Different expression patterns of intact forms of squamous cell carcinoma antigens between normal and malignant cervical squamous epithelial tissues: Nondenaturing polyacrylamide gel electrophoretic analysis

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Abstract. Squamous cell carcinoma antigen (SCCA), a 45-kDa tumor-associated serpin, mainly consists of two highly homologous molecules, SCCA1 and SCCA2, which possess unique proteinase inhibitory properties. Importantly, our previous study demonstrated that an intact structure of SCCAs, and not a cleaved form yielded by interacting with target proteinase, is essential for their function as a serpin. The aim of this study is therefore, to develop a simple method of analyzing expression patterns of intact forms of SCCAs (functional SCCAs) in cervical squamous epithelial tissues and to investigate whether there are any differences in the expression of intact forms of SCCAs between normal and malignant cervical squamous epithelial tissues. We used nondenaturing polyacrylamide gel electrophoresis (PAGE) with immunoblotting. The newly generated antibody, Pab Y2, recognizes only intact form of SCCAs, while the conventional antibody, Mab 27, reacts with the cleaved form of SCCA1 as well as intact forms of SCCAs. Nondenaturing PAGE using Pab Y2 showed that an intact form of SCCAs in the heat-treated tissue extract at 60˚C for 2 h was separated into at least five bands, termed as bands A-E from cathode to anode. By comparison with two-dimensional electrophoresis patterns of SCCAs, it was found that the first three bands, i.e. bands A-C, are derived from the intact form of SCCA1, while the other two bands, i.e. band D and E are from the intact form of SCCA2. Specifically, band E, but not band D, of SCCA2 is apparently increased in squamous cell carcinomas compared with normal squamous epithelium. In conclusion, this novel analytical approach will be useful for investigating the different expression patterns of functional SCCAs between normal and malignant cervical squamous epithelial tissues.

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Introduction

Squamous cell carcinoma antigen (SCCA) was originally purified from squamous cell carcinoma of the uterine cervix by Kato and Torigoe in our laboratory (1). At present, SCCA is clinically used as a serological tumor marker for the management of squamous cell carcinoma arising at various sites (2). However, it is well-known that SCCA is also expressed in normal squamous epithelium of the uterine cervix, besides cervical squamous cell carcinoma (3-5). Interestingly, some previous studies have suggested that specific molecular fractions of SCCA are increased in squamous cell carcinoma tissue compared with normal squamous epithelium (6,7).

As for the cause of its molecular heterogeneity, recent biochemical research has demonstrated that SCCA mainly consists of two highly homologous molecules, SCCA1 and SCCA2, which belong to the serine proteinase inhibitor (serpin) family (8,9). Until now, there are few reports on the characteristic expression of SCCA1 and SCCA2 in cervical squamous cell carcinoma tissue. For example, Murakami *et al* reported that the SCCA2 mRNA level, but not SCCA1, is significantly increased in squamous cell carcinoma tissue compared with normal squamous epithelial tissue (10). Hamada *et al* also reported that the SCCA1/SCCA2 mRNA ratio differs distinctly between squamous cell carcinoma and keratinocyte cell lines

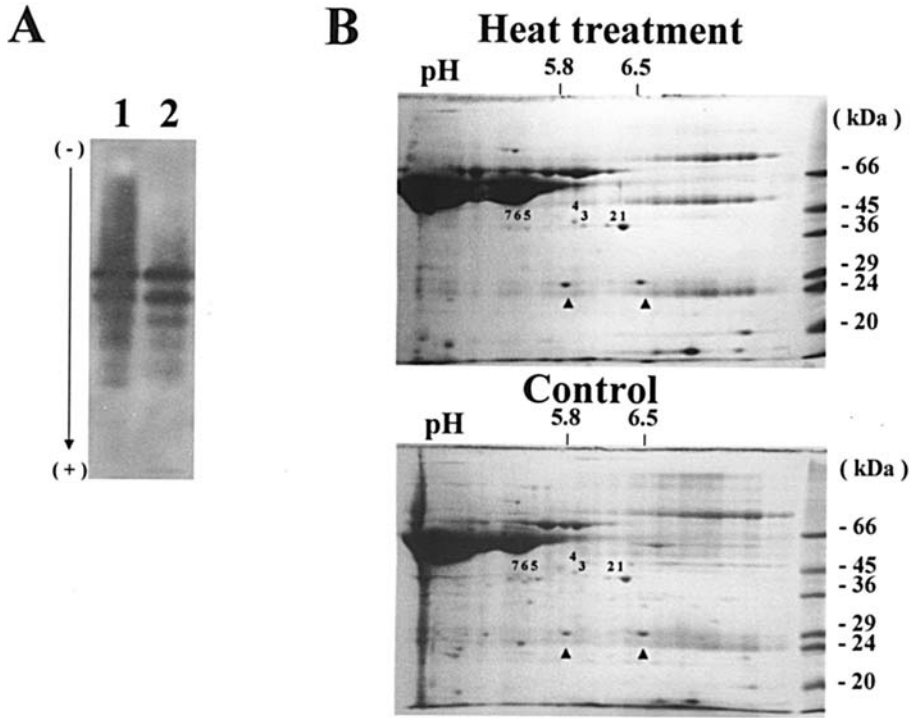

Figure 1. Effects on electrophoretic patterns of SCCAs by heat treatment. (A) Nondenaturing PAGE analysis. The cervical squamous cell carcinoma tissue extract containing 50 ng of SCCAs (lane 1) and the same tissue extract treated by heating at 60˚C for 2 h (lane 2) were subjected to nondenaturing PAGE. After electrophoresis, the proteins were transferred to the PVDF membrane, and then detected by immunostaining using Mab 27. (B) 2-DE analysis. 2-DE of normal squamous epithelial tissue extract showed 7 spots of SCCAs; spots 1-4 of SCCA1 and spots 5-7 of SCCA2 as reported previously (17,22). Heat treatment did not affect the 7 spots of SCCAs. The left triangle indicates bovine carbonic anhydrase B with p*I* 5.8 and the right triangle indicates human carbonic anhydrase B with p*I* 6.5 as the p*I* marker.

(11). On the contrary, it was reported that SCCA1 and SCCA2 are co-localized equally in normal and malignant squamous epithelial tissues by immunohistochemical study using their specific antibodies (12). To resolve this disagreement, further investigation is required into the protein expression of both SCCAs in normal and malignant cervical squamous epithelial tissues.

In recent years, much attention has been focused on the biological function of serpin in tumor progression of cervical squamous cell carcinomas. In fact, we have reported that SCCA has various biological functions in cancer behavioural patterns (13-15). We found that SCCA stimulates matrix metalloproteinase-9 (MMP-9) production and promotes cell invasion in cervical squamous cell carcinoma cells, and that an intact structure of SCCA, and not a cleaved form yielded by interacting with target proteinase, is required for this function of SCCA (15). Thus, this finding suggests that an intact form of SCCA plays roles in cancer behavioural patterns such as invasion or metastasis. This data, therefore, led us to focus on analyzing the expression of the intact form of SCCA1 and SCCA2 (functional SCCAs) between normal and malignant cervical squamous epithelial tissues.

Using two-dimensional electrophoresis (2-DE), we previously showed that there are at least four variants of SCCA1 and three variants of SCCA2 in cervical squamous cell carcinoma tissues (16,17). Although 2-DE is a powerful tool for analyzing the molecular heterogeneity of proteins, this technique containing isoelectric focusing gel electrophoresis (IEF) is regarded as a complicated method. In this study, we therefore developed a simple electrophoretic method of detecting intact forms of SCCA1 and SCCA2, but not cleaved forms, in cervical squamous epithelial tissue extracts. The present study shows for the first time that there are different expression patterns of functional SCCAs between normal and malignant squamous epithelial tissues using nondenaturing polyacrylamide gel electrophoresis (PAGE) with a novel antibody specific for intact forms of SCCAs.

Materials and methods

Materials. The molecular mass marker proteins were obtained from Sigma (St. Louis, MO, USA). The isoelectric focusing point (p*I*) markers and the ECL Western blotting system were purchased from Amersham Biosciences (Buckinghamshire, UK). Silver staining 2D-Silver Stain II 'Daiichi' was from Daiichi Pure Chemicals (Tokyo, Japan). The peroxidase-conjugated rabbit anti-mouse IgG antibody and swine anti-rabbit IgG were from Dako (Tokyo, Japan). The polyvinylidene difluoride (PVDF) membrane was from ATTO (Tokyo, Japan).

Preparation of tissue samples. The project was reviewed and approved by the committee on investigations involving human subjects from Yamaguchi University School of Medicine. Informed consent from patients was obtained before collection of any tissue samples for this study. Normal or malignant cervical squamous epithelial tissues were obtained at surgery from patients bearing myoma uteri or bulky squamous cell carcinoma of the uterine cervix. The tissues were dissected into small pieces, and suspended in distilled water. The suspension was homogenized in a glass homogenizer, and then the homo-

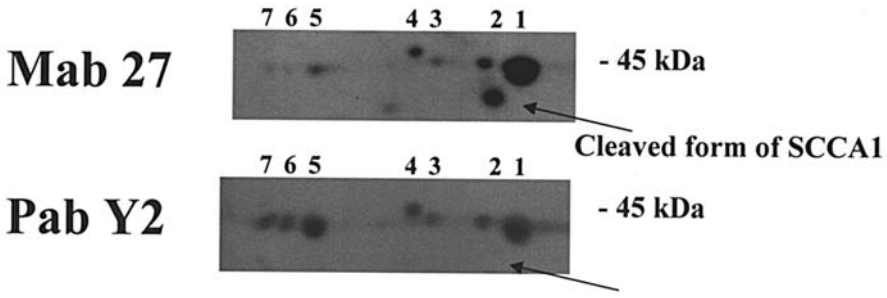

Figure 2. Characterization of a novel specific antibody, Pab Y2, by 2-DE combined with immunoblotting. Pab Y2 recognized intact forms of both SCCA1 and SCCA2 in normal cervical squamous epithelial tissue, but not the cleaved form of SCCA1 (arrow), which was detected by Mab 27, as reported previously (17).

genate was centrifuged at 10,000 x g and 4˚C for 10 min. The supernatant was stocked at -80˚C as the cervical squamous epithelial tissue extract. The concentration of SCCA was determined by the sandwich enzyme immunoassay system (IMx, Abbott Japan, Japan) (18).

Heat treatment of cervical squamous epithelial tissue extracts. As shown in Fig. 1A, nondenaturing PAGE combined with immunoblotting using Mab 27 showed that SCCAs migrated as smear bands, probably due to protein aggregations (lane 1). To improve this poor electrophoretic pattern of SCCA on nondenaturing PAGE, we first tested the heat treatment of cervical squamous epithelial tissue extract, as reported previously, with a modification (19). Briefly, the 50-μl solution of cervical squamous epithelial tissue extract in 20 mM Tris-HCl, pH 7.4, and 0.05% Tween-80 was heated at 60˚C for 2 h in a water bath. By heating, a marked increase of turbidity was observed in the sample solution due to the heat-induced protein degeneration. The solution was centrifuged at 10,000 x g at 4˚C for 10 min, and the super-natant was used as the heat-treated cervical squamous epithelial tissue extract. By heating the tissue extract at 60˚C for 2 h, the five bands of SCCAs were apparently recognized on nondenaturing PAGE (Fig. 1A, lane 2). To confirm that SCCA2, besides SCCA1, has a unique heat-stable property at 60˚C, we analyzed the 2-DE pattern of SCCAs in the heat-treated tissue extract. As reported previously (17), 2-DE of normal cervical squamous epithelial tissue extract showed four spots with p*I* 6.4, 6.3, 6.0 and 5.9, numbered from 1 to 4 of SCCA1 and three spots with p*I* 5.7, 5.6 and 5.5, numbered from 5 to 7 of SCCA2 (Fig. 1B; control, lower panel). Expectedly, the heat treatment at 60˚C for 2 h did not change the 2-DE patterns of SCCAs spots (Fig. 1B; heat treatment, upper panel). These data indicated that heat treatment of the tissue extract at 60˚C is essential for detection of SCCAs on nondenaturing PAGE.

Nondenaturing polyacrylamide gel electrophoresis (PAGE). Nondenaturing PAGE was run in 10% polyacrylamide gels without denaturants at a 10 mA/slab constant current as described previously (20), with a modification. Namely, nondenaturing PAGE in this study utilized a Tris/glycine buffer system with the same pH values i.e. pH 6.8, both for stacking and separating gels, instead of their different pH values.

Two-dimensional electrophoresis (2-DE). 2-DE was carried out as described previously (21), by using capillary-type polyacrylamide gels for isoelectric focusing in the first dimension under nondenaturing conditions, followed by slab-type polyacrylamide gels for SDS electrophoresis. As nondenaturing 2-DE, after IEF in the same conditions as 2-DE, the second dimensional electrophoresis was run in 10% polyacrylamide gels without denaturants at a 10 mA/slab constant current.

Specific antibodies for SCCA. We used specific monoclonal antibodies, Mab 27 and Mab 426. We previously reported that Mab 27 reacted with both intact and cleaved forms of SCCAs, while Mab 426 reacted with cleaved forms of SCCAs, but not with intact SCCAs (17). To yield the novel antibody specific for intact forms of SCCAs, Pab Y2 was newly generated by immunizing with keyhole limpet hemocyanine-conjugated to the synthetic peptide corresponding to a region from the C-terminal fragment of SCCA2 to rabbit and purified using the peptide affinity column.

Immunoblotting. After electrophoresis was completed, the proteins were transferred to a PVDF membrane with semidry type blotting apparatus (Horizblot, Atto, Japan). The PVDF membrane was stained by an immunochemical technique consisting of the following procedures. After blocking the membrane with skimmed milk dissolved in Tris-buffer saline (TBS), pH 7.5, the membrane was incubated with specific antibodies for SCCA, which were 500-fold diluted. Then, peroxidase-conjugated rabbit anti-mouse IgG antibody or swine anti-rabbit IgG was incubated with the membrane. Finally, the ECL Western blotting detection system was applied according to the protocol of Amersham, and the membrane was exposed to hyperfilm-ECL for 1 min.

Results

Identification of functional SCCA1 and SCCA2 on nondenaturing PAGE. We examined the specificity of the novel antibody, Pab Y2, with native SCCAs by 2-DE combined with immunoblotting. Fig. 2 demonstrated that Pab Y2 recognizes intact forms of both SCCA1 and SCCA2 equally, but not cleaved forms. Since we tried to focus on functional SCCAs in this study, we also checked the possibility that Pab Y2 could detect intact forms of SCCAs on nondenaturing PAGE. As shown in Fig. 3, Pab Y2 recognized at least five bands of SCCAs, termed as bands A-E from cathode to anode, like Mab 27. However, the second band recognized by Mab 27

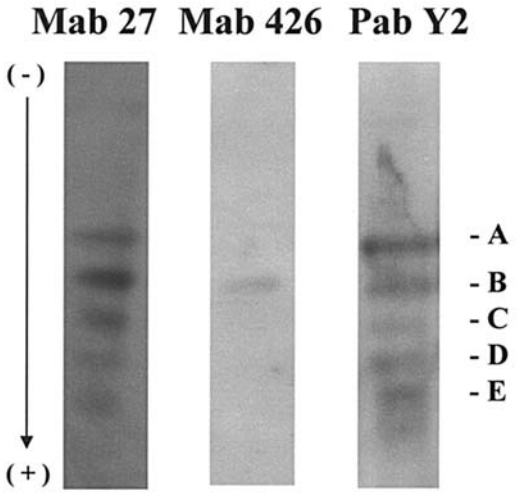

Figure 3. Identification of intact forms of SCCAs on nondenaturing PAGE. We tested the immunoreactivity of Pab Y2 using a heat-treated cervical squamous cell carcinoma tissue extract which contained an abundant amount of the cleaved form of SCCAs. Pab Y2 recognized at least five bands of intact forms of SCCAs, termed as bands A-E from cathode to anode.

contained the cleaved form of SCCA1, besides intact forms of SCCA (band B), from the results of immunoblotting with Mab 426. Namely, it was suggested that Mab 27 could not discriminate between intact forms of SCCAs and cleaved forms on nondenaturing PAGE. Then, by comparison with the nondenaturing 2-DE pattern of SCCAs as shown in Fig. 4, it was found that the three bands, A, B and C, are derived from the intact form of SCCA1, while the two bands, D and E, are from the intact form of SCCA2.

Comparison of nondenaturing PAGE patterns of functional SCCAs between normal and malignant squamous epithelial tissues. We analyzed nondenaturing PAGE patterns of the functional SCCAs in normal and malignant cervical squamous epithelial tissues. Although band A is a major band among the five bands in all tissue extracts, there are some distinct patterns of intact forms of SCCAs between normal and malignant cervical squamous epithelial tissues. Most remarkably, band E, but not band D, of SCCA2 is apparently increased in squamous cell carcinoma compared with normal squamous epithelium (Fig. 5). In addition, the minor band, which migrated faster than band E, as indicated by the arrow in Fig. 5, was detected in squamous cell carcinoma. Concerning SCCA1 bands, band C was apparently decreased compared with band B in all cases of squamous cell carcinoma, while band C was detected as densely as band B in normal squamous epithelium, except in case N3.

Discussion

In this study, we reported for the first time a simple analytical method with which to characterize the tissue expression pattern of two highly homologous functional serpins, SCCA1 and SCCA2. Namely, nondenaturing PAGE of heat-treated cervical squamous epithelial tissue extracts showed at least five bands of SCCAs. Bands A, B and C derived from an intact form of SCCA1 while bands D and E derived from an

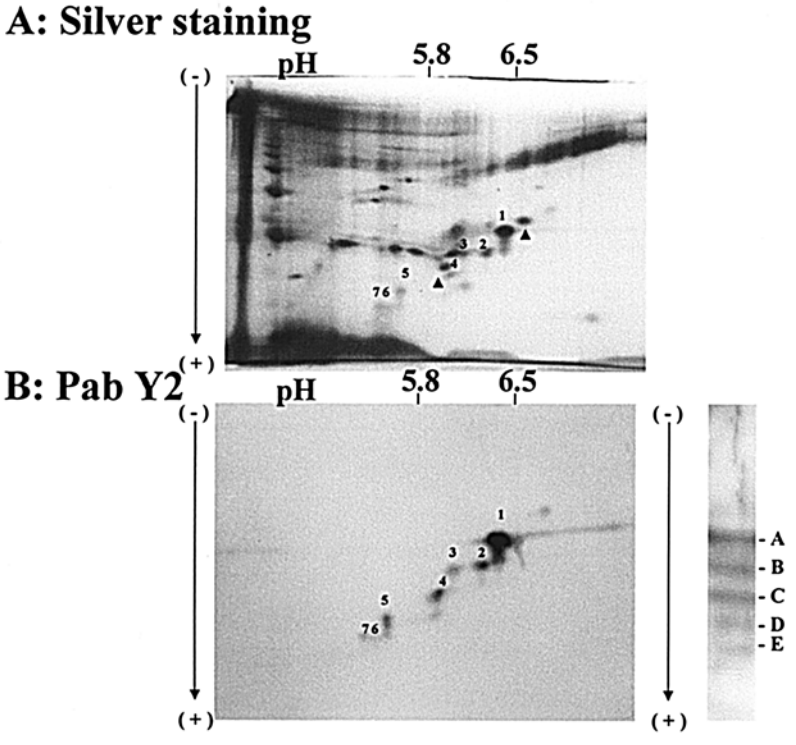

Figure 4. Comparison of SCCA bands on nondenaturing PAGE with SCCA spots on nondenaturing 2-DE. Nondenaturing 2-DE of a heat-treated normal squamous epithelial tissue extract showed 7 spots of SCCAs, i.e. spots 1-4 of SCCA1 and spots 5-7 of SCCA2. As indicated by the data of immunoblotting using Pab Y2, the cathodal three bands, i.e. bands A, B and C correspond to spot 1, spots 2-3 and spot 4 of SCCA1, respectively, while the anodal two bands, i.e. bands D and E correspond to spot 5 and spots 6-7 of SCCA2, respectively.

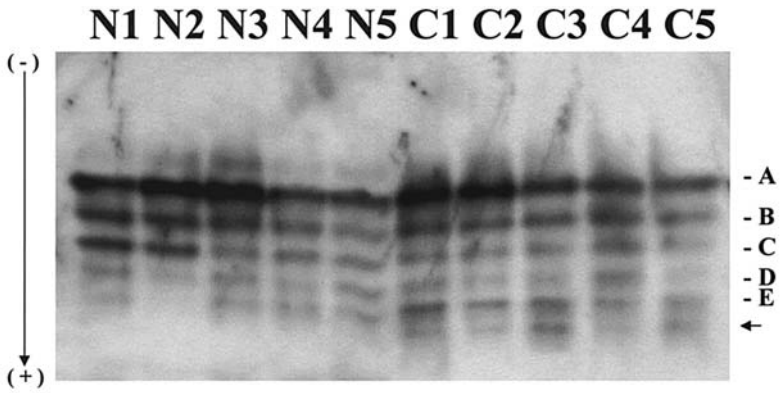

Figure 5. Nondenaturing PAGE of functional SCCAs in normal and malignant cervical squamous epithelial tissues. Heat-treated normal (N) and malignant (C) cervical squamous epithelial tissue extracts containing 100 ng of SCCAs were analyzed by nondenaturing PAGE combined with immuno-blotting using Pab Y2. In squamous cell carcinoma tissue, an additional band (arrow) was also detected.

intact form of SCCA2. It was also found that SCCA2, besides SCCA1, is a heat stable protein up to 60˚C. Importantly, it is speculated that these thermal properties could lead to an improvement in the separation of SCCA bands on nondenaturing PAGE.

In recent years, much attention has been focused on the biological functions of SCCAs in tumor progression and invasion of cervical squamous cell carcinoma (13-15). Remarkably, it was noted that an intact structure of SCCAs is essential for their function as a unique serpin, which posseses the property of stimulating MMP-9 production (15). For further elucidation of the biological significance of SCCAs, it is necessary to identify an intact form of SCCAs in cervical squamous cell carcinoma tissue. However, the conventional antibody for SCCA, Mab 27, recognizes not only intact forms of SCCAs but also the cleaved forms of SCCA1, which lost this function. Although several monoclonal antibodies for SCCAs have been characterized (22), Pab Y2 is a unique antibody which could react with intact forms of both SCCA1 and SCCA2 equally. Interestingly, it was suggested that multiple forms of serpin might contribute to the different functions of serpin under pathological conditions (23). Therefore, most likely, it is worthwhile to study tissue expression of SCCAs by distinguishing intact forms from cleaved forms using Pab Y2.

As shown in Fig. 5, we found the possibility that there are a few differences in nondenaturing PAGE patterns of intact forms of SCCAs between normal and malignant cervical squamous epithelial tissues. Specifically, band E, but not band D, of SCCA2 is apparently increased in squamous cell carcinomas compared with normal squamous epithelium. These data suggest that the increased SCCA2 variants, i.e. spots 6 and 7 on 2-DE, might be related to the malignant behavior of cervical squamous cell carcinoma. Furthermore, we detected the additional minor band of SCCA2 in squamous cell carcinoma tissue on nondenaturing PAGE. It is possible that this band, specific for squamous cell carcinoma, might be derived from the splicing variants of SCCAs, since it has been reported that splicing variants were detected in squamous cell carcinoma rather than normal squamous epithelium using RT-PCR analyses (24). However, the cause of molecular heterogeneity, including the additional band of SCCA2 remains unknown. Concerning the nondenaturing pattern of SCCA1, band C is decreased compared with band B in squamous cell carcinoma. This data is in agreement with a previous 2-DE report by Abe *et al* which states that, in cervical squamous cell carcinoma, spots 2 and 3 of SCCA1 are increased, but spot 4 is decreased (21). More recently, Bae *et al* demonstrated that SCCA1 and SCCA2 are up-regulated in cervical squamous cell carcinoma tissue (25). Our results also suggest that the analysis of tissue expression patterns of intact forms of SCCAs might be of great significance to the study of malignant behavioural patterns in cervical squamous cell carcinoma.

In conclusion, nondenaturing PAGE of heat-treated cervical squamous epithelial tissue is a simple method to identify intact forms of both SCCA1 and SCCA2. This unique analytical approach is useful to investigate the differences in expression of functional SCCAs between normal and malignant cervical squamous epithelial tissues. Gariboldi *et al* recently reported a significant association between SCCA2 protein expression and patient age at tumor presentation in skin squamous cell carcinomas (26), suggesting that SCCAs might play important roles in tumorigenesis, besides tumor progression. Therefore, further studies into the aberrant expression patterns of functional SCCAs might provide a novel insight into elucidating the molecular mechanism of cancer development of squamous cell carcinoma arising from various sites.

Acknowledgements

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References

1. Kato H and Torigoe T: Radioimmunoassay for tumor antigen of human cervical squamous cell carcinoma. Cancer 40: 1621-1628, 1977.
2. Kato H: Squamous cell carcinoma antigen. In: Serological Cancer Markers. Sell S (ed). Humana press, Clifton, pp437-451, 1992.
3. Crombach G, Scharl A, Vierbuchen M, Würz H and Bolte A: Detection of squamous cell carcinoma antigen in normal squamous epithelia and in squamous cell carcinomas of the uterine cervix. Cancer 63: 1337-1342, 1989.

4. Morioka H: Tumor-antigen (TA-4) of squamous cell carcinoma - its tissue distribution and its relationship to serum TA-4 concentrations. Asia-Oceania J Obstet Gynecol 6: 91-97, 1980.
5. Michioka T, Takeshima N, Tsunaga N, Suminami Y, Nawata S and Kato H: Expression of squamous cell carcinoma antigen, a serine protease inhibitor, in the integument of vertebrates: possible role in stratification of epidermis. Acta Histochem Cytochem 27: 435-440, 1994.
6. Kato H, Nagaya T and Torigoe T: Heterogeneity of a tumor antigen TA-4 of squamous cell carcinoma in relation to its appearance in the circulation. Gann 75: 433-435, 1984.
7. Tsuyama S, Hashimoto K, Nakamura K, Tamura H, Sasaki K and Kato H: Different behaviors in the production and release of SCC antigen in squamous-cell carcinoma. Tumour Biol 12: 28-34, 1991.
8. Suminami Y, Kishi F, Sekiguchi K and Kato H: Squamous cell carcinoma antigen is a new member of the serine protease inhibitors. Biochem Biophys Res Commun 181: 51-58, 1991.
9. Schneider SS, Schick C, Fish KE, Miller E, Pena JC, Treter SD, Hui SM and Silverman GA: A serine proteinase inhibitor locus at 18q21.3 contains a tandem duplication of the human squamous cell carcinoma antigen gene. Proc Natl Acad Sci USA 92: 3147-3151, 1995.
10. Murakami A, Suminami Y, Sakaguchi Y, Nawata S, Numa F, Kishi F and Kato H: Specific detection and quantification of SCC antigen 1 and SCC antigen 2 mRNAs by Fluorescence-based asymmetric semi-nested reverse transcription PCR. Tumour Biol 21: 224-234, 2000.
11. Hamada K, Hanakawa Y, Hashimoto K, Iwamoto M, Kihana T, Hirose S, Nakamura M and Ito M: Gene expression of human squamous cell carcinoma antigens 1 and 2 in human cell lines. Oncol Rep 8: 347-354, 2001.
12. Cataltepe S, Gornstein ER, Schick C, Kamachi Y, Chatson K, Fries J, Silverman GA and Upton MP: Co-expression of the squamous cell carcinoma antigens 1 and 2 in normal adult human tissues and squamous cell carcinomas. J Histochem Cytochem 48: 113-122, 2000.
13. Suminami Y, Nawata S and Kato H: Biological role of SCC antigen. Tumour Biol 19: 488-493, 1998.
14. Suminami Y, Nagashima S, Murakami A, Nawata S, Gondo T, Hirakawa H, Numa F, Silverman GA and Kato H: Suppression of a squamous cell carcinoma (SCC)-related serpin, SCC antigen, inhibits tumor growth with increased intratumor infiltration of natural killer cells. Cancer Res 61: 1776-1780, 2001.
15. Sueoka K, Nawata S, Nakagawa T, Murakami A, Takeda O, Suminami Y, Kato H and Sugino N: Tumor-associated serpin, squamous cell carcinoma antigen stimulates matrix metalloproteinase-9 production in cervical squamous cell carcinoma cell lines. Int J Oncol 27: 1345-1353, 2005.
16. Nawata S, Murakami A, Hirabayashi K, Sakaguchi Y, Ogata H, Suminami Y, Numa F, Nakamura K and Kato H: Identification of squamous cell carcinoma antigen-2 in tumor tissue by two-dimensional electrophoresis. Electrophoresis 20: 614-617, 1999.
17. Nawata S, Nakamura K, Hirakawa H, Sueoka K, Emoto T, Murakami A, Umayahara K, Ogata H, Suminami Y, Numa F and Kato H: Electrophoretic analysis of the cleaved form of serpin, squamous cell carcinoma antigen-1 in normal and malignant squamous epithelial tissues. Electrophoresis 24: 2277-2282, 2003.
18. Takeshima N, Nakamura K, Takeda O, Morioka H, Tamura H, Takasugi N and Kato H: Individualization of the cutoff value for serum squamous-cell carcinoma antigen using a sensitive enzyme immunoassay. Tumour Biol 11: 167-172, 1990.
19. Nawata S, Suminami Y, Hirakawa H, Murakami A, Umayahara K, Ogata H, Numa F, Nakamura K and Kato H: Electrophoretic characterization of heat-stable squamous cell carcinoma antigen. Electrophoresis 22: 3522-3526, 2001.
20. Nawata S, Suminami Y, Hirakawa H, Murakami A, Ogata H, Numa F, Fujimoto M, Tanaka T, Nakamura K and Kato H: Nondenaturing two-dimensional electrophoretic analysis of loop-sheet polymerization of serpin, squamous cell carcinoma antigen-2. Electrophoresis 22: 161-164, 2001.
21. Abe H, Okuno N, Takeda O, Suminami Y, Kato H and Nakamura K: Analysis on heterogeneity of squamous cell carcinoma antigen by two-dimensional electrophoresis. Electrophoresis 15: 988-991, 1994.
22. Nustad K, Dowell BL, Davis GJ, Stewart K, Nilsson O, Röijer E, Suminami Y, Nawata S, Cataltepe S, Silverman GA, Kato H and de Bruijn HWA: Characterization of monoclonal antibodies directed against squamous cell carcinoma antigens: Report of the TD-10 Workshop. Tumour Biol 25: 69-90, 2004.
23. Janciauskiene S: Conformational properties of serine proteinase inhibitors (serpins) confer multiple pathophysiological roles. Biochim Biophys Acta 1535: 221-235, 2001.
24. Suminami Y, Kishi F, Murakami A, Sakaguchi Y, Nawata S, Numa F and Kato H: Novel forms of squamous cell carcinoma antigen transcripts produced by alternative splicing. Biochim Biophys Acta 1519: 122-126, 2001.
25. Bae SM, Lee CH, Cho YL, Nam KH, Kim YW, Kim CK, Han BD, Lee YJ, Chun HJ and Ahn WS: Two-dimensional gel analysis of protein expression profile in squamous cervical cancer patients. Gynecol Oncol 99: 26-35, 2005.
26. Gariboldi M, Peissel B, Fabbri A, Saran A, Zaffaroni D, Falvella FS, Spinola M, Tanuma J, Pazzaglia S, Mancuso MT, Maurichi A, Bartoli C, Cataltepe S, Silverman GA, Pilotti S, Hayashizaki Y, Okazaki Y and Dragani TA: SCCA2-like serpins mediate genetic predisposition to skin tumors. Cancer Res 63: 1871-1875, 2003.