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Expression pattern of heat shock protein 90 in patients with oral squamous cell carcinoma in northern Taiwan

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Abstract

Heat shock protein 90 (HSP90), which is expressed in cancer cells, profoundly affects progression, invasion, and metastasis. However, to our knowledge, in East Asia, the correlation between the expression of HSP90 and clinicopathological variables has seldom been discussed. We therefore investigated this and its prognostic value in 36 patients newly diagnosed with oral squamous cell carcinoma (SCC) in northern Taiwan. Samples of tumour and normal samples from the patients were compared immunohistochemically. HSP90 was expressed mainly in the samples of tumour, and was significantly higher in these than in the normal epithelium ($p < 0.001$). Metastases to the lymph nodes in the 36 patients also correlated with expression of HSP90. Correlation between expression of HSP90 and the size of the tumour or pathological staging was not significant, but strong expression correlated with poor survival. In general, expression was low among our samples (30/36). It was significantly higher in the tumour samples than in normal samples, and correlated with metastases to lymph nodes in the neck.

Keywords: Expression pattern; Heat shock protein 90; Oral squamous cell carcinoma

Introduction

Oral squamous cell carcinoma (SCC) is the sixth most prevalent cancer worldwide and the fourth most common in Taiwanese men.[1] In most cases, operation, with or without concurrent chemotherapy or radiotherapy, remains the treatment of choice.[2–4] Long-term prognosis and decisions about treatment are related to the TNM system, and several risk factors affect prognosis and treatment.[5] The identification of molecular signatures can be adapted to the patient's long-term prognosis.[6]

Heat shock protein 90 (HSP90) is a molecular chaperone protein essential in tumour progression for the post-translational maturation and stability of client proteins.[3,6,7] It is a cytoplasmic protein that is constitutively expressed in several normal tissues and cancers, and is associated with malignant transformation, invasion, and metastasis. It chaperones hypoxia-inducible factor 1-alpha and vascular endothelial growth factor receptor (EGFR) to influence angiogenesis, and can induce antiapoptosis by interacting with various client proteins such as protein kinase B and survivin.[8,9] It also promotes metastasis through the activation of matrix metalloproteinase 2 (MMP-2).[8] As each of these pathways or proteins is independently associated with

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poor outcomes in oral SCC,[10,11] HSP90 is recognised as a crucial facilitator of oncogene addiction and survival of cancer cells.[12] Recent studies have focused on its inhibition as a strategy for the treatment of cancer.[12,13]

We therefore aimed to investigate the immunohistochemical expression of HSP90 in patients with oral SCC in northern Taiwan and to explore the clinical variables that correlate with conventional clinicopathological variables and survival.

Material and methods

Tissue samples and ethics statement

All tissues were obtained from the Department of Oral and Maxillofacial Surgery at the Tri-Service General Hospital, Taiwan. The Ethics Committee of the Tri-Service General Hospital approved all the experiments (IRB protocol: 2-103-05-045), and all patients provided informed consent. The investigation followed the guidelines of the Declaration of Helsinki.

Inclusion and exclusion criteria

Taiwanese (Mongoloid) patients who were aged between 20 and 65 years of age and newly diagnosed with oral SCC, were included. Those who had had operations for cancer, chemotherapy, or radiotherapy, and had an Eastern Cooperative Oncology Group Performance Status score of 2 or more, or who had a concurrent second primary or distant metastases, were excluded.

Tissue microarray

Thirty-six patients were enrolled. We prepared 36 paraffin-embedded tissue slides of the primary tumours (from resected specimens) and 10 of normal oral mucosal tissues, which were randomly obtained from healthy sites in the 36 patients. One core sample 2 mm in diameter was extracted from a representative area of each paraffin-embedded tissue (each representative core sample in the tissue microarray slide was the same size). Lymph nodes were also dissected.

Immunohistochemical staining

We used the avidin-biotin complex method of immunohistochemical staining. Tissue sections were deparaffinised in xylene and rehydrated in alcohol. Antigen retrieval was done by incubating the sections in 0.01 mol/L citrate buffer (pH 6.0) at 95 °C for 40 minutes in a water bath. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide for 30 minutes. The sections were then incubated with 5% normal horse serum in phosphate-buffered saline for 30 minutes at room temperature (25 °C) to block any non-specific antibody reactions. The slides were washed in Tris-buffered saline with 0.1% Tween® 20 (Sigma-Aldrich) and incubated overnight at 4 °C with the anti-human HSP90 monoclonal antibody clone 3H5 (1:1000 dilution, DAKO, Osaka, Japan). After rinsing in Tris-buffered saline with 0.1% Tween® 20, the sections were incubated for 40 minutes at room temperature (25 °C) with biotinylated anti-mouse IgG, then with an avidin-biotin-peroxidase complex (Vectastain Elite ABC kit, Vector Laboratories Inc, Burlingame, USA) for 40 minutes. They were then stained with 0.003% 3, 3-diaminobenzidine tetrahydrochloride and 0.005% hydrogen peroxide in 0.05 mol/L Tris-hydrochloride (pH 7.2), counterstained with Mayer's haematoxylin, dehydrated, and mounted.

Evaluation of immunohistochemical staining

All the slides were evaluated by a single pathologist who was blinded to the patients' data. For each specimen, we prepared a section without the addition of the primary antibody as a negative control, and used HSP90 sections from immunogenic, transgenic mice as positive controls to confirm the immunoreactivity of HSP90 in each series of experiments. HSP90-stained samples were defined as those that showed a cytoplasmic pattern in the tissue. The intensity of HSP90 immunoreactivity in the tumour cells was standardised to the staining of the internal control (0 = none; 1 = weak; 2 = moderate; and 3 = strong), and the distribution of HSP90 labelling was measured as the percentage of stained tumour cells in the total tumour volume of each section. The percentage of tumour cells with cytoplasmic staining was graded on a five-point scale (0 = 0%; 1 = up to 10%; 2 = 11%-25%; 3 = 26%-50%; 4 = over 50%). To evaluate HSP90 immunoexpression in each case, the grade of the percentage of HSP90-stained cells (0 - 4) at each intensity was multiplied by the corresponding intensity (0 -3) to obtain an immunostaining score (0 - 12).[14]

Statistical analyses

All results are expressed as median (range) or mean (SD). Scores for HSP90 immunostaining were compared between the samples of tumour and those of normal tissues. Any correlations between the HSP90 immunostaining scores for tumour specimens and metastases to lymph nodes in the neck, size of tumour, and pathological staging, were also assessed. Statistical analysis was done with the help of IBM SPSS Statistics for Windows version 20.0 (IBM Corp, Armonk, USA). Spearman's rank correlation coefficient was calculated to assess the associations between variables. Because the scores for intensity, percentage, and immunostaining were not normally distributed, the Wilcoxon X and Mann-Whitney U test were used for comparison. The Kaplan-Meir estimator was used to evaluate survival. An immunostaining score of six was chosen as the cut-off to distinguish between low