyr Gln Thr Ser Val Glu Ser Val Asp Phe Ala Asn Ala Pro
GAT GCC ATC AAG AAA TTT TAC CAG ACC AGT GTG GAA TCT GTT GAT TTT GCA AAT GCT CCA  481
                                   150                                         160
Glu Glu Ser Arg Lys Lys Ile Asn Ser Trp Val Glu Ser Gln Thr Asn Glu Lys Ile Lys
GAA GAA AGT CGA AAG AAG ATT AAC TCC TGG GTG GAA AGT CAA ACG AAT GAA AAA ATT AAA  541
                                   170                                         180
Asn Leu Ile Pro Glu Gly Asn Ile Gly Ser Asn Thr Thr Leu Val Leu Val Asn Ala Ile
AAC CTA ATT CCT GAA GGT AAT ATT GGC AGC AAT ACC ACA TTG GTT CTT GTG AAC GCA ATC  601
                                   190                                         200
Tyr Phe Lys Gly Gln Trp Glu Lys Lys Phe Asn Lys Glu Asp Thr Lys Glu Glu Lys Phe
TAT TTC AAA GGG CAG TGG GAG AAG AAA TTT AAT AAA GAA GAT ACT AAA GAG GAA AAA TTT  661
                                   210                                         220
Trp Pro Asn Lys Asn Thr Tyr Lys Ser Ile Gln Met Met Arg Gln Tyr Thr Ser Phe His
TGG CCA AAC AAG AAT ACA TAC AAG TCC ATA CAG ATG ATG AGG CAA TAC ACA TCT TTT CAT  721
                                   230                                         240
Phe Ala Ser Leu Glu Asp Val Gln Ala Lys Val Leu Glu Ile Pro Tyr Lys Gly Lys Asp
TTT GCC TCG CTG GAG GAT GTA CAG GCC AAG GTC CTG GAA ATA CCA TAC AAA GGC AAA GAT  781
                                   250                                         260
Leu Ser Met Ile Val Leu Leu Pro Asn Glu Ile Asp Gly Leu Gln Lys Leu Glu Glu Lys
CTA AGC ATG ATT GTG TTG CTG CCA AAT GAA ATC GAT GGT CTC CAG AAG CTT GAA GAG AAA  841
                                   270                                         280
Leu Thr Ala Glu Lys Leu Met Glu Trp Thr Ser Leu Gln Asn Met Arg Glu Thr Arg Val
CTC ACT GCT GAG AAA TTG ATG GAA TGG ACA AGT TTG CAG AAT ATG AGA GAG ACA CGT GTC  901
                                   290                                         300
Asp Leu His Leu Pro Arg Phe Lys Val Glu Glu Ser Tyr Asp Leu Lys Asp Thr Leu Arg
GAT TTA CAC TTA CCT CGG TTC AAA GTG GAA GAG AGC TAT GAC CTC AAG GAC ACG TTG AGA  961
                                   310                                         320
Thr Met Gly Met Val Asp Ile Phe Asn Gly Asp Ala Asp Leu Ser Gly Met Thr Gly Ser
ACC ATG GGA ATG GTG GAT ATC TTC AAT GGG GAT GCA GAC CTC TCA GGC ATG ACC GGG AGC 1021
                                   330                                         340
Arg Gly Leu Val Leu Ser Gly Val Leu His Lys Ala Phe Val Glu Val Thr Glu Glu Gly
CGC GGT CTC GTG CTA TCT GGA GTC CTA CAC AAG GCC TTT GTG GAG GTT ACA GAG GAG GGA 1081
                                   350                                         360
Ala Glu Ala Ala Ala Ala Thr Ala Val Val Gly Phe Gly Ser Ser Pro Ala Ser Thr Asn
GCA GAA GCT GCA GCT GCC ACC GCT GTA GTA GGA TTC GGA TCA TCA CCT GCT TCA ACT AAT 1141
                                   370                                         380
Glu Glu Phe His Cys Asn His Pro Phe Leu Phe Phe Ile Arg Gln Asn Lys Thr Asn Ser
GAA GAG TTC CAT TGT AAT CAC CCT TTC CTA TTC TTC ATA AGG CAA AAT AAG ACC AAC AGC 1201
                                   390
Ile Leu Phe Tyr Gly Arg Phe Ser Ser Pro End
ATC CTC TTC TAT GGC AGA TTC TCA TCC CCG TAG ATGCAATTAGTCTGTCACTCCATTTGGAAAATGTT 1269

CACCTGCAGATGTTCTGGTAAACTGATTGCTGGCAACAACAGATTCTCTTGGCTCATATTTCTTTTCTTTCTCATCTTG 1348
ATGATGATCGTCATCATCAAGAATTTAATGATTAAAATAGCATGCCTTTCTCTCTTTCTCTTAATAAGCCCACATATAA 1427
ATGTACTTTTTCTTCCAGAAAAATTCTCCTTGAGGAAAAATGTCCAAAATAAGATGAATCACTTAATACCGTATCTTCT 1506
AAATTTGAAATATAATTCTGTTTGTGACCTGTTTTAAATGAACCAAACCAAATCATACTTTTTCTTTGAATTTAGCAAC 1585
CTAGAAACACACATTTCTTTGAATTTAGGTGATACCTAAATCCTTCTTATGTTTCTAAATTTTGTGATTCTATAAAACA 1664
CATCATCAATAAAATAGTGACATAAAATCAAAAAAAAAAAAAAAAAA                                  1711
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FIG. 3. cDNA sequence and deduced amino acid sequence of SCC antigen. Boxes show the positions of each lysil endopeptidase fragment. The thin underlines indicate the location of the primers used for PCR (from 5'-end, primer 6, 5, 1 and 2, 3 and 4) and thick underlines indicate N-glycosylation consensus sequences. Dotted line indicates the poly(A) signal.

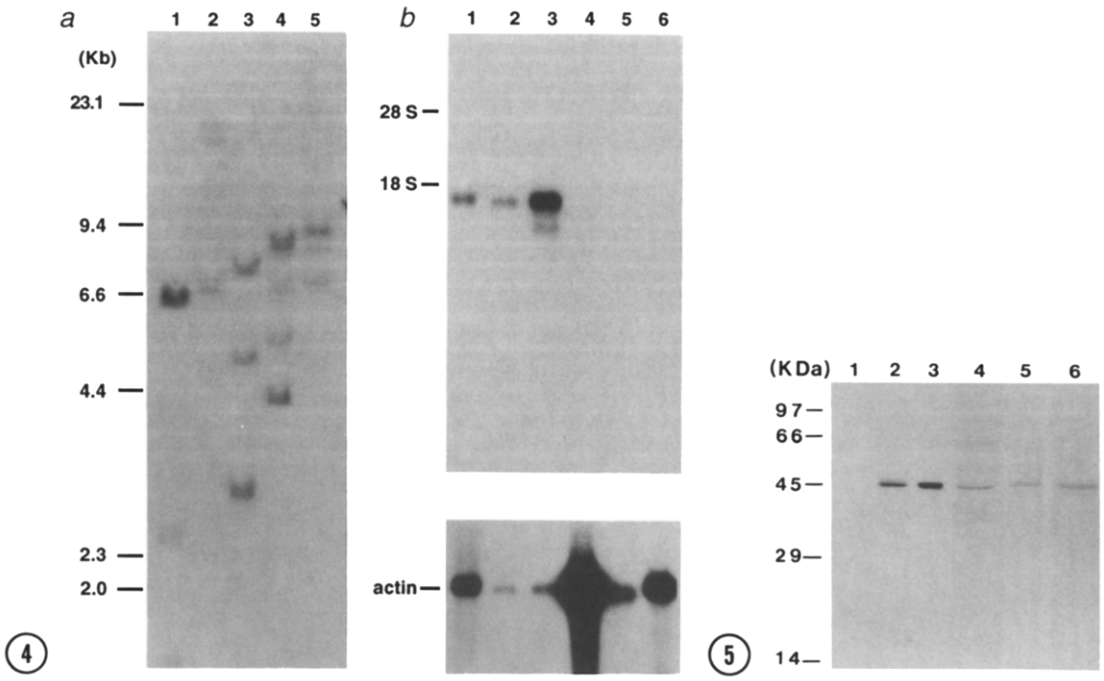

FIG. 4. Southern and Northern blot analysis. *a*, Southern blot analysis of genomic DNA. Lane 1, *Eco*RI; Lane 2, *Bam*HI; Lane 3, *Hin*dIII; Lane 4, *Bgl*II; Lane 5, *Sac*I. *b*, Northern blot analysis of poly $(A)^+$ RNA. Lane 1, SKGIIIa cell; Lane 2, cervical cancer tissue; Lane 3, normal squamous epithelium; Lane 4, normal uterine muscle; Lane 5, placenta; Lane 6, liver. About 5 μg (Lane 1, 4, 5 and 6) or 1 μg (Lane 2, 3) of poly $(A)^+$ RNA was electrophoresed. Blots were hybridized with the SCC antigen probe derived from nucleotide 64-1014 (Fig. 3). β-actin cDNA was used as the control probe for Northern blot analysis.

FIG. 5. Immunoblot analysis of SCC antigen. Lane 1, *E. coli* lysate with pKK233-2 plasmid; Lane 2, *E.coli* lysate with pKK-SCC-1; Lane 3, purified SCC antigen; Lane 4, SKGIIIa cell extract; Lane 5, cervical cancer tissue extract; Lane 6, normal squamous epithelium extract. Molecular weight size markers are indicated.

of carbohydrate on SCC antigen is, if any, minimal in spite of four possible N-glycosylation sites (Asn-X-Thr/Ser) as deduced from amino acid sequence (Fig. 3).

Homology of SCC antigen with serpins

A comparison of amino acid sequence to sequences contained in NBRF-PIR Protein Database revealed an unexpectedly close homology of SCC antigen with serpins; chicken gene Y protein (16) (45%), plasminogen activator inhibitor-2 (17) (43%), ovalbumin (18) (42%), antithrombin III (19) (39%), indicating that SCC antigen was a new member of the serpins family (Fig. 6). Although some members of the serpins family, such as ovalbumin, have lost their original inhibitory function, the target enzyme specificity of the inhibitory serpins is largely determined by the nature of reactive site P_1-P_1' peptide bond (20). However, the P_1-P_1' portion of SCC antigen, Ser-Ser (Fig. 6), has yet to be found in other members of the serpins family. In the inhibitory serpins, the flexibility of the stalk (P_{15}-P_9) which consists of a dominance of residues with small side chain such as Ala or Gly may contribute to the inhibitory function (21). In the case of SCC antigen, this region is abundant with Ala residues (Fig. 6). Furthermore there is a

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ATIII:  mysnvigtvtsgkrkvyllsllligfwdcvtcHGSPVDICTAKPRDIPMNPMCIYRSPEKKATEDEGSEQKIPEATNRR

        1       10        20          30        40        50        60
SCC:    MNSLSEANTKFMFDLFQQFR-KSKE-NNIFYSPISITSALGMVLLGAKDNTAQQIKKVLHFDQVTEN----------
GeneY:  MDSISVTNAKFCFDVFNEMKVHHVN-ENILYCPLSILTALAMVYLGARGNTESQMKKVLHFDSIT----------
PAI-2:  MEDLCVANTLFALNLFKHLAKASPT-QNLFLSPWSISSTMAMVYMGSRGSTEDQMAKVLQFNEVGANAVTPMTPENFTS
Oval:   mGSIGAASMEFCFDVFKELKVHHAN-ENIFYCPIAIMSALAMVYLGAKDSTRTQINKVVRFDKLP----------
ATIII:  VWELSKANSRFATTFYQHLADSKNDNDNIFLSPLSISTAFAMTKLGACNDTLQQLMEVFKFDTIS----------

                70        80        90        100       110       120       130
SCC:    ---------TTGKAATYHVDRSGNVHHQFQKLLTEFNKSTDA-YELKIANKLFGEKTYLFLQEYLDAIKKFYQTSVESV
GeneY:  ---------GAGSTTDSQCGSSEYVHNLFKELLSEITRPNAT-YSLEIADKLYVDKTFSVLPEYLSCARKFYTGGVEEV
PAI-2:  CGFMQQIQKGSYPDAILQAQAADKIHSSFRSLSSAINASTGD-YLLESVNKLFGEKSASFREEYIRLCQKYYSSEPQAV
Oval:   ---------GFGDSIKAQCGTSVNVHSSLRDILNQITKPNDV-YSFSLASRLYAEERYPILPEYLQCVKELYRGGLEPI
ATIII:  ------------------EKTSDQIHFFFAKLNCRLYRKANKSSKLVSANRLFGDKSLTFNETYQDISELVYGAKLQPL

          140       150       160       170       180       190       200       210
SCC:    DFANAPEESRKKINSWVESQTNEKIKNLIPEGNIGSNTTLVLVNAIYFKGQWEKKFNKEDTKEEKFWPNKNTYKSIQMM
GeneY:  NFKTAAEEARQLINSWVEKETNGQIKDLLVSSSIDFGTTMVFINTIYFKGIWKIAFNTEDTREMPFSMTKEESKPVQMM
PAI-2:  DFLECAEEARKKINSWVKTQTKGKIPNLLPEGSVDGDTRMVLVNAVYFKGKWKTPFEKKLNGLYPFRVNSAQRTPVQMM
Oval:   NFQTAADQARELINSWVESQTNGIIRNVLQPSSVDSQTAMVLVNAIVFKGLWEKAFKDEDTQAMPFRVTEQESKPVQMM
ATIII:  DFKENAEQSRAAINKWVSNKTEGRITDVIPSEAINELTVLVLVNTIYFKGLWKSKFSPENTRKELFYKADGESCSASMM

            220       230       240       250           260       270       280
SCC:    RQYTSFHFASLEDVQAKVLEIPYKGKDLSMIVLLPNEID----GLQKLEEKLTAEKLMEWTSLQNMRETRVDLHLPRFK
GeneY:  CMNNSFNVATLPAEKMKILELPYASGDLSMLVLLPDEVS----GLERIEKTINFDKLREWTSTNAMAKKSMKVYLPRMK
PAI-2:  YLREKLNIGYIEDLKAQILELPYA-GDVSMFLLLPDEIADVSTGLELLESEITYDKLNKWTSKDKMAEDEVEVYIPQFK
Oval:   YQIGLFRVASMASEKMKILELPFASGTMSMLVLLPDEVS----GLEQLESIINFEKLTEWTSSNVMEERKIKVYLPRMK
ATIII:  YQEGKFRYRRVAE-GTQVLELPFKGDDITMVLILPKPEK----SLAKVEKELTPEVLQEWLDEL--EEMMLVVHMPRFR

        290       300       310       320       330       340       350       360
                                                          P15   10    5    11'   5'
SCC:    VEESYDLKDTLRTMGMVDIFNGD-ADLSGMTGSR--GLVLSGVLHKAFVEVTEEGAEAAAATAVVGFG-SSPASTNEEF
GeneY:  IEEKYNLTSILMALGMTDLFSRS-ANLTGISSVD--NLMISDAVHGVFMEVNEEGTEATGSTGAIGNIKHSLEL--EEF
PAI-2:  LEEHYELRSILRSMGMEDAFNKGRANFSGMSERN--DLFLSEVFHQAMVDVNEEGTEAAAGTGGVMTG-RTGHGG-PQF
Oval:   MEEKYNLTSVLMAMGITDVFSSS-ANLSGISSAE--SLKISQAVHAAHAEINEAGREVVGSAEAGVDA-ASVS---EEF
ATIII:  IEDGFSLKEQLQDMGLVDLFSPEKSKLPGIVAEGRDDLYVSDAFHKAFLEVNEEGSEAAASTAVVIAG-RSLNPNRVTF

            370       380       390
SCC:    HCNHPFLFFIRQNKTNSILFYGRFSSP
GeneY:  RADHPFLFFIRYNPTNAILFFGRYWSP
PAI-2:  VADHPFLFLIMHKITKCILFFGRFCSP
Oval:   RADHPFLFCIKHIATNAVLFFGRCVSP
ATIII:  KANRPFLVFIREVPLNTIIFMGRVANPCVK
```

FIG. 6. Alignment of SCC antigen with other serpins. SCC, SCC antigen; GeneY, GeneY protein; PAI-2, plasminogen activator inhibitor-2; Oval, ovalbumin; ATIII, antithrombin III. Amino acids of SCC antigen are numbered. P_n-numbered portion enclosed by a box indicates the reactive center loop of serpins and the arrow indicates the reactive site.

unique hydrophobic hexapeptide (Ala-P_7 through Gly-P_2, Fig. 6) which is found in antithrombin III, that may contribute stability to a serpin-serine protease complex by apolar associations with a complementary hydrophobic site in serine protease (22).

Detailed studies (23, 24) indicate that the expression of SCC antigen is closely related to cellular differentiation in both normal and malignant squamous cells, and that a certain type of serpin-protease complex may be involved in tissue differentiation. Therefore, it is possible that SCC antigen is a novel inhibitor for a protease such as tissue plasminogen activator. It is also interesting that two different serine proteases have been identified so far from human cytotoxic T lymphocyte (CTL) (25, 26). Exocytosis of CTL granules containing serine proteases is thought to induce lysis of cells that present foreign antigens mediated by MHC class 1 antigens (27) and a number of low molecular weight protease inhibitors can block CTL-mediated lysis (28). Furthermore, the cultured medium of mononuclear cells from the peripheral blood of the cancer patient stimulated the production of SCC antigen in SKGIIIa cells (data not shown). Considering that cancer tissue is more apt to release SCC antigen than normal squamous epithelium (1, 5), we

may speculate that SCC antigen may act, as a novel protease inhibitor, to modulate the host immune response against tumor cells. Further analysis including the search of the target protease is now in progress.

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