Overexpression of SENP5 in oral squamous cell carcinoma and its association with differentiation

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Abstract. Small ubiquitin-like modifiers (SUMOs) play an important role in tumors. The SUMOylation can be reversed by SUMO-specific proteases (SENPs). As one of the crucial members of the SUMO system, SENP5 is essential in mitosis and/or cytokinesis. In the present study, we analyzed the expression of SENP5 in human oral squamous cell carcinoma and investigated the relationship between its expression level and clinicopathological parameters. Expression levels of SENP5 in 48 patients with oral squamous cell carcinoma were investigated by immunohistochemistry. Immunoreactivity was evaluated semiquantitatively using German immunoreactive score (IRS). The relationship between SENP5 expression status and the clinical and pathological characteristics of patients was analyzed and survival curves were calculated using the Kaplan-Meier method and log-rank tests. We found that SENP5 predominantly localized to cytoplasm of OSCC cells and the expression of SENP5 in OSCC is not the same as in paracarcinoma epithelia. The SENP5 expression status was associated with differentiation of OSCC and not with any other clinicopathological parameters. These findings suggest altered subcellular localization of SENP5 in OSCC cells and the correlation between SENP5 expression and the differentiation of OSCC.

Introduction

Squamous cell carcinoma is the most common malignant tumor in oral cavity. It is related to lymphogenous spread, local recurrence and distant metastasis. Survival is associated with tumor size, nodal involvement, distant metastasis, staging, differentiation and betel quid chewing (1).

SUMOylation is one of the most important post-translational modifications. The small ubiquitin-like modifiers (SUMOs) are ubiquitin-like proteins and as with ubiquitin, these modifiers are conjugated by a serial of enzymes to cellular regulators. Consequently, the localization, activity and stability of the substrates are changed (2). The SUMOylation can be reversed by SUMO-specific proteases (SENPs) which can mediate processing of SUMO precursors, remove SUMO from modified proteins and depolymerize poly-SUMO chains (3). Many oncoproteins and tumor suppressor proteins have proven to be the substrates for SUMOylation (4-7). Given the substrates involved, SUMOylation is important in the course of tumorigenesis, and is altered in human cancer types (8). Evidence to support this notion, and research on SUMOylation related to oral squamous cell carcinoma (OSCC) is scarce.

Humans have seven SENPs (SENP-1, -2, -3, -5, -6, -7 and -8), and several of these have been characterized as SUMO (or Nedd8) endopeptidases or isopeptidases. However, almost no information exists on the function of SENP5 in tumorigenesis. In the present study, we investigated the expression of SENP5 in oral squamous cell carcinoma and paracarcinoma relative to normal epithelium tissue using immunohistochemistry. We investigated the possible correlations among SENP5 expression, pathological characteristics and clinical prognosis.

Materials and methods

Clinical study. Archived paraffin-embedded tissue specimens from 48 previously untreated patients were obtained from the Department of Oral Pathology, The 9th People's Hospital, Shanghai Jiaotong University, Shanghai, P.R. China. The patients included 26 males and 22 females, with a mean age of 57.65 years (range 24-81). The number of primary tumor sites were: tongue, 34; gingiva, 7; buccal mucosa, 3; oral floor, 3; and palate, 1. The sections from the cases were stained with hematoxylin and eosin (H&E). The histological grades,

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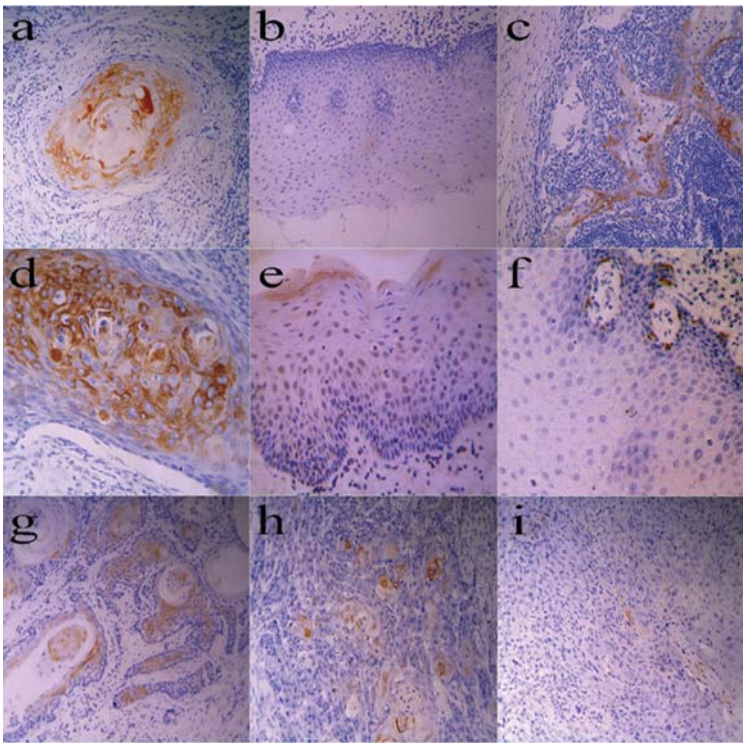

Figure 1. Expression of SENP5 in oral squamous cell carcinoma and paracarcinoma epithelial tissues. Immunohistochemical detection of SENP5 protein in (a) cancer and (b) paracarcinoma epithelial tissue. The expression of SENP5 in the cancer is significantly higher than in the paracarcinoma relative to normal mucosa. (c) Positive staining tumor cells in the lymphatic vessels of the lymph nodes. (d) Cytoplasmic expression was predominantly observed in tumor cells. (e) Nuclear staining in paracarcinoma epithelium occurs throughout the epithelium layer and (f) cytoplasmic staining is sparse in the basal layer of the paracarcinoma epithelium. (g) SENP5 expression in well-differentiated and (h) moderately differentiated tumors is higher than in (i) poorly differentiated tumors.

diagnosed according to Broder's classification system, consisted of well-, 17; moderately, 27; and poorly differentiated carcinomas, 4. Details of the lymph node metastasis were gathered from patient records.

The follow-up studies were obtained from the hospital charts. At least 5-year cumulative survival was observed among the 48 patients and 36 are still alive.

Immunohistochemical study. Immunohistochemical staining was carried out by means of the avidin-biotin complex (ABC) immunoperoxidase method. Sections (4 μm) were cut from each tissue block, including the tumor and adjacent epithelial tissue. The sections were deparaffinized in xylene, rehydrated in decreasing concentrations of ethanol and treated with 3% hydrogen for 20 min at room temperature to remove endogenous peroxidase activity. After antigen retrieval performed by microwave in citrate buffer (pH 6.0) for 20 min, sections were immunostained for SENP5 (1:50, Abgent, CA, USA) and incubation was carried out overnight at 4˚C. After washing in phosphate saline buffer (PBS), sections were incubated with secondary antibody for 1 h. Staining was developed using ABC reagents (Dako, Glostrup, Denmark) and 3'3-diaminobenzidine tetrahydrochloride (DAB) as a substrate. The sections were counterstained with hematoxylin for 5 min, dehydrated and mounted. Negative controls were prepared by replacing the primary antibody with PBS and no reactive products were detected.

Each case was evaluated in terms of staining intensity as positive and negative cells. Quantity scores were given as 0-4 if 0%, and 0-5%, 5-35%, 35-70% and 70-100% cells were positive, respectively. Staining intensity was judged in a semiquantitative way (0 negative, 1 weak, 2 moderate and 3 strong staining). The raw data were then converted into German immunoreactive score (IRS) by multiplying quantity and staining intensity scores. An IRS score of 6 or higher was considered to be a strong reactivity, 4-5 moderate, 2-3 weak and 0-1 negative.

Statistical analysis. Statistical analyses were performed using SPSS 11.0 software. Relationships between SENP5 expression and clinicopathological factors were examined with Fisher's exact or Wilcoxon signed-ranks tests. The cumulative survival of patients was assessed by the Kaplan-Meier method and log-rank test. $P<0.05$ was considered statistically significant.

Results

Expression patterns of SENP5 in oral squamous cell carcinoma. SENP5 was mainly expressed in the well-differentiated cells located at the inner layer of carcinoma nests. No staining was found in the peripheral cells of the nests (Fig. 1a). Cytoplasmic expression was predominantly observed in tumor cells (Fig. 1d). Only two cases had some positive staining in the nuclei of tumor cells while positive staining in cytoplasm was chiefly detected in these two cases. Some

Table I. Correlations between SENP5 expression and clinicopathological findings.

	Cases	SENP5, No. (%)				P
		0	1	2	3	
OSCC	48	12 (25.0)	19 (39.6)	4 (8.3)	13 (27.1)	
Differentiation						0.019
Well	17	1 (5.9)	6 (35.3)	4 (23.5)	6 (35.3)	
Moderate	27	10 (37.0)	10 (37.0)	0 (0)	7 (25.9)	
Poor	4	1 (25.0)	3 (75.0)	0 (0)	0 (0)	
Pathological T stage						0.459
T1+T2	27	8 (29.6)	12 (44.4)	2 (7.4)	5 (18.5)	
T3+T4	11	4 (19.0)	7 (33.3)	2 (9.5)	8 (38.1)	
Pathological N stage						0.557
pN0	37	9 (24.3)	15 (40.5)	2 (5.4)	11 (29.7)	
pN1+pN2+pN3	11	3 (27.3)	4 (36.4)	2 (18.2)	2 (18.2)	
Stage classification						0.520
I+II	23	7 (30.4)	10 (43.5)	2 (8.7)	4 (17.4)	
III+IV	25	5 (20.0)	9 (36.0)	2 (8.0)	9 (36.0)	

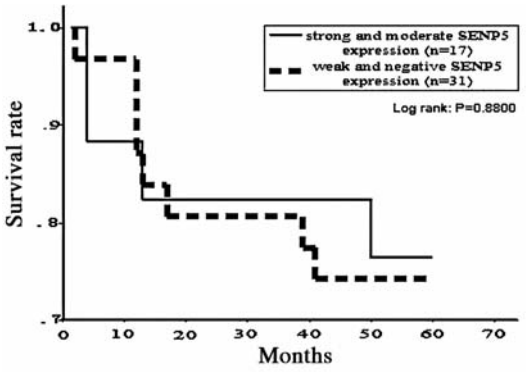

Figure 2. Kaplan-Meier survival curves of patients with oral squamous cell carcinoma subdivided according to SENP5 status. No significant difference exists between the two groups.

tumors exhibited weak or no SENP5 expression. We observed positively stained tumor cells in the lymphatic vessels of the lymphonodes (Fig. 1c).

SENP5 was expressed sparsely in paracarcinoma relative to normal epithelial cells (Fig. 1b) as we only detected epithelial staining in 8 (17%) cases. Among these cases, cytoplasmic staining was observed in 5 cases, nuclear in 2 and both of them in 1. Cytoplasmic staining was sparsely observed in the basal cell layer (Fig. 1f) or in mass in the granular and spinous layers while nuclear staining occurred throughout the whole epithelial layer (Fig. 1e). There was no detected correlation between SENP5 expression and the degree of epithelial dysplasia in the paracarcinoma epithelium. Brown staining was seen in the cytoplasm of certain inflammatory, filament and nerve cells.

SENP5 expression in the tumor and its association with the clinicopathological characteristics in patients with OSCC. In the 48 cases, 12 (25%) had a negative expression of SENP5 (IRS score 0-1), 23 (48%) had a weak or moderate (IRS score 2-5) and 13 (27%) had a high expression (IRS score ≥6). Pathological grading was associated with SENP5 expression. Sixteen of 17 well-differentiated cases had SENP5 expression (10 cases had a high or moderate expression). Seventeen of 27 moderately differentiated cases had SENP5 expression (7 cases had a high or moderate expression). Three of four poorly differentiated cases had weak SENP5 expression. There was no expression difference between primary tumors and lymph node metastatic lesions. SENP5 expression was not associated with lymph node metastasis and cumulative survival (Fig. 2). No statistically significant associations were detected among SENP5 expression and other clinicopathological characteristics (Table I).

Discussion

Many studies have revealed the relationship between the SUMOylation system and tumors. As the essential members of the SUMOylation system, the SENP family is undoubtedly involved in tumors even though there are only a few studies on SENP expression in tumors. Some investigations have proven that SENP1 is related to the development of prostate cancer and SENP1 overexpression has been found in thyroid oncocytic tumors (9,10). Fusion of the genes SENP1 and MESDC2 were detected in a patient with an infantile teratoma and a constitutional t(12;15)(q13;q25) (11). However, the association among other members of the SENP family and tumors have yet to be investigated.

As one of the essential members of the SENP family, SENP5 is localized to the nucleolus by sequences in its N-terminal regions and appears to have a distinct preference for deconjugating and processing SUMO-2 and -3 over SUMO-1

Table II. Immunohistochemical evaluation of SENP5 in oral squamous cell carcinoma (OSCC) and paracarcinoma epithelium (PE) (Wilcoxon signed-ranks test).

	Cases	SENP5, No. (%)				P
		0	1	2	3	
Tissue						0.000
OSCC	48	12 (25)	19 (40)	4 (8)	13 (27)	
PE	48	40 (83)	8 (17)	0 (0)	0 (0)	

Table III. SENP5 localization in OSCC and paracarcinoma epithelium (PE) (Fisher's exact test).

	Cases	SENP5, No. (%)			P
		Nucleus	Cytoplasm	Both	
Tissue					0.01
OSCC	43	0 (0)	41 (95.3)	2 (4.7)	
PE	8	2 (25.0)	5 (62.5)	1 (12.5)	

(12-14). The small interfering RNA (siRNA)-mediated ablation of SENP5 expression resulted in the inhibition of cell proliferation with the appearance of binucleate cells and cells with an altered nuclear architecture. Thus, SENP5 appears to have an essential role in mitosis and/or cytokinesis (13,15). In addition to the essential role in cell division, SENP5 is required to maintain the normal mitochondrial morphology and the intracellular levels of reactive oxygen species (16). The cytosolic pool of SENP5 catalyzes the cleavage of SUMO-1 from a number of mitochondrial substrates. The cytosolic SENP5 maintains the mitochondrial morphology, in part, through its effect on DRP1 deSUMOylation.

In the present study, we investigated SENP5 expression in OSCC. A much higher expression in tumor tissue than adjacent normal epithelia indicated a role of SENP5 in OSCC (Table II). Although a large number of studies in the last decade have shown the role of SUMOylation system in several types of tumors other than SCC, only very recently was the function of Piasy (one of SUMOylation factors) in human skin squamous cell carcinoma (SCC) hypothesized and over-expression of SUMO-1 proved to be detected in OSCC (17,18). This raised the question of whether the overexpression of SUMO-1 leads to overexpression of SENP5 in order to maintain the mitochondrial morphology or the reverse. The relationship between overexpression of SUMO-1 and SENP5 in OSCC has yet to be clarified. Katayama *et al* found that SUMO-1 was possibly involved in tumor proliferation and prognosis and the dual-high expression of SUMO-1 and Mdm2 was associated with local occurrence in early stage OSCC (17). We failed to show any statistically significant correlation between SENP5 expression and cumulative survival, or SENP5 expression and lymph node metastasis in OSCC. However, the correlation between SUMOylation and OSCC was clear.

SUMOylation may play an important role in keratinocyte differentiation. In a human keratinocyte cell line model (HaCaT cells), it was proven by Wilson *et al* recently that Ca^{2+}-induced differentiation led to the transient transcriptional activation of genes encoding crucial SUMOylation system components including Ubc9 and SENP1 (19). Squamous cell carcinoma may be considered as the tissue mainly composed of non-differentiated keratinocytes. We found that SENP5 was mainly expressed in the cells located at the inner layer of carcinoma nests (Fig. 1a). These positively stained cells were usually larger and better-differentiated than the negatively stained periphery cells which were morphologically similar to basal cells. However, SENP5 had not been included in the study conducted by Wilson *et al*, nor were the results suitable for comparison. A significant correlation between SENP5 immunoreactivity and pathological grades ($P<0.05$) was found in our investigation. The correlation implied that better-differentiated cases had more SENP5 expression. This suggested the possibility that SENP5 immunoreactivity is the marker of differentiation in oral squamous cell carcinoma (Fig. 1g-i) and has implications in the induced differentiation therapy of OSCC.

SENP5 was confirmed to localize predominantly to the nucleolus and the N-terminal portion of SENP5 contained sequences essential for nucleolar localization (3,13,14,16). Deletion of the N-terminal domain of SENP5 led to loss of nucleolar localization and increased deSUMOylation activity *in vivo* (13). Herein, we used a commercial anti-SENP5 antisera and found SENP5 expression was chiefly detected in the cytoplasm of tumor cells and in the cytoplasm and/or nucleus of normal epithelium cells (Table III). Conceivably, SENP5 is mainly cytoplasmic in cancer tissues because SENP5 is also largely cytoplasmic in breast cancer tissue according to the manufacturer. The shift of SENP5 location from the nucleus to cytoplasm may reflect the need of metabolism and homeostasis of tumor cells because alteration of the substrates of SENP5 occur when localization of SENP5 changes. An increase of cytosolic SENP5 may help to rescue the SUMO-1-induced mitochondrial fragmentation by catalyzing the cleavage SUMO-1 from a number of mitochondrial substrates (13). However, the reason why, and the manner in which SENP5 change its localization in OSCC cells, remain unknown as does the relationship between increased SUMO-1 and SENP5. The mechanism of the alteration is being investigated in our laboratory.

In summary, the present study showed the expression pattern of SENP5 in OSCC and suggested the correlation

between SENP5 expression and the differentiation of OSCC. More studies must be conducted to clarify the function of the overexpression of SENP5 in OSCC and the mechanism of the subcellular distribution of SENP5 in OSCC.

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