Tumor-associated ov-serpin, SCC Antigen, is involved in apoptosis

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Introduction

SCC antigen (SCCA), a tumor-associated protein, is a member of the ovalbumin serine proteinase inhibitor (ov-serpin) subgroup of the serpin superfamily. Serum levels of SCCA have been used as a marker of squamous cell carcinoma progression, and elevated serum levels of SCCA are considered a risk factor for disease relapse. However, the biological significance of this cytoplasmic protein in cancer cells remains unknown. This review briefly describes the ov-serpins and summarizes what is presently known about SCCA.

Characteristics of the ov-serpin family

The serpin superfamily includes proteins composed of 350-500 amino acids that fold into a conserved secondary structure of three β-sheets and nine α-helices. The reactive site loop is an exposed, flexible stretch of approximately 17 residues located 30-40 amino acids from the carboxyl terminus. Serpins inhibit serine proteinases yielding cleaved, inactive serpins (suicide substrate-like inhibitory mechanism). The specificity of the target proteinase is dependent on the amino acids in the P1-P1' positions in the reactive site loop. Ov-serpins are a subset of serpins. They have N- and C-termini, that are shorter than those of the prototypical serpin, α1-antitrypsin, and they lack a classical secretory signal peptide.[1] The genes encoding the human ov-serpins, which number 13 at present (Table 1), are located on chromosomes 6p25 and 18q21, and all have well-defined gene structures comprising eight exons with conserved exon-intron boundaries, although the 6p25 genes lack one exon.[2] These findings suggest that ov-serpins arose from duplication of a common ancestral gene. Most ov-serpins are intracellular proteins, whereas other serpin family members are located extracellularly. Furthermore, some of ov-serpins, such as SCCAs, plasminogen activator inhibitor-2 (PAI-2), maspin and monocyte/neutrophil elastase inhibitor, are released from cells under specific conditions. Ov-serpins are involved in regulation of cell growth and differentiation (megsin), tumor cell invasiveness and motility (maspin), hematopoiesis (bomapin), microbial or viral infection (proteinase inhibitor-8, -9), extracellular matrix remodeling (PAI-2) and apoptosis (proteinase inhibitor-9, PAI-2).

Characteristics of SCCA

SCCA was first identified as a squamous cell carcinoma-related protein[3], and

Table 1 ov-serpin family

ov-serpin	P1-P1' in reactive site loop	target proteinase	homology (%)	chromosome	inhibition of apoptosis
SCC Antigen - 1	Ser - Ser	cathepsin L,S,K	—	18q21.3	+
SCC Antigen - 2	Leu - Ser	cathepsin G, chymase	92	18q21.3	+
PAI 2	Arg - Thr	uPA	44	18q21.3	+
PI (proteinase inhibitor) 8	Arg - Cys	furin	46	18q21.3	
PI 10 (bomapin)	Arg - Ile	thrombin, trypsin	46	18q21.3	
PI 5 (maspin)	His - Lys		36	18q21.3	
megsin	Lys - Gln		41	18q21.3	
epipin	Lys - Ser			18q21.3	
yukopin	Arg - Ser	trypsin	47	18q21.3	
PI 13 (headpin)	Thr - Ser		59	18q21.3	
elastase inhibitor	Cys - Met	elastase	51	6p25	
PI 6	Arg - Cys	cathepsin G, chymotrypsin thrombin, trypsin	48	6p25	
PI 9	Glu - Cys	granzyme B	47	6p25	+

isoelectric focusing analyses indicated that it comprises several fractions.[4] SCCA1 was later isolated by cDNA cloning [5], and chromosomal analysis of 18q21.3 indicated the presence of tandem array of two homologous genes, *SCCA1* and *SCCA2*.[6] Both SCCA1 and SCCA2 are composed of 390 amino acids with high homology (92%). Two-dimensional analyses have shown four SCCA1 species (pI 5.9-6.4) and two or three SCCA2 species (pI 5.5-5.7).[7] The origin of this heterogeneity is unclear, but one species of SCCA1 is a phosphorylated form of another species. The P1-P1' positions in the reactive site loops of SCCA1 and SCCA2 are Ser-Ser and Leu-Ser, respectively, suggesting a different target specificity. Indeed, *in vitro* analyses have indicated that SCCA1 inhibits chymotrypsin and that SCCA2 inhibits the cathepsin G and chymase.[8,9] Furthermore, SCCA1 can inhibit cysteine proteinases such as cathepsin L and also inhibit papain as a cross-class inhibitor[10] such as a viral serpin, CrmA. Alternative splicing variants of SCCA1 (SCCA1b) and SCCA2 (SCCA2b) have recently been identified.[11] Because these variants lack all (SCCA1b) or part (SCCA2b) of exon 7, that codes Sheets B and C of

rigid framework of serpin molecule, their inhibitory function against proteinases is doubtful.

SCCA and apoptosis

Although a possible target proteinase *in vitro* is known, the physiological function of SCCA remains unclear. Because SCCA expression is linked to cancerous status and because some ov-serpins inhibit apoptosis, we analyzed the role of SCCA in apoptosis. Apoptosis induced by 7-ethyl-10-hydroxycamptothecin, TNF α, IL-2-activated natural killer (NK) cells, or radiation is inhibited significantly in tumor cells transduced with *SCCA1* cDNA. Inhibition of SCCA1 production by tumor cells transfected with antisense *SCCA1* cDNA results in significantly increased sensitivity of these cells to etoposide- or TNF α-induced apoptosis.[12] A similar effect was observed with *SCCA2* cDNA.[13] This anti-apoptotic effect of SCCA involves inhibition of caspase-3 activity and/or upstream proteinases. In the case of radiation-induced apoptosis, SCCA1 and SCCA2 inhibit expression of activated p38 MAPK and MKK 3/6.[13]

SCCA is also expressed by normal squamous epithelium. This epithelium con-

sists of several layers of cells, and it was recently reported that differentiation of squamous epithelium occurs through an apoptotic mechanism. For example, Bcl-2 is expressed by the basal layer, possibly to inhibit apoptosis at the lowest layer of the squamous epithelium.[14] SCCA is expressed in the suprabasal layer and may contribute to differentiation of squamous epithelium through inhibition of apoptosis.

Other functions of SCCA

In vivo, tumor cells that overexpress SCCA1 form significantly large tumors in nude mice with reduced infiltration of large mononuclear cells in comparison to SCCA1-negative control tumors.[12] If SCCA1 expression is suppressed by local administration of antisense SCCA into the tumor site, tumor growth is inhibited, and increased numbers of apoptotic tumor cells and large mononuclear cells in the tumor are observed.[15] The data suggest that extracellular SCCA1 inhibits migration of NK cells into the tumor. Indeed migration of NK cells induced by monocyte-chemoattractant protein-1 in vitro is inhibited completely by addition of SCCA1, and this inhibitory effect is lost when the reactive site loop of SCCA1 is mutated.[15] These functions of SCCA1 may contribute to the escape of squamous cell carcinoma from the host immune system.

Although SCCA is reported to be squamous-cell specific, recent analyses indicate that both SCCA1 and SCCA2 are expressed in several organs.[11] Recently Thakker-Varia et al.[16] reported that LPS-activated microglia show increased expression of SCCA genes. SCCA1 alters microglia morphology and increases cell numbers in vitro; therefore, it may affect microglia activation and brain inflammation. De Falco et al.[17] identified an HBV-binding protein (HBV-BP) on HepG2 cells by affinity purification with the preS1 peptide of HBV. The nucleotide sequence of HBV-BP was nearly identical to that of SCCA1, with only four nucleotide differences and three amino acid substitutions. Although HBV-BP may be a novel ov-serpin, it is possible that an allelic variant of SCCA1 participates in HBV infection of hepatocytes.

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