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Does securin expression have significance in prognostication of oral tongue cancer? A pilot study

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Abstract The proliferation marker, securin, is involved in the progression of many carcinomas. However, its expression in oral tongue squamous cell carcinoma (OTSCC) has not been previously studied. We examined securin expression by immunohistochemistry in OTSCC. A total of 93 cases treated for OTSCC were included in this study. Expression of securin in OTSCC was studied by immunohistochemistry of tissue microarrays (52 cases) and routine tumor sections (41 cases). Securin overexpression is significantly associated with higher tumor grade ($P = 0.03$). Overexpression of securin was observed more frequently in advanced stages of OTSCC than in earlier stages but the difference was not statistically significant. These findings suggest that overexpression of securin in OTSCC may be important during progression of this cancer. No significant association was found between securin expression and the prognosis of OTSCC.

Introduction

Oral tongue squamous cell carcinoma (OTSCC) is the most common epithelial malignancy of the oral cavity. The incidence rates of OTSCC have increased over the last few decades. Despite advances in cancer therapy, the prognosis of OTSCC remains poor. This is mainly because of high frequency of regional lymph node metastases that are not uncommon even in early stages of the disease [1]. The outcome of OTSCC varies greatly between different stages, and cervical lymph node metastasis is probably the most important prognostic marker so far [1–4]. During the last decade, numerous molecular markers have been examined

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by immunohistochemistry in different cancers [5]. However, in OTSCC the expression of several important markers remains to be determined. Therefore, further studies on the molecular background of OTSCC are required to understand tumor progression, to identify prognostic markers and to recognize therapeutic targets [6].

Cell proliferation is an important prognosticator in many cancers, and there are ongoing efforts to recognize new markers for it [7]. Securin is a proliferation marker encoded by pituitary tumor transforming gene (*PTTG1*), and it plays an important regulatory role in the cell cycle through blocking of mitosis [8]. Securin regulates the transition into M-phase and it also plays a role in the adhesion of sister chromatids, in the regulation of p53 and in DNA repair [9, 10]. Expression of securin has been studied in the development, invasion and metastasis of various cancers. Overexpression of securin has been suggested to promote genetic instability in normal cells and in cancer cells [8, 11]. Genetic instability is one of the most important driving forces in carcinogenesis [6]. In several human cancers, overexpression of securin has been associated with poor prognosis [8, 9, 12]. In oral squamous cell carcinoma (OSCC), specifically, *PTTG1* (which is identified as a mammalian securin) was involved in early oral tumorigenesis [13], and its overexpression promotes invasion [14]. Thus, we carried out this study to find out whether securin could provide prognostic power in OTSCC. The relationship of securin expression with clinical stage and histopathological grade were also evaluated.

Materials and methods

Patients and tissue materials

Paraffin blocks of cancer tissues from 104 patients diagnosed with OTSCC at the University hospitals of Helsinki ($n = 60$) and Oulu ($n = 44$) between 1981 and 2005 were retrieved for this study.

Tissue microarrays (TMAs) were available from a previous study [15] and sections were cut from the paraffin blocks. Six 1 mm cores were obtained from each tumor with the tissue microarray instrument (Beecher Instruments, Silver Spring, MD, USA). The cores were punched from the central tumor area and from the most invasive front of the tumor. Seventeen TMA slides with a total of 56 cores in 15 slides and 72 cores in two slides were prepared. For the other 44 cases where TMA blocks were not available, routine sections of normal size were cut for immunohistochemistry, chosen from representative sections of each case. Tissue cores with <100 cancer cells were excluded from this study as previously described [16]. Eleven cases were excluded due to unavailability of

clinical data or lack of tissue specimens; thus 93 cases were included in the final analysis. The use of patient samples and follow-up information were approved by the ethics committees of Helsinki and Oulu university hospitals; and by the National Supervisory Authority for Welfare and Health (VALVIRA). Our study is retrospective, and for this type of study formal consent is not required.

Securin immunohistochemistry

For immunohistochemistry, 4-µm thick sections were cut from the tissue cores and treated according to the protocol. Deparaffinization in xylene and rehydration in a graded ethanol series and distilled water were performed. Antigen recovery was performed in Tris–HCl buffer (pH 8.5) in a PT-module (LabVision UK Ltd, UK) for 20 min at 98 °C. Staining was performed in Autostainer 480 (LabVision) with Dako REAL EnVision Detection System, Peroxidase/DAB+, Rabbit/Mouse (Dako, Glostrup, Denmark). Sections were incubated in mouse monoclonal anti-securin antibody (securin, clone DCS-280, ab3305; Abcam, Cambridge, UK) with a dilution of 1:70 for 1 h; followed by 30-min incubation in peroxidase-conjugated [Dako REAL EnVision/HRP, Rabbit/Mouse (ENV)] reagent. Incubation in Dako REAL DAB+ Chromogen for 10 min was used to visualize the antigen. Slides were counterstained with Meyer's hematoxylin. Finally they were covered with xylene-based mounting medium in a glass coverslipper (Dako Ltd, Copenhagen, Denmark).

Evaluation of securin immunoexpression

The expression of securin was seen as a light or dark brown staining reaction in the cytoplasm or in the nuclei of cancer cells. In the evaluation, we included both nuclear and/or cytoplasmic expression and calculated the average fraction of stained cells. Intensity of staining reaction was not included in the scoring. The samples were classified into five categories, based on the proportion of cancer cells stained positively by securin antibodies. Negative immunoexpression was scored as (0). If less than 5 % of cancer cells were stained, the sample was scored (1); ≥5 to <10 % was scored (2); ≥10 to <30 % was scored (3); and ≥30 % was scored (4). The cores with the highest percentage of stained cancer cells were considered representative for the expression of securin in a particular tumor.

Two investigators (I.H. and A.A.) evaluated the samples independently using Olympus BH-2 light microscope and their scoring was reviewed by an experienced oral pathologist (J.H.). In case of discrepancy, the samples were re-evaluated for consensus. All observers were blinded to patient data during evaluation.

Statistical analysis

The analyses were executed with IBM SPSS Statistics (version 20). Evaluated samples were divided into low- and high-risk categories according to expression of securin at different percentage cut-off points (5, 10 and 30 %). The differences in the expression of securin between early vs. late stages of OTSCC and between low grade vs. intermediate or high-grade tumors were calculated using Chi square test.

Survival analysis was performed including overall survival (OS): the time from surgery to death or last follow-up; disease-free survival (DFS): the time from surgery to local and/or regional recurrence and/or distant metastasis; disease-specific survival (DSS): the time from surgery to death from OTSCC or to last follow-up.

Survival curves were constructed using the Kaplan–Meier method and compared with log-rank test. Univariate and multivariate analyses were performed with Cox-proportional hazard model, using 95 % confidence interval (CI). The analyses were carried out separately in early (T1/T2) and advanced (T3/T4) cases as well as in all patients jointly (T1 to T4). P values under 0.05 were considered statistically significant.

Results

In clinical staging, the patients were distributed as follows: T1 (23 patients), T2 (29 patients), T3 (36 patients) and T4 (5 patients). Fifty-one men and 42 women were included in this study (Table 1). At the end of follow-up, 5 patients had developed local recurrences, 10 had regional recurrences and 9 had both local and regional recurrences. At that time, 38 patients were alive, 30 had died of OTSCC and 25 had died of other causes.

Relationship between securin expression, histopathological grade, clinical stage and survival of OTSCC

In cases where securin was greatly overexpressed ($\geq$30 %) (Fig. 1), its expression was consistently found to be more common in moderately and poorly differentiated tumors than in well-differentiated tumors (Table 2). This difference was found to be statistically significant ($P = 0.03$). When our cases were divided based on a cut-off at 5 %, the expression of securin $\geq$5 % was associated with tumors of higher grade (Table 2), but this tendency did not reach statistical significance ($P > 0.05$). The same applied to using 10 % as the cut-off point.

When the expression of securin was compared between early and late stages of OTSCC, the latter showed higher expression of securin (Table 3). However, the association between overexpression of securin and advanced stages of OTSCC did not reach statistical significance ($P > 0.05$).

At any of the cut-off points (5, 10 or 30 %) of securin expression, there was no statistically significant association between the expression of securin and overall survival, disease-specific survival or disease-free survival (using Cox-proportional hazard and log-rank test, $P > 0.05$) (Fig. 2).

In multivariate analysis, the presence of cervical lymph node metastases (pN-stage) was the only statistically significant prognosticator for disease-specific survival (DSS) with a hazard ratio (HR) of 3.89 (95 % CI 1.59–9.48, $P = 0.003$). Similarly for overall survival (OS), the presence of recurrent tumor in the lymph nodes was a significant predictor of poor OS (HR of 2.36, 95 % CI 1.26–4.43, $P = 0.007$).

Discussion

The expression of securin has been examined in many carcinomas (e.g. breast [8], esophageal [17] and rectal [18] cancer). To the best of our knowledge, this is the first study to examine the expression of securin in OTSCC. We

Table 1 Clinicopathologic characteristics of 93 patients with OTSCC

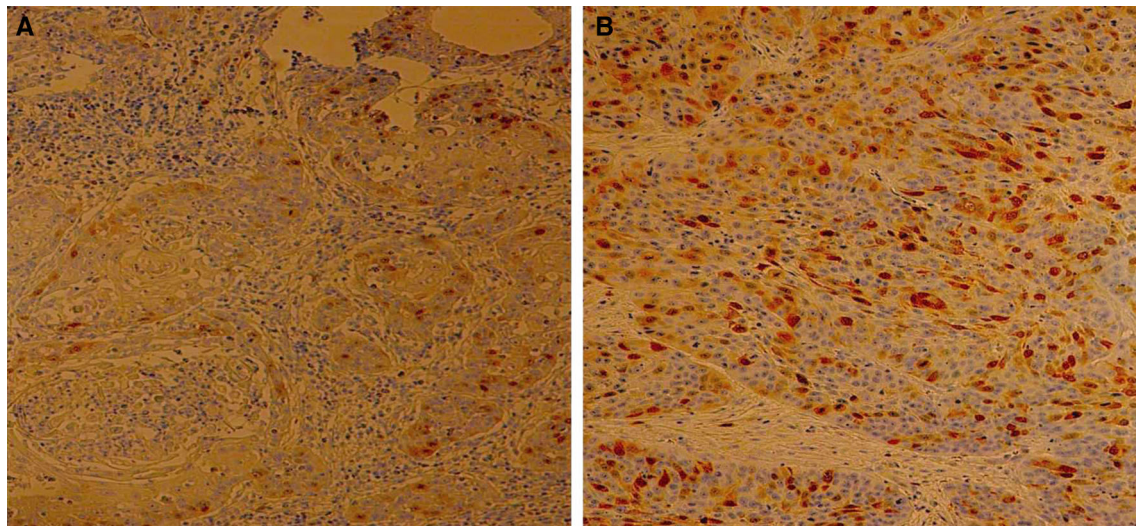| Variable	Number of cases	%
Age		
≤60 years	52	55.9
>60 years	41	44.1
Gender		
Male	51	54.8
Female	42	45.2
cT stage		
T1	23	24.7
T2	29	31.2
T3	36	38.7
T4	5	5.4
Grade		
Grade I	31	33.3
Grade II	44	47.3
Grade III	18	19.4
Securin (5 % expression)		
Low (<5 %)	48	51.6
High (≥5 %)	45	48.4
Securin (10 % expression)		
Low (<10 %)	66	71.0
High (≥10 %)	27	29.0
Securin (30 % expression)		
Low (<30 %)	84	90.3
High (≥30 %)	9	9.7

Fig. 1 Securin expression in OTSCC. **a** Low: <30 % of cancer cells were stained with securin; **b** high: ≥30 % of cancer cells were stained

Table 2 Distribution of tumor grades (I, II, III) in cases with high securin expression

	Grade I (31 cases)	Grade II (44 cases)	Grade III (18 cases)
High securin expression (≥5 %)	12 (38.7 %)	22 (50 %)	11 (61.1 %)
High securin expression (≥10 %)	5 (16.1 %)	15 (34.1 %)	7 (38.9 %)
High securin expression (≥30 %)	0 (0 %)	5 (11.4 %)	4 (22.2 %)

The percentages refer to the proportion of high securin expression cases among each tumor grade category

hypothesized that securin might have a prognostic value in OTSCC. We found, however, no significant differences in the survival between patients with low and high expression of securin. Interestingly, we found that overexpression of securin is significantly associated with higher tumor grade. Overexpression of securin was also common in advanced clinical stages of OTSCC.

The pituitary tumor transforming gene 1 protein, securin, is an essential protein for separation of sister chromatids [18, 19], and it is an inhibitor of separase activity [10]. In normal tissues, securin was shown to have a low level of expression [20], while overexpression of securin has been reported in various cancers. Although the role of securin in cancer progression is not well-understood, it has been reported to play a role in apoptosis, aneuploidy, angiogenesis, and regulation of tumor microenvironment [16, 21].

The prognostic value of traditional proliferation markers such as Ki-67 and Mitotic Activity Index (MAI) has been widely studied [22]. Recently, securin has been used as a proliferation marker in a number of cancers. Interestingly, Karra et al. [8] reported a strong prognostic power of securin in predicting breast cancer mortality, and they found that high expression of securin is a predictor of aneuploid DNA content. In a later report [16], they showed that a combination of high Cdc20 and high securin expression may aid in the identification of breast cancer patients with aneuploid DNA and poor prognosis. In prostate cancer, securin overexpression has been reported to cause an elevation in mitotic cells and apoptosis [23]. In colorectal cancer, depletion of securin is reported to enhance cell death and apoptosis [24]. Of note, Talvinen et al. [22] reported that MAI and Ki-67 provide a better reproducibility between observers than securin (although

Table 3 Distribution of early and late stages of OTSCC in cases with high securin expression

	Early stage (52 cases)	Late stage (41 cases)
High securin expression (≥5 %)	21 (40.4 %)	24 (58.5 %)
High securin expression (≥10 %)	13 (25.0 %)	14 (34.1 %)
High securin expression (≥30 %)	3 (5.8 %)	6 (14.6 %)

The percentages refer to the proportion of high securin expression cases among stage categories

Fig. 2 Kaplan–Meier curves show survival of OTSCC patients divided into groups according to immunoexpression of securin at 30 % cut-off point (<30 vs. ≥30 %). **a** Overall survival; **b** disease-specific survival; **c** disease-free survival. At cut-off points of 5 and 10 % of securin expression, there were no significant associations with tumor grade, stage, or patient survival
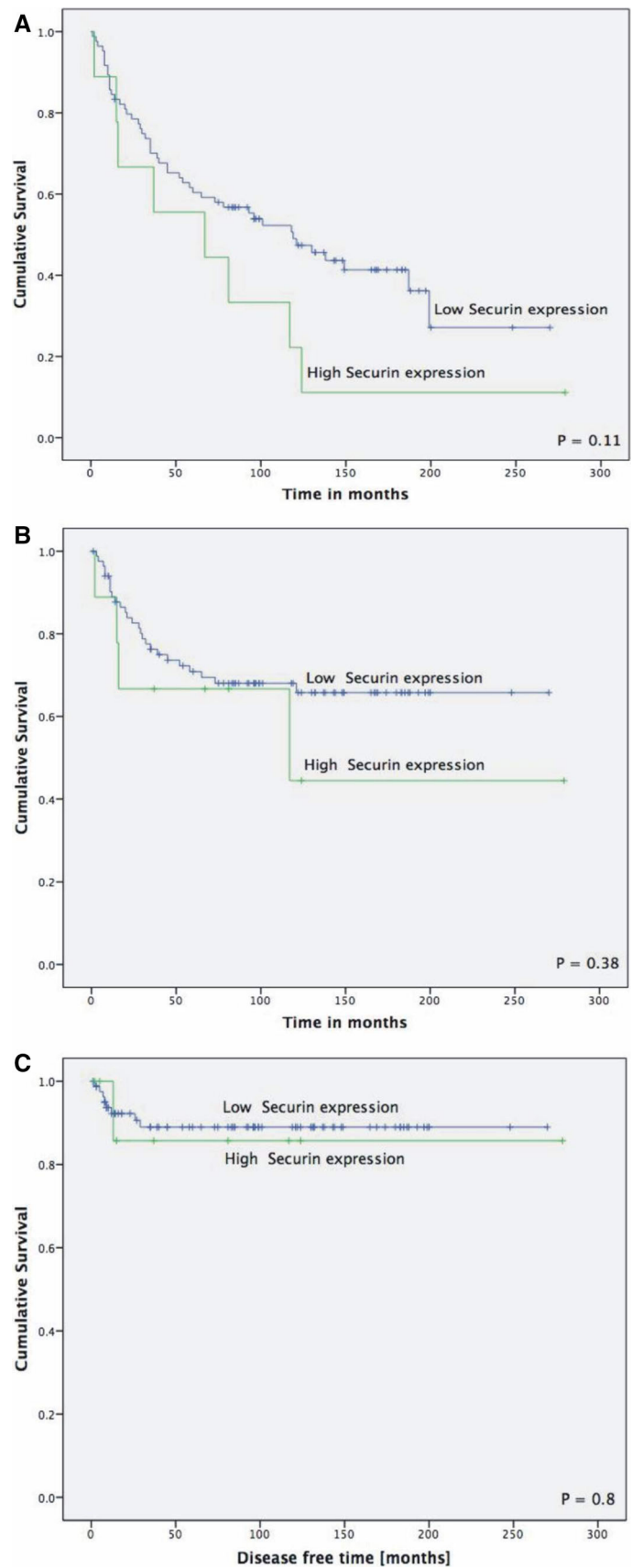
securin was a promising prognosticator for breast cancer in their analysis).

PTTG1 protein overexpression was reported as an indicator of transformation of precancerous lesions into OSCC [13]. Moreover, Zhang et al. [14] reported that PTTG1 promotes invasion and epithelial–mesenchymal transition in OSCC. They also reported that high expression of PTTG1 correlates with expression of matrix metalloproteinase 2; lymph node metastasis and TNM stage [14].

In breast cancer, Ogbagabriel et al. [20] have reported that securin overexpression was noticed in tumors with the highest degree of nuclear pleomorphism and also in tumors with the highest mitotic rates compared to low grade tumors. Similarly, we did not find any cases with low grade OTSCC having highest securin expression ($\geq$30 %), while we found such expression in high-grade tumors. Moreover, we found that overexpression of securin was less common in early stage (cT1–2) OTSCC compared to advanced stages (cT3–4). However, we could not find any prognostic power of securin in predicting survival of patients with OTSCC.

In conclusion, securin is overexpressed in high-grade OTSCC, which indicates that securin may have a role in progression of OTSCC. Here, we did not find a prognostic role for securin in OTSCC. Further evaluation of securin in other cohorts of OTSCC might be indicated.

Compliance with ethical standards

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