

EDITORIAL

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Squamous Cell Carcinoma Antigen (SCCA) Isomers – Markers for Squamous Cell Carcinoma

Izomery antygenu raka płaskonabłonkowego (SCCA) jako markery raka płaskonabłonkowego

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Serum tumor markers that accurately reflect the active tumor status of patients with squamous cell carcinomas can be particularly useful. Squamous cell carcinoma antigen (SCCA) is a serological marker for squamous cell carcinomas of the uterine cervix, lung, head and neck and esophagus. SCCA isomers are cytoplasmic proteins found in normal squamous epithelia. Serum levels have been reported to correlate with clinical stage, prognosis and clinical outcome in squamous cell carcinoma patients. SCCA is associated with squamous cell carcinomas of different carcinomas, but the clinical utility for this marker has been best documented in cervical cancer.

Biochemistry of SCCA

SCCA was first isolated from a cervical squamous cell carcinoma by applying conventional protein purification methods; ammonium sulfate precipitation, gel filtration, ionexchange chromatography and polyacrylamide gelelectrophoresis [1]. Biochemical characterization of the originally protein fraction (TA-4), from squamous cell cancer tissue of the uterine cervix showed it to be a group of glycoproteins with a molecular weight of approximately 45 kDa [1, 2]. This protein fraction was used to raise polyclonal antibodies for further characterization and for development of assays for serological studies. The TA-4 fraction could be further separated in two subfractions by isoelectric focusing (acidic fraction pI < 6.25; neutral fraction pI > 6.25) and these subfractions consisted of at least ten different protein components [2]. These studies also included clinical serological investigations and they demonstrated that there were differences

in the expression of the acidic and neutral form of the SCCA antigen in cervical cancer patients. The acidic fraction was more associated with cervical cancer than the neutral SCCA fraction.

Molecular Diagnostics

Molecular cloning of cDNA of SCCA demonstrated that SCCA is transcribed by two almost identical gene products named SCCA1 and SCCA2 [3, 4]. The two SCCA genes are tandemly arranged and located on chromosome band 18q21.3 [5]. The more telomeric SCCA gene was designated SCCA1, whereas the more centromeric was designated SCCA2. Both the genomic organisation of SCCA1 and SCCA2, with eight exons, and the putative intron-exon boundaries, splice sites and terminal codons, are identical. The gene products, SCCA1 and SCCA2, are characterised as cysteine and serine protease inhibitors [4, 6]. The encoded proteins, SCCA1 and SCCA2, demonstrate a very high degree of homology (98% at the nucleotide level and 92% at the amino acid level). SCCA1 corresponds to the neutral protein fraction and SCCA2 to the acidic protein fraction, and both belong to the family of serine proteinase inhibitors (serpins).

Exons 2–8 are translated into mature protein and exon 8 contains the reactive site loop, which determines the specificity of the serpins [4]. SCCA1 and SCCA2 protease inhibitors constitute approximately 390 amino acids each. The specificity of SCCA1 and SCCA2 is related to the differences in the amino acid composition (4–6 amino acid residues) in the reactive site loop [6]. SCCA1 is an inhibitor of papain-like cysteine proteases, such as cathe-

psin K, L and S, whereas SCCA2 inhibits chymotrypsin-like serine proteases, cathepsin G and mast cell chymase [7]. SCCA1 and SCCA2 may exist in a free form and also in complexes with serine and cysteine proteases.

Cellular Localization and Function

Immunohistochemical localisation of SCCA has been shown to be cytoplasmic with the greatest intensity of staining in large cell non-keratinizing squamous cell carcinomas [8]. The mRNA of SCCA is located in the basal and parabasal layer of normal squamous epithelium and in the stratified epithelium of carcinoma tissue [9]. Applying discriminatory monoclonal antibodies in immunohistochemistry and RT-PCR, the localisation of SCCA1 and SCCA2 has been demonstrated in normal tissues as well as in carcinoma tissues [10]. Differential expression could not be demonstrated for SCCA1 and SCCA2 using immunohistochemistry. Conversely, studies of mRNA levels of SCCA1 and SCCA2 have indicated that the relative expression of SCCA2 compared to SCCA1 is certainly increased in cancer cell lines, cervical cancer tissues and head and neck cancers [11]. The targets and functions of serpins are not fully understood. The primary functions for most serpins are regulation of proteolytic events associated with coagulation, fibrinolysis, apoptosis and inflammation [12].

Clinical Applications of First Generation SCC Assays

From the clinical use of SCCA it was proposed that there might be differences in tumor expression of the acidic and neutral protein fractions. Studies have supported the hypothesis that the acidic SCCA fraction was more commonly associated with cancer as compared to the neutral SCCA protein fraction [2, 6, 13–15]. The clinical utility of serum SCC assays has been best documented for prognosis, monitoring therapy and follow-up of patients with SCCA expressing squamous cell carcinomas of the cervix, lung and head and neck [17, 25–28]. Reported clinical data from the application of SCC assays are mostly from studies using radioimmunoassay or IMx SCC assays (Abbott Diagnostics) [13]. Both SCCA1 and SCCA2 protease inhibitors are measured in these assay systems [2].

Serum SCC antigen levels correlate with clinical stage and treatment outcome in various carcino-

mas [2,16,17]. High pretreatment levels indicate extensive disease and poor prognosis for cervical cancer patients with squamous cell carcinoma histotype, but not for adenocarcinomas. Good correlation between serum SCC antigen levels and extent of disease have been published [17]. Most SCC data has been reported for cervical cancer patients, but due to low sensitivity and specificity SCC has limited clinical utility in early stage disease. In particular, more than 50% of patients with cervical cancer have loco-regional disease (stage Ib and stage IIa) and at the time for diagnosis 38% and 51%, respectively, show elevated serum SCC levels [17]. Similar results have also been reported for non-small cell lung cancer patients with squamous cell carcinomas [18, 19]. SCC demonstrated a clear relationship with tumor stage and was a prognostically significant factor for survival in squamous cell lung cancer patients [29–31]. Even in patients with head and neck cancer, low sensitivity of SCC has been reported for patients with early disease [16]. Conversely, postoperative monitoring of SCC levels in patients with head and neck cancer showed increased ability to identify patients with high risk for recurrence and early detection of recurrent disease [16]. Elevated serum SCC levels in patients with head and neck cancer have also related to nodal involvement (node positive patients) and multivariate analysis showed that SCC was a significant independent predictor of disease-free survival [20]. New methods have been developed to measure serum SCCA1 or SCCA2 separately and by measuring recombinant SCC preparations. It has been shown that recombinant SCCA2 is underestimated using the IMx SCC assay (Abbott Diagnostics) [6]. In serum samples from cervical cancer patients, the SCC antigen consists mainly of the SCCA2 isomer and this might be one reason for the limited sensitivity of the commercially available SCC assays.

Elevated serum levels have also been reported in various benign conditions (skin diseases, pulmonary infections, renal dysfunction) [32, 33]. Another concern in SCC testing is that contamination of serum samples with secretions (saliva or sweat) may result in increased serum SCCA levels. In saliva the dominating isomer appears to be SCCA1 and in sweat both SCCA1 and SCCA2 have been detected.

Monoclonal Antibodies Specific for SCCA1 and SCCA2 Antigens

The discovery of two distinct SCCA related serine proteinase inhibitors and the hypothetical differences in cancer specificity of SCCA1 and

SCCA2 underscores the need for specific assays that can discriminate between SCCA1 and SCCA2 and further recognize all serological forms of SCCA with similar sensitivity. Monoclonal antibodies, specific for different forms of SCC antigens, were raised by intra peritoneal immunization of Balb/c mice with recombinant SCC antigen and fused to mouse myeloma cells [21]. The generated monoclonal antibodies were purified by Protein A affinity chromatography. The reactivity of the monoclonal antibodies and epitope specificities were established by determination of the reactivity with free and complexed SCC antigens, reduced SCC antigens and different fusion transcripts positioned between exon 2–7 and exon 8 of SCCA1 and SCCA2. Determination of dose-response curves for different sandwich immunoassays was the final confirmation of the assay specificity [22].

Despite the high degree of sequence homology of the gene products SCCA1 and SCCA2, it has been possible to establish monoclonal antibodies specific for SCCA1 and SCCA2 (differences in the reactive site loop of each inhibitor) based upon antibody reactivity.

Thirteen monoclonal antibodies directed against SCCA1 and SCCA2 were characterized in an international collaborative study (ISOBM TD-10 Workshop) using both native and recombinant forms of SCCA [34]. The major conclusion from this study was that the antibody specificities found might have application for antibody measurement of all forms of squamous cell carcinoma in serum.

Selection of Monoclonal Antibodies for Specific Immunoassays

More than 20 new monoclonal antibodies with specificity towards SCCA antigens have been raised and characterized at CanAg Diagnostics [21, 22]. It was possible to group the monoclonal antibodies based upon their reactivity with recombinant SCCA into different epitope groups; group A antibodies reacted with both SCCA1 and SCCA2, group B antibodies reacted only with SCCA1 and group C antibodies reacted only with SCCA2. The cross-reactivity between group B and C was low. Based upon this information, it has been possible to design new sensitive and specific immunoassays for measuring different serological forms of SCCA. However, only the application of such immunoassays to clinical samples can prove if any of these monoclonal antibody combinations have a greater discriminatory capacity and clinical utility for the circulating tu-

mor markers SCCA1 and SCCA2, than the present first generation assays.

Total SCC Antigen Assay

Two antibodies both recognising SCCA1 and SCCA2 are mandatory to create a sandwich assay for measuring total SCC or Pan SCC. Optimal reactivity in the ELISA assay for measuring total SCC was obtained with the antibody pair SCC140 (catcher antibody) and SCC107 (detector antibody).

Total SCCA1 Assay

Combination of monoclonal antibodies from Group B and from Group A permitted the design of specific immunoassays for SCCA1 antigen. A monoclonal antibody characterised by high sensitivity for SCCA1 antigen and low cross-reactivity with SCCA2 antigen was selected. Optimal reactivity in the immunoassay for measuring total SCCA1 was obtained with the antibody pair SCC111 (catcher antibody) and SCC107 (detector antibody).

Total SCCA2 Assay

Different antibodies from Group A and Group C could be selected for measuring total SCCA2 antigen. Three monoclonal antibodies recognising free SCCA2 and two monoclonal antibodies recognising total SCCA2 antigen were established. Optimal reactivity for measuring total SCCA2 antigen (free SCCA2 and SCCA2 in complex with proteases) was obtained with the antibody pair SCC103 (catcher antibody) and SCC107 (detector antibody).

Free SCCA2 Assay

Antibodies from Group C were specific for SCCA2 antigen. All antibodies demonstrated low cross-reactivity with SCCA1 antigen. The preferred configuration for measuring free SCCA2 antigen was based upon the pair SCC 104 (catcher antibody) and SCC107 (detector antibody).

Clinical Evidence for the Second Generation SCCA Assays

The pretreatment serum SCC levels in patients with cancer of the uterine cervix have been reported to help to distinguish between patients with

and without a high risk for lymph node metastases. Furthermore, SCC has shown to correlate with the clinical course following treatment and to predict relapse [2, 16, 17, 23]. Deep stromal infiltration and positive lymph nodes increased the SCC level significantly in many patients. However, about one-third to one-half of patients with cervical carcinoma have produced normal SCCA concentrations. Thus determination of SCCA alone is not optimal for clinical purposes.

The first generation SCC biomarker is not sensitive enough for early diagnosis of cervical cancer. Using the newly developed ELISA assays for measuring the different serological forms of SCCA, it was shown that SCCA2 was the dominating serological form of SCCA in healthy subjects and in most cervical cancer patients (FIGO stage II–IV) [24]. However, both SCCA1 and SCCA2 followed the clinical course of the disease, and could be used for monitoring treatment outcome in cervical cancer patients and also to predict disease progression. In most patients SCCA2 antigen showed the most pronounced elevation of the SCCA antigens in clinically confirmed progressive disease, but in some patients the SCCA1 antigen was the earliest predictor of progressive disease. Optimal clinical sensitivity and specificity would be obtained using an assay measuring all serological forms of SCCA [24].

Perspectives with SCCA1 and SCCA2

SCC antigen is an established biomarker for patient management in cervical cancer, lung cancer and head and neck cancer patients; for prognostic information, treatment outcome and for prediction of recurrence. Cancer markers are needed that will further enhance the ability to diagnose, prognose and predict treatment response. The second generation SCCA assays may be useful for early detection of disease and prediction of disease progression. Based upon studies with SCCA1 and SCCA2 it can be concluded that for optimal clinical sensitivity, an assay recognising both SCCA1 and SCCA2 with similar sensitivity should be recommended. However, specific determination of SCCA1 antigen and SCCA2 antigen may provide additional clinical information compared to the determination of "Total SCC". Thus, it might be possible to increase the sensitivity and specificity for measuring SCC in early cancer diseases, e.g. cervical cancer, lung cancer and head and neck cancer, by applying the new second generation monoclonal antibodies. The SCCA isoforms might also be useful in order to characterise the native proteases associated with SCCA1 and SCCA2 in squamous cell carcinoma and normal tissues.

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