Heat Shock Protein 27 Serves as a Differentiation Marker in Oral Squamous Cell Carcinoma

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ABSTRACT

We examined the expression of heat shock protein (HSP) 27 in normal oral squamous epithelium and oral squamous cell carcinoma with an immunohistochemical method and compared the results wiht the expressions of proliferation cell nuclear antigen (PCNA), and transglutaminase (TG). HPS27 was expressed in good correlation with the expression of TG in the differentiated and dyskeratotic cells, expect for the completely keratinized cells composing the cancer pearl of squamous cell carcinoma. HSP27 expression was very low in the cells positive for PCNA. These results suggest that HSP 27 is useful as a differentiation marker in squamous cell carcinoma.

Key words: HSP27, TG, PCNA, Squamous cell carcinoma, Differentiation

INTRODUCTION

Squamous cell carcinoma is the most common malignant tumor of the upper digestive, and respiratory tracts, and the uterine cervix. It frequently shows various degrees of differentiation, which is one of the most important biologic features, as well as the proliferative, invasive and metastatic potentials of cancer. Keratin[1], desmoplakin[2], transglutaminase (TG)[3], involucrin[4] and filaggrin[5] are useful markers for morphological investigation of differentiation of squamous cell carcinoma, but further new markers are required to study the differentiation of squamous cell carcinoma in detail.

Heat shock protein (HSP) 27 is a member of the HSP family which is synthesized in cells undergoing heat and other environmental and physicochemically induced pathologic stimuli, enabling the cell to resist stress and heat[6, 7]. HSP27 has been also found to be expressed in a variety of malignant tumors in humans[8–16]. Expression of HSP27 are of particular clinical interest

because it often occurs in association with disease stage and prognosis in breast carcinomas, for example, HPS27 indicates rapid recurrence[11, 14], while in malignant fibrous histiocytoma[15] and neuroblastoma[16], in contrast, it indicates a more favorable prognosis. HSP27 is also involved in the regulation of cell growth[17]. In view of these roles, it is clear that HSP27 must be more widely examined in many malignant tumors. It has not been examined in oral squamous cell carcinoma as yet, however.

In this paper an immunohistochemical analysis of HSP27 expression was performed in oral squamous cell carcinoma as compared with those of markers of cell proliferation and differentiation, proliferation cell nuclear antigen (PCNA)[18] and TG. The present study revealed that HSP27 is a novel differentiation marker of oral squamous cell carcinoma.

MATERIALS AND METHODS

Tissue samples

Tissue samples from oral cancer were collected from 12 patients undergoing surgical procedures such as biopsy and resection of tumor at our clinic. Samples of cancer tissue and adjacent non-tumorous oral mucosa were obtained simultaneously. Part of the tissue was formalin-fixed and paraffin-embedded and the remainder was frozen immediately for storage at -80°C until histologic sectioning.

Immunohistochemistry

Two known markers were used in parallel to compare their results with HSP27 expression: Proliferation cell nuclear antigen (PCNA)[18], a cell proliferation marker, and transglutaminase (TG)[3], a differentiation marker for squamous epithelium. The immunohistochemical expressions of the three agents were observed on 6μm paraffin-embedded or frozen formalin-fixed sections and avidin -biotin immunoperioxidase technique using antibodies against these marker proteins and Histofine kit (Nichirei, Tokyo). The primary agents used in the present study were an anti-HSP27 monoclonal antibody (Funakoshi, Tokyo), an anti -human PCNA monoclonal antibody PC10 (Dako Japan, Tokyo) and an anti-human transglutaminase monoclonal antibody (Biomedical Technologies, Stoughton).

Briefly, the sections were deparaffinized, dehydrated with graded alkohol and washed in phosphate-buffered saline. Endogenous peroxidase and nonspecific binding were blocked by sequential incubation of the sections in 2% hydrogen peroxide solution followed by incubation in normal serum. Proper dilutions of the primary antibodies were applied to each section; 1: 200 for HSP27, 1: 50 for PCNA, and 1 : 100 for TG. After incubation of the sections with the second

biotinylated antibody and avidin-biotin complex reagent, the peroxidase reaction was developed using diaminobenzidine. The sections were weakly counterstained with methyl green or hematoxylin and mounted with coverglass. For negative control, a few sections were incubated with a solution lacking each antibody and put through the routine immmunoistochemical processes.

RESULTS

Expressions of PCNA, TG and HSP27 in Non-tumorous Oral Squamous Epithelium

PCNA activities were expressed only in the nuclei of proliferative basal cells and in a few prickel cells adjacent to the basal cells (Fig. 1). On the other hand, the staining results for TG activities were just the opposite of those for PCNA (Fig. 2). TG activities were only seen in suprabasal differentiated layers, exclud-

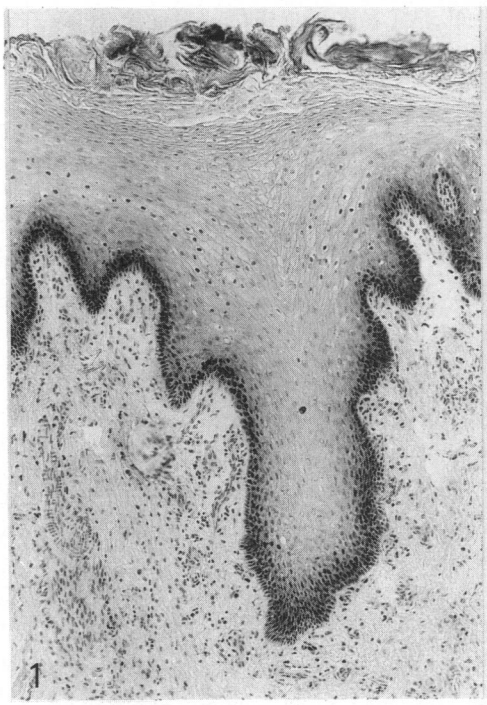

Fig. 1 Normal tongue mucosa. PCNA-positivities are found in the proliferative basal cells (hematoxylin counter staining. X 170).

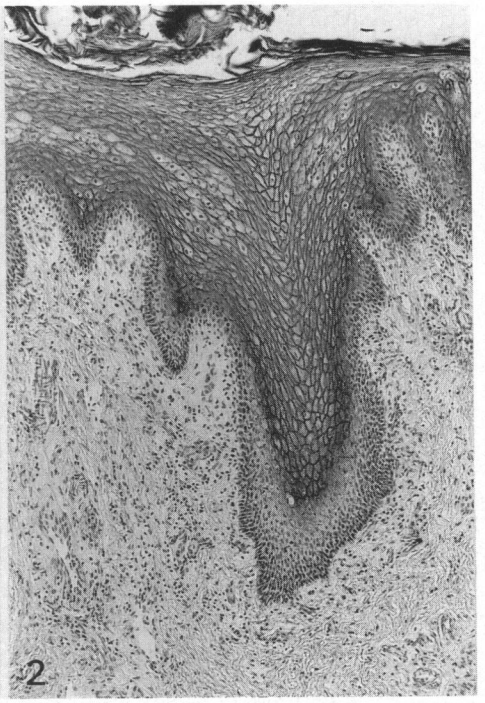

Fig. 2 Normal tongue mucosa. Intercellular reactivities of TG are expressed in the suprabasal differentiated layers (hematoxylin counter staining. X 170).

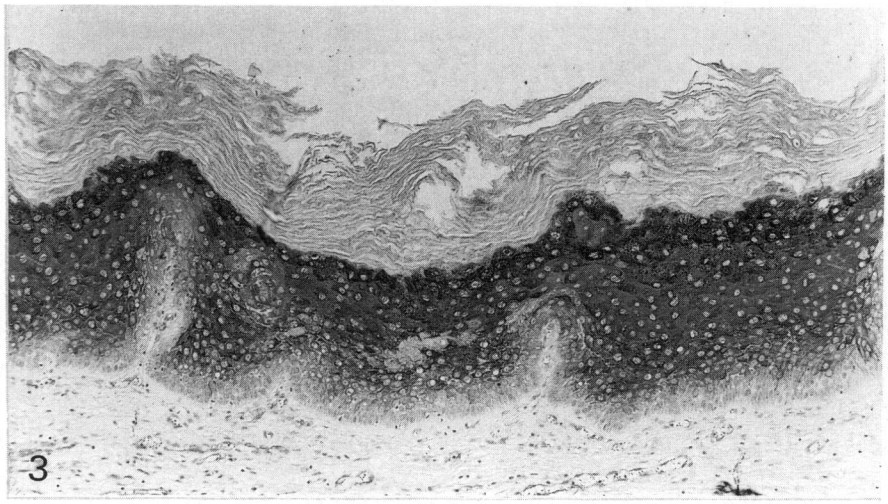

Fig. 3 Hyperkeratinized oral squamous epithelium. Cytoplasmic expressions of HSP27-immuno-positive deposits are seen in the comparatively differentiated suprabasal layers and most intensive in subcornified layer (hematoxylin counter staining. X 170).

ing cornified cells, and was especially prominent in the granular cell layers (Fig. 2). The intracellular localization of the activities of TG were in the cell membranes. HSP27 activities, like TG, were expressed in the same differentiated cells as in TG, but localized in the cytoplasm (Fig. 3).

Expressions of PCNA, TG and HSP27 in Oral Well Differentiated Squamous Cell Carcinoma

PCNA activity was only seen in cancers which occupied positions at the periphery of the cancer cell nests; it was not seen in differentiated cancer cells, including singly keratinized dyskeratotic cells (Figs. 4, 6). However, TG activity was negative in the cancer cells at the periphery of cancer cell nests where PCNA activity was positive: it was only expressed in the differentiated cells of cancer cell nests, excluding cancer pearls formed with amorphous cornified structure (Fig. 5). On the other hand, the HSP27 activity showed almost the same distribution pattern as TG, excluding the difference in localization, as seen in normal epithelium (Fig. 7). Dyskeratotic cancer cells with acantholysis, in which cytoplasm was markedly stained by eosin and the nuclei were deeply stained by hematoxylin, were negative for PCNA, but positive for both HSP27 and TG, as in normal epithelium (Figs. 5, 7).

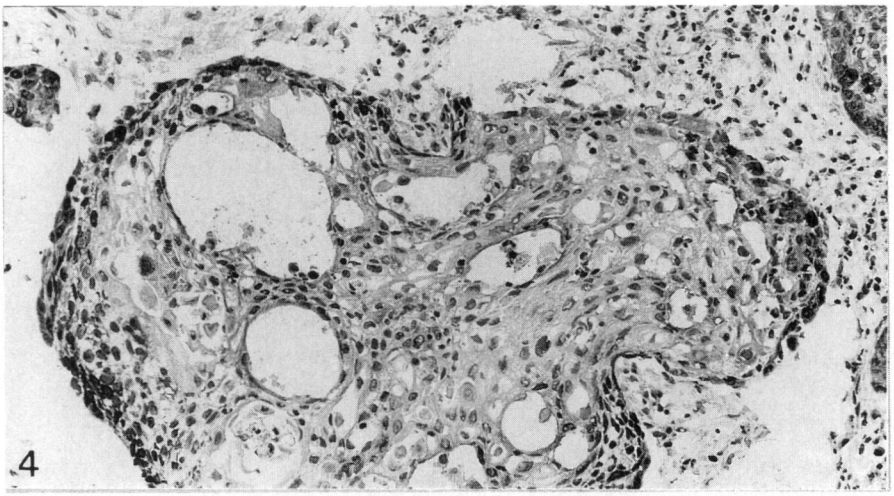

Fig. 4 Moderately differentiated squamous cell carcinoma. PCNA-positive cells were seen only in the proliferative peripheral area of cancer cell nest (hematoxylin counter staining. X 170).

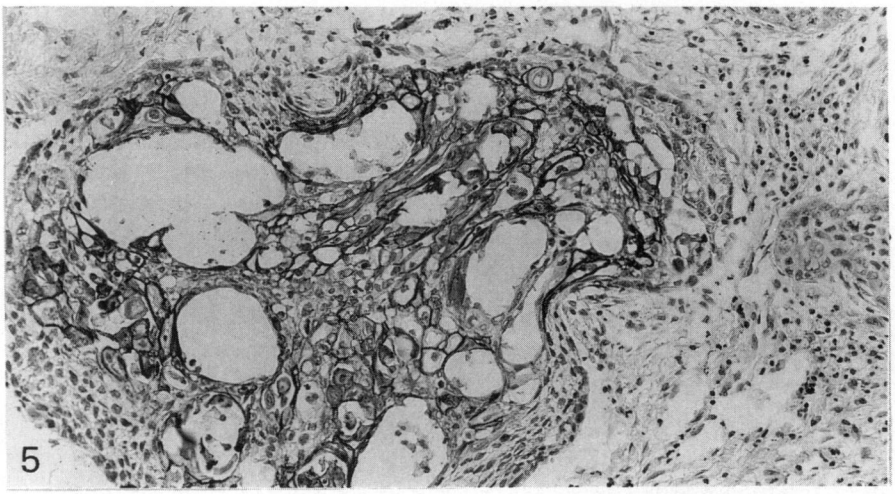

Fig. 5 Serial section from the same block as in Figure 4. TG immunoreactivities are expressed in the comparatively differentiated areas, but not in the proliferative peripheral area of the cancer cell nest (hematoxylin counter staining. X 170).

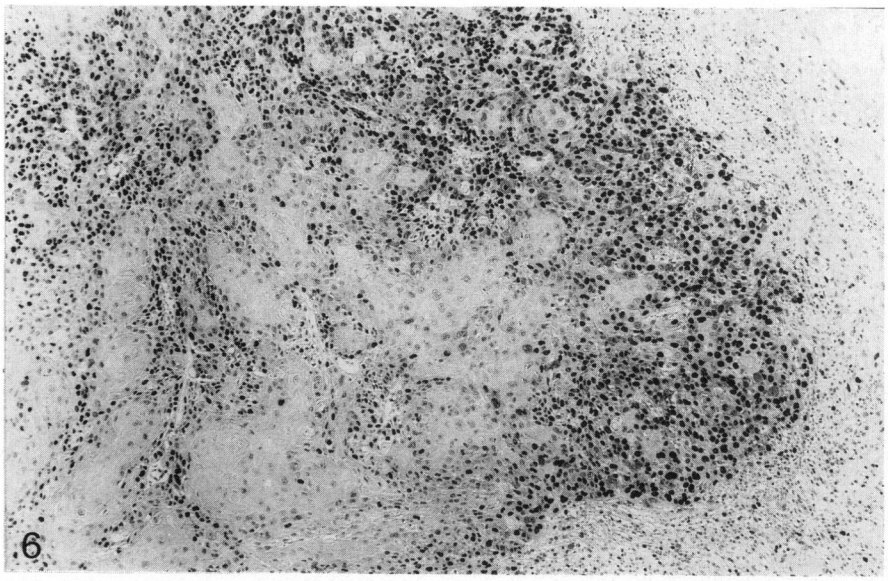

Fig. 6 Well differentiated squamous cell carcinoma. PCNA-positive cells were distributed only in the proliferative peripheral area of cancer cell nest (hematoxylin counter staining. X 170).

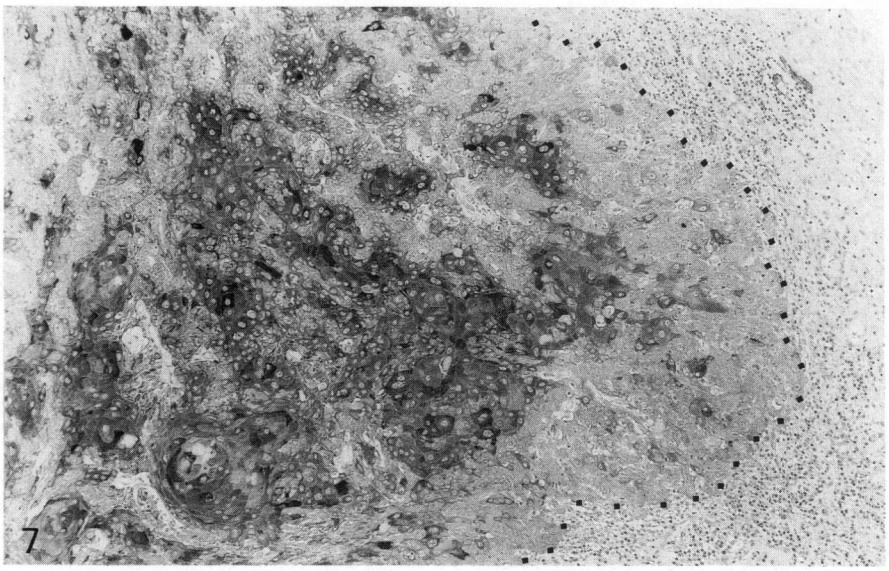

Fig. 7 Serial section from the same block as in Figure 6. Comparatively differentiated areas in cancer cell nest display positive reaction for HSP27 immunostaining. HSP27 is not expressed in the periphery of the cancer cell nest. The dotted line shows epithelio-stromal junction (hematoxylin counter staining. X 170).

Expressions of PCNA, TG and HSP27 in Poorly Differentiated Squamous Cell Carcinoma

In poorly differentiated squamous cell carcinoma, most of the cancer cells were made up of PCNA-positive cancer cells and TG and HSP activities were hardly seen.

DISCUSSION

This study has clearly shown that the immunohistochemical expression of HSP27 activity almost exactly matches that of TG in normal squamous epithelium and squamous cell carcinoma: PCNA activities were exclusively expressed in undifferentiated or proliferative cells in basal layers of normal squamous epithelia and the peripheral regions of cancer cell nests of differentiated cancers, while HSP27 activities were found in the differentiated cells in these tissues, including singly differentiated dyskeratotic cells, like TG activities. These HSP27-and TG -positive cancer cells were all negative for PCNA. Cancer cell nests from poorly differentiated cancers consisted of almost completely of PCNA-positive cancer cells with no expressions of HSP27 or TG. These findings revealed that HSP27 is a differentiation maker in squamous cell carcinoma.

HSP27 is highly expressed mainly in estrogen-regulated endometrium[8, 19], endometrial carcinoma[8, 9] and breast carcinoma[8-11], suggesting that it seems to be under hormonal control. It has also been found in gastric cancer[12] and leukemia[13], though it has not shown any relation with hormone regulation. Thus, the expressions of HSP27 seem to correlate with different biological features in different tissues including tumors.

This is the first study to show expression of HSP27 in normal squamous epithelium and squamous cell carcinoma. Aside from its significance as a novel differentiation marker in these tissues, however, neither its role in the differentiation of these tissues nor its correlations with the known differentiation markers are yet understood. Recently, roles of HSP27 in signal transduction pathways of physiological cell regulator[20] and as a molecular chaperone in thermotolerance[21] have been advanced. The expression of HSP27 in squamous cell carcinoma may be investigated in connection with such possible functions of HSP27 in future.

In conclusion, although this study did not elucidate the role of HSP27 in the differentiation of squamous cell carcinoma, it may provide a new clue to the analysis of differentiation of squamous cell carcinoma throughout the known informations on HSP27. Further detailed biochemical examination on the role of HSP27 in the differentiation of squamous cell carcinoma are recommended.

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