9

Involvement of Squamous Cell Carcinoma Antigen in Invasion and Metastasis of Squamous Cell Carcinoma of Uterine Cervix

Akihiro Murakami*, Keiko Yoshidomi and Norihiro Sugino
Yamaguchi University Graduate School of Medicine,
Department of Obstetrics and Gynecology, Ube
Japan

1. Introduction

A tumor-related protein, squamous cell carcinoma antigen (SCCA) was first discovered in uterine cervical squamous cell carcinoma [1], and subsequently has been used as a useful tumor marker for squamous cell carcinoma of various organs [2-4]. Cloning and characterization of *SCCA* cDNA has revealed that SCCA belongs to serine proteinase inhibitor (serpin) family [5]. Since SCCA is present not only in squamous cell carcinomas but also in normal squamous epithelium, the biological function of SCCA is of great interest. The present paper reviews the current understanding of SCCA, focusing on its biological function in uterine cervical squamous cell carcinoma.

2. Characteristics of SCCA

SCCA consists of more than 10 protein fractions with different isoelectric points, ranging from 5.9 to 6.6, which are roughly divided into two groups: the acidic SCCAs with pIs of less than 6.25 and the neutral SCCAs with pIs of 6.25 or higher [6]. The neutral SCCAs are generally present inside the cell, whereas the acidic SCCAs are often increased in squamous cell carcinomas and is easily secreted by the cell [6]. In 1991, our laboratory reported the cloning of *SCCA* cDNA, which consist of 1,170 nucleotides coding for 390 amino acids [5]. Schneider et al. also found two *SCCA* genes (*SCCA1* and *SCCA2*) and these two genes were tandemly arrayed at the human chromosome 18q21.3 locus [7, 8]. The predicted amino acid sequences of SCCA1 and SCCA2 are 92% identical and have identical predicted secondary structures, which suggests that *SCCA1* gene encodes the neutral SCCA, while *SCCA2* gene encodes the acidic SCCA [7]. SCCA1 inhibits the activities of serine proteinases, e.g. chymotrypsin and cysteine proteinases, e.g. cathepsin K, L, S and papain, whereas SCCA2 inhibits serine proteinases such as cathepsin G and chymase *in vitro* [9-12] (Table 1). For these reasons, SCCA1 and SCCA2 are thought to have different biological functions. It is thus of interest to better understand the biological behaviors of SCCAs in normal squamous epithelium and squamous cell carcinomas.

*Corresponding Author

group of proteinases	proteinases	inhibitors	
		SCCA1	SCCA2
Serine proteinase	chymotrypsin	+	-
	chymase	-	+
	cathepsin G	-	+
	plasmin	-	-
	plasminogen activator	-	-
	thrombin	-	-
	trypsin	-	-
Cysteine proteinase	cathepsin B	-	-
	cathepsin H	-	-
	cathepsin K	+	-
	cathepsin L	+	-
	cathepsin S	+	-
	papain	+	-

Table 1. Inhibitory effects of SCCAs on proteinases.

3. Evaluation of SCCA in clinical practice

Serum SCCA levels have been used as an indicator of a variety of squamous cell carcinomas, including skin cancers, head and neck cancers, esophageal cancers, lung cancers, bladder cancers, epidermoid cancers of the anal canal, and malignant transformation of mature cystic ovarian teratoma [13]. Serum SCCA levels are especially useful for monitoring treatment efficacy, disease progression and recurrence. In general, increased serum SCCA levels reflect disease progression and poor prognosis in squamous cell carcinomas [13]. In advanced cancers, pretreatment serum SCCA levels are associated with clinical stages, tumor sizes, and lymph node involvement. Furthermore, over 6 ng/ml of serum SCCA level shows a significant independent effect on survival and disease-free survival [14]. Even in the early stage of uterine squamous cell carcinomas, elevated serum SCCA levels predict pelvic lymph node involvement and are associated with a poor prognosis [15]. Recently, patients with elevated *SCCA2/SCCA1* mRNA ratios in uterine squamous cell carcinoma tissues were found to be at higher risk for recurrence in early stage uterine cervical cancers, suggesting SCCA2 is increased during cervical carcinogenesis [16]. In addition to malignant diseases, several benign and chronic inflammatory skin diseases, such as psoriasis, pemphigus, or eczema are often characterized by elevated SCCA levels [13]. SCCA will be a useful marker for monitoring the status of these diseases not only for malignant diseases but also for non-malignant diseases.

4. Role of SCCA in normal squamous epithelial cells

Human squamous epithelium is composed of four compartments; *stratum germinativus*, *stratum spinosum*, *stratum granulosum* and *stratum corneum*. Immunohistochemical staining

shows that SCCA is present in the spinous and granular compartments, but not in the basal and parabasal cells [17] (Fig. 1). SCCA is not present in the epithelial region adjacent to the squamo-columnar junction of the uterine cervix. Interestingly, SCCA levels begin to increase at 18-20 weeks of pregnancy for the first time when the fetal epidermis begins to cornify during the development of human fetal skins [18]. *SCCA* genes has been found in most of the eutheria (placental mammals), but not in other vertebrates [19]. Furthermore, several eutherian species show heterogeneous patterns of *SCCA* nucleotides in Southern blot analyses [19]. This suggests that SCCA has had a role in the stratification and differentiation of integuments during evolutional change.

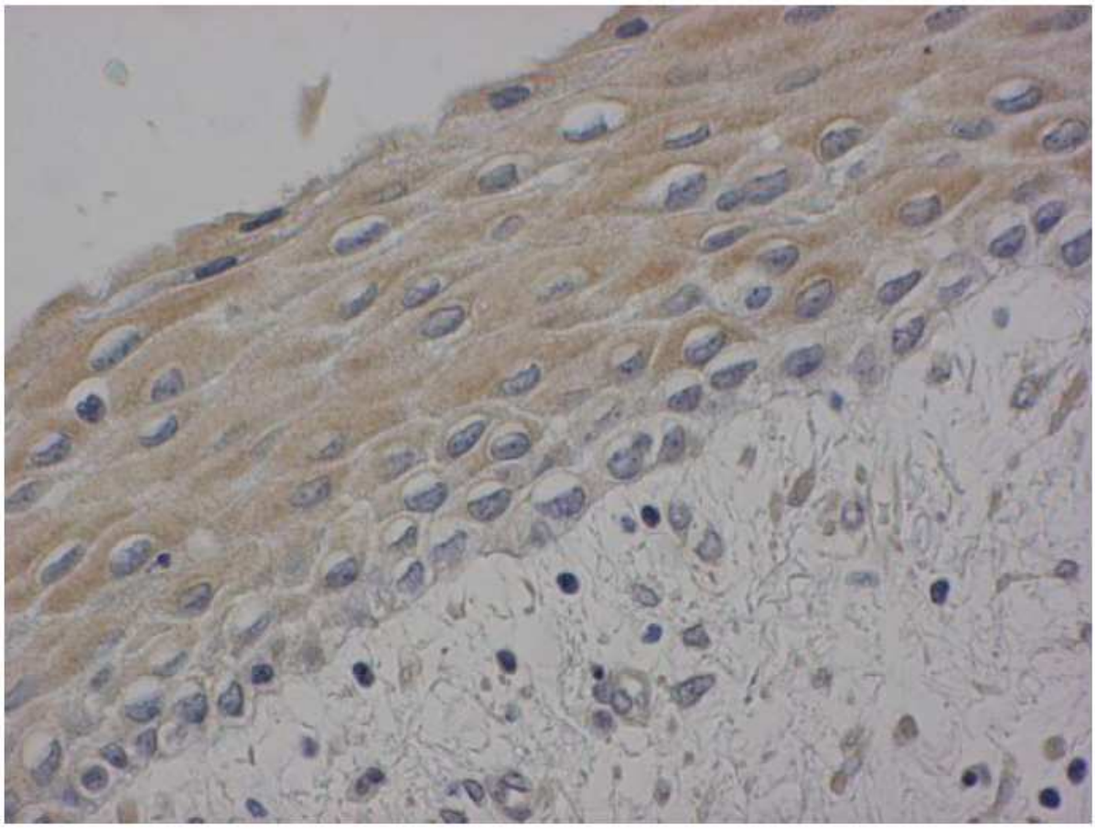


Fig. 1. Immunohistochemistry for SCCA expression in normal cervical squamous epithelium. SCCA is expressed in all epithelial layers except the basal layer (original magnification: X 100).

The stratification and cornification of normal squamous epithelial cells are influenced by extracellular calcium concentrations. Calcium concentrations are low in the parabasal layer but high in the granular layers. Keratinocytes begin to stratify and cornify in the presence of high concentrations of calcium [20]. High concentrations of calcium stimulate the production of neutral SCCA, whereas low concentration of calcium stimulate the production of acidic SCCA [21].

The final stage of differentiation of squamous epithelial cells is modulated by several cysteine proteinases, such as cathepsin L, calpain, and epidermal transglutaminase [20].

SCCA1 inhibits cathepsin L and some of the proteinases in the spinous and granular layers, suggesting that SCCA1 inhibits UV-induced apoptosis of squamous epithelial cells to maintain barrier functions in the squamous epithelium. On the other hand, SCCA2 may act outside of the cells to enhance the cell adhesion system in the parabasal layer [22, 23], suggesting that SCCA2 may play important roles to maintain the structure of the normal squamous epithelium, particularly structure of the thick stratum corneum in mammalian species.

5. Role of SCCA in squamous cell carcinoma of uterine cervix

Anti-tumor therapeutics inhibits the cancer cell proliferation and induce necrotic and apoptotic cell death. However, some cancer cells acquire the ability to resist anti-tumor therapeutics. Thus, proliferation, cell invasion and migration are the most crucial biological events in the progression of cancer.

Recently, much attention has been focused on the role of proteinases and their inhibitors in the malignant behavior of cancer cells. Proteinase inhibitors are thought to suppress the apoptotic process of cancer cells. Apoptosis involves complicated mechanisms with multistep pathways. Some serpins are involved in the apoptotic process. In squamous cell carcinoma tissues, the expression levels of SCCA2 are higher than those in normal squamous epithelial tissues, suggesting that SCCA2 plays a role in suppressing apoptotic cell death [24, 25]. Both SCCA1 and SCCA2 belong to the ov-serpin family, and some of the ov-serpins have been reported to inhibit apoptosis [5]. In fact, SCCA1 inhibits both serine proteinases and cysteine proteinases, and SCCA2 inhibits serine proteinases [9-12]. Although the target proteinases are different, both SCCA1 and SCCA2 inhibit apoptosis. SCCA1 suppresses apoptosis induced by activated natural killer cells, TNF-α, irradiation and anti-tumor agents, while SCCA2 suppresses apoptosis induced by irradiation and TNF-α [26-28]. Both SCCAs suppress the activity of caspase-3 and caspase-9 via down-regulation of p38 MAPK and/or MKK3/MKK6 [27]. These results suggest that SCCAs in tumor cells help to protect cancer cells from apoptotic cell death, both from therapeutic modalities and the immune systems. Proteinase inhibitors are also thought to suppress the invasion and metastasis of cancer cells by inhibiting proteinase activities that disrupt the cell-to-cell adhesion system. In the first step of cancer metastasis, loss of E-cadherin expression causes detachment of cancer cells from the primary tumor lesion. After the detachment from the primary tumor, cancer cells migrate, attach to vessels, and move to other organs through blood and lymph fluid flow. In fact, suppression of SCCA2 expression promoted cell invasion and cell migration with the decreased expression of E-cadherin [29, 30]. Blockage of E-cadherin action suppressed SCCA production in squamous cell carcinoma cell lines [31]. Our immunohistochemical study on cervical squamous cell carcinoma revealed that SCCA2 expression was significantly related with E-cadherin expression and that mixed pattern with loss and positive stained of SCCA2 and E-cadherin in primary lesions was strongly associated with high incidence of lymph node metastasis [32]. These facts strongly suggest that cancer cells with loss of SCCA2 expression, as well as loss of E-cadherin expression, metastasize to other organs including the lymph nodes. In contrast, increased expression of E-cadherin induces the increase of SCCA2 expression through a PI3K - Akt pathway in uterine squamous cell carcinoma cells [33]. These results suggest that the decrease in E-cadherin expression causes cancer cells to detach from the primary tumor, and acquire the

activated E-cadherin – SCCA system, which leads to their aggregation, survival, and growth into metastatic tumors (Fig. 2).

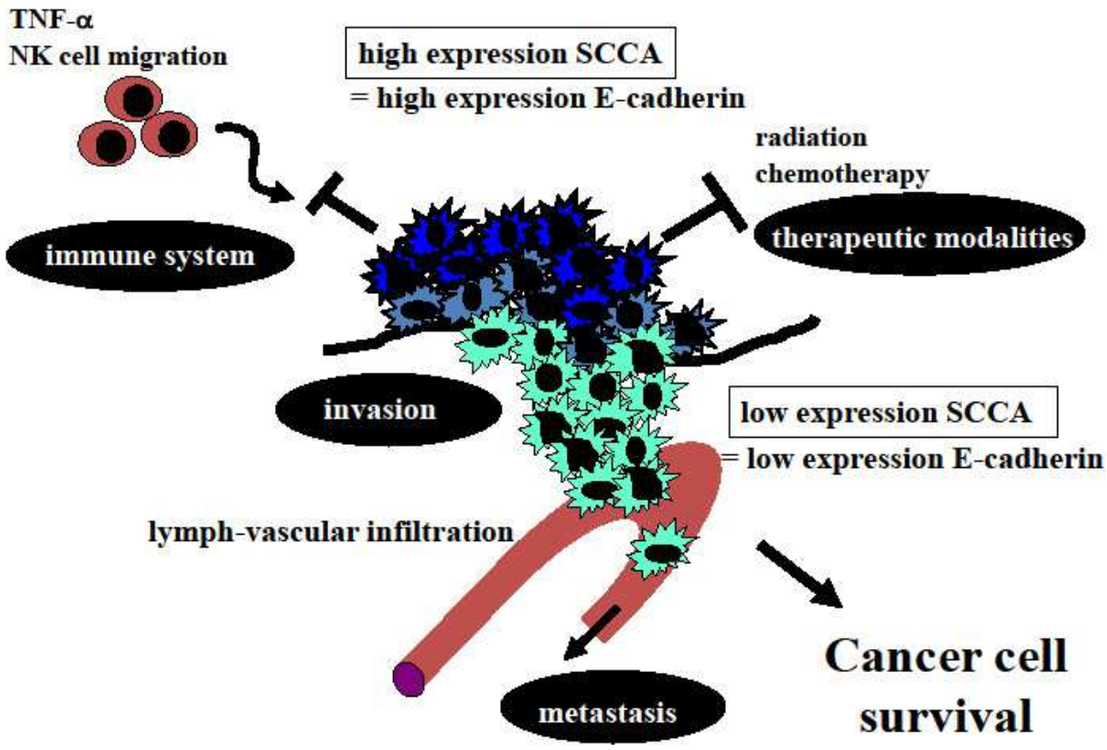


Fig. 2. Possible roles of SCCA in tumor cell survival and metastasis in uterine cervical squamous cell carcinoma. Cancer cells with abnormally high expression of SCCA are resistant to apoptosis induced by the immune system and therapeutic modalities. In contrast, cancer cells with abnormally low expression of SCCA show loss of E-cadherin expression, resulting in detachment from the primary tumor lesion. These cells migrate, attach to the vessels, and metastasize in other organs through blood and lymph fluid flow.

6. Conclusions

SCCAs have been regarded as a useful tumor marker for squamous cell carcinoma in clinical practice. Furthermore, they have some interesting biological functions. SCCAs are regarded as a useful tumor marker for squamous cell carcinoma in clinical practice. In normal squamous epithelium, SCCA may have roles in the stratification, cornification, barrier functions and structure of the epithelium. In squamous cell carcinomas, both SCCA1 and SCCA2 suppress apoptosis by inhibiting serine and cysteine proteinases concerned that function in the apoptotic pathway, resulting in the proliferation of cancer cells. Furthermore, suppression of SCCA2 promoted cancer cell invasion and migration with the decreased expression of E-cadherin, resulting in cancer cell metastases. Thus, SCCA appears to have roles not only in the normal squamous epithelium but also in the squamous cell carcinomas.

7. Conflict of interest

The authors declare no conflict of interest.

8. References

[1] Kato H and Torigoe T: Radioimmunoassay for tumour antigen of human cervical squamous cell carcinoma. Cancer 40: 1621-1628, 1977

[2] Kato H, Tamai K, Morioka H, Nagai M, Nagaya T, and Torigoe T: Prognostic significance of the tumour antigen TA-4 in squamous cell carcinoma of the uterine cervix. Am J Obstet Gynecol 145: 350-354, 1983

[3] Maruo T, Shibata K, Kimura A, Hoshina A, and Mochizuki M: Tumour-associated antigen, TA-4, in the monitoring of the effects of therapy for squamous cell carcinoma of the uterine cervix. Cancer 59: 302-308, 1985

[4] Brioschi PA, Bischof P, Delafosse C and Krauer F: Squamous cell carcinoma antigen (SCC-A) values related to clinical outcome of pre-invasive and invasive cervical carcinoma. Int J Cancer 47: 376-379, 1991

[5] 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 Comm 181: 51-58, 1991

[6] Kato H, Nagaya T, and Torigoe T: Heterogeneity of a tumor antigen TA-4 of squamous cell carcinoma in regulation to its appearance in circulation. Gann 75: 433-435, 1984

[7] 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

[8] Kuwano A, Kondo I, Kishi F, Suminami Y, and Kato H: Assignment of the squamous cell carcinoma antigen locus (SCC) to 18q21 by in situ hybridization. Genomics 30: 626, 1995

[9] Nawata S, Tsunaga N, Numa F, Tanaka T, Nakamura K, and Kato H: Serine protease inhibitor activity of recombinant squamous cell carcinoma antigen towards chymotrypsin, as demonstrated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Electrophoresis 16: 1027-1030, 1995

[10] Nawata S, Nakamura K, Tanaka T, Numa F, Suminami Y, Tsunaga N, Kakegawa H, Katsunuma N, and Kato H: Electrophoretic analysis of the“cross-class” interaction between novel inhibitory serpin, squamous cell carcinoma antigen-1 and cystein proteinases. Electrophoresis 18: 784-789, 1997

[11] Schick C, Kamachi Y, Bartuski A J, Çataltepe S, Schechter NM, Pemberton PA, and Silverman GA: Squamous cell carcinoma antigen 2 is a novel serpin that inhibits the chymotrypsin-like proteinases cathepsin G and mast cell chymase. J Biol Chem 17: 1849-1855, 1997

[12] Schick C, Pemberton PA, Shi GP, Kamachi Y, Çataltepe S, Bartuski AJ, Cornstein ER, Brömme D, Chapman HA, and Silverman GA: Cross-class inhibition of the cysteine proteinases cathepsin K, L, S by the serpin squamous cell carcinoma antigen 1: A kinetic analysis. Biochemistry 37: 5258-5266, 1998

[13] Kato H: Squamous cell carcinoma antigen, in: Sell S (ed), Serological Cancer markers. Human Press, Totowa, NJ, pp 437-451, 1992

[14] Ogino I, Nakayama H, Okamoto N, Kitamura T, and Inoue T: The role of pretreatment squamous cell carcinoma antigen level in locally advanced squamous cell carcinoma of the uterine cervix treated by radiotherapy. Int J Gynecol Cancer 16: 1094-1100, 2006

[15] van de Lande J, Davelaar EM, von Mendorff-Pouilly S, Water TJ, Berkhof J, van Baal WM, Kenemans P, and Verheijen RH: SCC-Ag, lymph node metastases and sentinel node procedure in early stage squamous cell cervical cancer. Gynecol Oncol 112: 119-125, 2009

[16] Hsu KF, Huang SC, Shiau AL, Cheng YM, Shen MR, Chen YF, Lin CY, Lee BH, and Chou CY: Increased expression level of squamous cell carcinoma antigen 2 and 1 ratio is associated with poor prognosis in early-stage uterine cervical cancer. Int J Gynecol Cancer 17: 174-181, 2007

[17] Suehiro Y, Kato H, Nagai M, Torigoe T: Flow cytometric analysis of tumor antigen TA-4 in cervical cytological specimens. Cancer 57: 1380-1384, 1986

[18] Takeshima N, Suminami Y, Takeda O, Abe H, and Kato H: Origin of CA125 and SCC antigen in human amniotic fluid. Asia Oceania J Obstet Gynecol 19: 199-204, 1993

[19] Michioka T, Takeshima N, Tsunaga N, Suminami Y, Nawata S, Kato H: Expression of squamous cell carcinoma antigen, a serine proteinase inhibitor, in the integment of vertebrates: possible role in stratification of epidermis. Acta Histochem Cytochem 27: 435-440, 1994

[20] Yuspa SH: The pathogenesis of squamous cell cancer: Lessons learned from studies of skin carcinogenesis-Thirty-third G.H.A. Clowes Memorial Award Lecture. Cancer Res 54: 1178-1189, 1994

[21] Tsunaga N: Effects of calcium on the production of squamous cell carcinoma antigen in normal human keratinocytes. Yamaguchi Igaku 43: 419-426, 1994 (abstract in English)

[22] Katagiri C, Nakanishi J, Kadoya K, and Hibino T: Serpin squamous cell carcinoma antigen inhibits UV-induced apoptosis via suppression of c-JUN NH_2-terminal kinase. J Cell Biol 172: 983-990, 2006

[23] Katagiri C, Negishi K, and Hibino T: c-JUN N-terminal kinase-1 (JNK1) but not JNK2 or JNK3 is involved in UV signal transduction in human epidermis. J Dermatol Sci 43: 171-179, 2006

[24] 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

[25] Murakami A, Suminami Y, Sakaguchi Y, Nawata S, Numa F, Kishi F, and Kato H : Specific detection and quantitation of *SCC antigen 1* and *SCC antigen 2* mRNAs by fluorescence-based asymmetric semi-nested reverse transcription PCR. Tumour Biol 21: 224-234, 2000

[26] Suminami Y, Nagashima S, Vujanovic NL, Hirabayashi K, Kato H, and Whiteside TL: Inhibition of apoptosis in human tumour cells by the tumour-associated serpin, SCC antigen-1. Br J Cancer 82: 981-989, 2000

[27] Murakami A, Suminami Y, Hirakawa H, Nawata S, Numa F, and Kato H: Squamous cell carcinoma antigen suppresses radiation-induced cell death. Br J Cancer 84: 851-858, 2001

[28] McGettrick AF, Barnes RC and Worrall DM: SCCA2 inhibits TNF-mediated apoptosis in transfected HeLa cells. Eur J Biochem 268: 5868-5875, 2001

[29] Iwasaki M, Nishikawa A, Akutagawa N, Fujimoto T, Teramoto M, Sakaguchi Y, Kato H, Ito M, Yoshida K, Kudo R: E1AF/PEA3 reduces the invasiveness of SiHa cervical

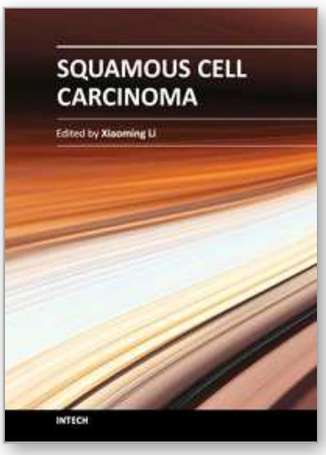
cancer cells by activating serine proteinase inhibitor squamous cell carcinoma antigen. Exp Cell Res 299: 525-532, 2004

[30] Murakami A, Nakagawa T, Kaneko M, Nawata S, Takeda O, Kato H, Sugino N: Suppression of SCC antigen promotes cancer cell invasion and migration through the decrease in E-cadherin expression. Int J Oncol 29: 1231-1235, 2006

[31] Hirakawa H, Nawata S, Sueoka K, Murakami A, Takeda O, Numa F, Kato H, and Sugino N: Regulation of squamous cell carcinoma antigen production by E-cadherin mediated cell-cell adhesion, ion in squamous cell carcinoma cell line. Oncol Rep 11: 415-419, 2004

[32] Murakami A, Nakagawa T, Fukushima C, Torii M, Sueoka K, Nawata S, Takeda O, Ishikawa H, Sugino N: Relationship between decreased expression of squamous cell carcinoma antigen 2 and E-cadherin in primary cervical cancer lesions and lymph node metastasis. Oncol Rep 19: 99-104, 2008

[33] Nakagawa T, Murakami A, Torii M, Nawata S, Takeda O, and Sugino N: E-cadherin increases squamous cell carcinoma antigen expression through phosphatidylinositol-3 kinase-Akt pathway in squamous cell carcinoma cell lines. Oncol Rep 18: 175-179, 2007



Squamous Cell Carcinoma
Edited by Prof. Xiaoming Li

ISBN 978-953-51-0024-9
Hard cover, 302 pages
Publisher InTech
Published online 03, February, 2012
Published in print edition February, 2012

This book points to some new areas for investigation on squamous cell carcinoma (SCC). Firstly, the features and management of some specific SCC is discussed to give the readers the general principles in dealing with these uncommon and sophisticated conditions. Some new concepts in adjuvant therapy including neoadjuvant therapy and gold nanoparticle-based photo dynamic therapy are introduced. Secondly, a detailed discussion of molecular aspects of tumor invasion and progression in SCC is provided with the emphasis on the roles of some important factors. The role of tumor microenvironment in head and neck SCC is specifically discussed. Thirdly, the roles of cancer stem cells (CSC) in cancer therapy of SCC are described. Molecular mechanisms involving therapeutic resistance and new therapeutic strategies targeting CSC are discussed in detail. Finally, other aspects concerning SCC are included, which involve the assessment, genetic manipulation and its possible clinical implications for the treatment of SCC.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen