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Original article

Caveolin-1 expression in oral lichen planus, dysplastic lesions and squamous cell carcinoma

Zohreh Jaafari-Ashkavandi [a,*], Ehsan Aslani [b]

[a] Department of Oral and Maxillofacial Pathology, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran
[b] School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran

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ABSTRACT

Caveolin-1(Cav-1), the main part of caveolae structure, is supposed to play a role in pathogenesis of many human tumors. Since oral lichen planus (OLP) is considered as a potential premalignant disease, this study evaluated Cav-1 expression in OLP in comparison with benign hyperkeratosis, dysplastic epithelium and oral squamous cell carcinoma (OSCC), to investigate its possible role in pathogenesis and malignant transformation of OLP. In this cross-sectional retrospective study, immunohistochemical expression of Cav-1 in the epithelial component and stroma was evaluated in 81 samples, including 12 cases of hyperkeratosis, 24 OLP, 22 epithelial dysplasia, and 23 OSCC samples. Correlations between Cav-1 expression and clinicopathological variables were evaluated statistically. Positive Cav-1 staining was found in 58% of OLP, 91% of hyperkeratosis, 100% of epithelial dysplasia, and 95% of OSCC samples. OSCC showed the highest Cav-1 expression and OLP had the lowest ($P = 0.001$). The intensity of staining was significantly increased in stepwise manner from OLP to OSCC ($P = 0.001$). Expression of Cav-1 was related to the grade of samples in OSCC and dysplastic samples ($P = 0.04$). Based on the findings, it was concluded that Cav-1 may play a role in the pathogenesis of OLP and carcinogenesis of SCC, but its role in malignant transformation of OLP is not confirmed. Further studies are needed to evaluate its potential therapeutic function in OLP and SCC.

1. Introduction

Oral lichen planus (OLP) is a common muco-cutaneous condition whose etiological factors are not completely still recognized. World health organization (WHO) announces OLP as a potentially oral premalignant disorder [1,2]. There are some controversies over the malignant transformation chance of OLP, but its malignant transformation rate is about 0.5- 12% and the mechanisms responsible for its malignant transformation are still unknown. Alterations in the expression of proteins that regulate cell kinetics are necessary for malignant transformation. Some oncogenes and tumor suppressor genes have been attributed to this transformation; however, the main mechanism is still unknown [1,3].

Caveolins are a family of proteins that generally form caveolae structures that are plasma membrane invaginations [4]. Caveolae have one transmembrane and two cytoplasmic microdomains and are critical components for the interactions between integrin receptors and intracellular signaling molecules. The main proteins required for caveolae formation are three caveolins: caveolin-1 (Cav-1), -2, and -3. Cav-1 is widely expressed in human tissues. In the context of cancer, Cav-1 is the major isoform and many researchers suggests that Cav-1 expression is related to malignant transformation, differentiation, angiogenesis, tumor stage and metastasis, chemotherapeutic response, and other clinical characteristics. It has been shown that its prognostic value is strongly cancer- specific [4,5].

Hung et al. in 2003 [6] and Xue et al. in 2010 [7] reported that an increased Cav-1 expression correlates with the carcinogenesis in a stepwise manner from the normal oral mucosa and oral precancerous lesions to primary oral SCC (OSCC); however, the decrease in the expression from the primary OSCC to metastatic OSCC did indicate the necessity of exploring its dual functions in oral carcinogenesis [8]. Since this protein is a target of treatment in some tumors, evaluation of its function in any specific pathologic lesion is suggested [5]. To the best of our knowledge, there is no research focusing on the evaluation of Cav-1 expression in specific oral precancerous lesions like OLP, and since Cav-1 is known to be an oncogene in oral carcinogenesis, we aimed to evaluate the Cav-1 expression in epithelial dysplasia (ED), OLP and OSCC lesions, and then to inves-

* Corresponding author.
E-mail address: jaafariz@sums.ac.ir (Z. Jaafari-Ashkavandi).

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tigate its possible roles as a prognostic and predictive marker, and evaluate possible premalignant characteristics of OLP.

2. Material and methods

2.1. Patients and tissue selection

In this cross-sectional and analytical study, 81 cases were retrieved, including 12 cases of oral hyperkeratosis (HK), 24 OLP, 22 ED and 23 OSCC from the archive of Oral and Maxillofacial Pathology Department, from 1998 to 2015. All samples had definitive diagnosis. Related hematoxylin- eosin (H&E) slides were rechecked to confirm the diagnosis. Specimens without definitive diagnosis and insufficient epithelial component were excluded. Erosive and ulcerative OLP samples were also excluded.

Demographic data and clinical characteristics of each lesion, including the patients' age, gender, and location of the lesion were collected from the patients' medical files. Histopathologic grades of dysplastic and tumoral specimens were noted.

2.2. Immunohistochemistry

IHC staining was performed using Envision Labeled Peroxidase System (DAKO, Carpentaria, CA, USA). Formalin-fixed and paraffin-embedded blocks were cut with 4 μm thickness. Tissue sections were deparaffinized and hydrated following standard procedures and then washed with distilled water. Antigen retrieval was performed using DAKO cytomation target retrieval solution at pH = 9 and 100 °C for 20 min. Internal peroxidase activity was inhibited using 3% H2O2 solution. The sections were incubated with anti-Cav-1 polyclonal rabbit antibody (1:200, Novocastra, Newcastle, UK) for 60 min. Then, the samples were stained with 3, 3 diaminobenzidine (DAB liquid) as chromogen. Myer's hematoxylin was used for background staining. Colon tissue was used as positive control. Endothelial and adipose cells were also considered as internal positive control. Negative control sections were provided by replacing the primary antibody with phosphate-buffered saline solution (PBS).

Antibodies were detected using a light microscope. Cells with distinct brown staining of cell membrane and/or cytoplasm were considered as positive. For the slides that showed heterogeneous staining, more stained area was evaluated. The percentage of positive cells was estimated in 1000 cells of each lesion at x400 magnification. Stained samples were classified in five scores: negative (<10% staining), 1+ (10%–25% staining), 2+ (26%–50% staining), 3+ (51% –75% staining), 4+ (76%–100% staining). The mean of positive cells was also calculated in the basal layer of epithelium or peripheral cells of tumoral nests, and suprabasal layer or central cells of tumoral nests, separately. Intensity of staining was estimated in each sample, in comparison with the stained vessels and classified as: weak (+), moderate (++), or strong (+++). The final score for each specimen was obtained by multiplying overall mean (the mean of basal and supra-basal percentages) with the intensity of each specimen. The final scores less than 6 were considered as low-expression and those higher or equal to 6 were considered as high-expression [9]. The mean of stained stromal vessels was evaluated in 3 microscopic fields at x400 magnification just adjacent to the epithelial component of the specimens.

2.3. Statistical analysis

Data were analyzed by SPSS version 18 software using one-way ANOVA, Kruskal- Wallis, and post-hoc Tukey test for comparing the mean of Cav-1 expression in the study groups. Intensity of staining and final scores of all study groups were compared with Chi-square test. Also, Spearman's correlation and Mann-Whitney tests were used for detecting any correlation between Cav-1 expression and clinical variables. P-values less than 0.05 were considered as significant.

3. Results

81 patients in this study included 43 males and 38 females, with a mean age of 52.3 years (min = 19, max = 91). Baseline data including the patients' age and male to female ratio as well as the grades of dysplastic and tumoral samples, and the number of positive stained samples in each group are shown in Table 1.

Positive membranous and/or cytoplasmic Cav-1 expression was observed in epithelial component of 85.2% of the study samples. Table 2 shows the mean percentage of Cav-1 expression in the epithelial component and stroma of all study groups. In the stroma, staining was limited to the endothelial cells of all specimens (100%) and stromal fibroblasts did not show staining, except a weak and focal staining in the limited number of non-tumoral specimens.

58.33% of OLP samples showed a positive but non-continuous Cav-1 staining in basal cell layer, and only one case showed positive staining in the suprabasal cells. Details about percentage and intensity of staining in the epithelium as well as stromal vessels are shown in Table 2. One case (4.1%) of OLP samples showed high expression of Cav-1 and 96% of them showed low expression (Fig. 1a).

91.7% of HK samples showed positive Cav-1 staining (16.6% high and 83.4% low- expression). The staining was continuous and limited to the basal layer in most of the samples (Fig. 1b).

In the ED group, all samples had positive Cav-1 staining; 99% of these samples showed basal layer and 30% of them showed suprabasal layer staining (Fig. 2). The staining intensity was 2+ (69.2%) in most of the samples. Almost, 54.5% of the samples had a high score and 45.5% of them had a low score (Table 3).

In OSCC samples, staining of the epithelial cells was observed in 95.6% of the samples and only one specimen did not show any staining in the tumoral cells. More staining percentage was observed in the peripheral cell layers in comparison with central portions. Also, 92% of these samples had a high score. Central area of large sheets

Table 1
Baseline data of all study samples.

Sample (n)	Age Mean ± SD	Gender Male:Female	Grade (n)	Positive Cav-1 n (%)
Hyperkeratosis (12)	47.1 ± 15.6	9:3		11 (91.6)
OLP (24)	45.2 ± 14	11:13		14 (58.3)
Dysplasia (22)	57.6 ± 16.3	9:13	Mild = 10 Moderate = 10 Severe = 2	22 (100)
SCC (23)	56.8 ± 19	14:9	Well = 16 Moderate = 7 Poor = 0	22 (95.6)
Total (81)	52.3 ± 17.1	43:38		69 (85.2)