Dental Research Journal

Original Article

HSP27 and HSP70 expression in squamous cell carcinoma: An immunohistochemical study

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ABSTRACT

Background: Heat shock proteins (HSPs) are a family of proteins that are known to play a significant role in the repair of denatured proteins in the cell. It seems that cytoprotective properties of HSPs may help in malignant progression by facilitating tumor cell growth and survival. The purpose of this study is to evaluate HSP27 and HSP70 expression in various histopathological grades of squamous cell carcinoma (SCC).

Materials and Methods: In this retrospective-analytical study, the sections of 51 formalin-fixed paraffin-embedded biopsy specimens of SCC from various sites of oral and paraoral regions and 10 normal oral mucosa were immunostained by Novolink Polymer technique to determine the expression of HSP27 and HSP70. Then the data were analyzed according to the Kruskal–Walis, Mann–Whitney and the Spearman correlation tests (P<0.05).

Results: The expression of HSP27 in well-differentiated SCC was significantly higher than normal epithelium (P=0.007) and in moderately differentiated SCC higher than poorly-differentiated SCC (P=0.023). Inverse correlation was observed between HSP27 expression and SCC's histopathological grade (P=0.001, r=-0.448). There was no significant difference between HSP70 staining of specimens (P=0.38).

Conclusions: The present study revealed that the expression level of HSP27 was inversely related to histopathological grade of SCC and it may provide prognostic value for patients with SCC, but there was no significant relationship between the expression of HSP70 and histopathological grades of SCC.

Key Words: Histopathological grade, HSP27, HSP70, squamous cell carcinoma

Received: August 2011
Accepted: September 2011

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INTRODUCTION

Head and neck cancer is one of the most important cancers worldwide, and it is the third common malignancy in developing countries.[1,2] Among these cancers, squamous cell carcinoma (SCC) with 54% mortality rate has the highest frequency. So a vast part of oncologic studies has been done on this neoplasm.[1,2]

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Despite many improvements in treatment over the past 30 years, little progress has been made in improving survival rates. Therefore, the prevention and any innovation that facilitates early detection of this neoplasm have the potential to improve survival and quality of life.[1,2]

Microscopically SCC consists of invasive cords and islands of dysplastic squamous epithelial cells. Evaluation of histopathological similarity of these tumors to their parent tissue and the amount of their keratin production is called grading. The histopathological grade of the tumor is related to its biologic behavior.[3]

In addition to histopathological features that can affect on its prognosis, many efforts have been done to find molecular markers that can predict its biologic

behavior. Furthermore, many studies have evaluated the molecular signals that initiate carcinogenesis, but they are still unidentified.[1,2,4]

Heat shock proteins (HSPs) are a family of cytoprotective proteins vary in molecular size from 8 to 150 KDa that are known to play a role in the repair of denatured proteins in the cell.[5] It seems that cytoprotective properties of HSPs may help in malignant development by facilitating tumor cell growth and survival.[6] Among the several HSPs, HSP27 and 70 have been reported to have a strong relationship with cancer and either increase or decrease of their levels have been shown during carcinogenesis.[2,7] For example Lo Muzio,[4] Wan-Yu Lo[2] and Anxun Wang and colleagues[8] reported that expression of HSP27 in SCC was significantly higher than normal mucosa. Moreover studies of Lee[9] and Xiaoping Wang[10] revealed that expression levels of HSP70 in SCC were higher than normal epithelium; but unlike HSP27, it had reverse correlation with differentiation.

The present study was performed with the aim of estimating the amount of the immunohistochemical expression of HSP27 and HSP70 in the histopathological grades of SCC

Original hypothesis in this study is that there is a correlation between expression of HSP27 and HSP70 and histopathological grades of SCC.

MATERIALS AND METHODS

This retrospective, analytical study involved the following four procedures:

Tissue samples

The achieved tissue samples from 51 cases of SCC from various sites of oral and paraoral regions such as alveolar mucosa, tongue, oropharynx, buccal mucosa, larynx and esophagus (17 well differentiated, 17 moderately differentiated, 17 poorly differentiated) and 10 normal oral epithelium specimens from normal mucosa over the third impacted molar were used in this study. The patients consist of 38 men and 19 women with a mean age of 66.9 years who had undergone excisional biopsy between 2005 and 2010. Metastatic tumors and small samples were eliminated in this study. In addition, samples with insufficient fixation and those containing hemorrhage or necrosis were excluded. Diagnosis was based on histological examination of hematoxylin and eosin stained sections and the tumor grade was classified according to the Bryne's[11] classification

Immunohistochemistry

3-4 micron sections from paraffin embedded specimens were mounted on poly-L-lysine-coated glass slides.

After rinsing with 3 changes of xylol for deparaffinization, the sections were rehydrated with alcohol at different discending concentrations (100%, 100%, 95%, 85%, and 75%). In order to inactivate endogenous peroxidase, sections were incubated for 5 min in 3% H2O2, and were then rinsed with phosphate-buffered saline (PBS).

Specimens were incubated for 1 h with the lyophilized monoclonal anti-HSP27 (NCL-HSP27. Clone 2B4; Novocastra, Germany) at a dilution of 1:20 and the lyophilised monoclonal anti-HSP70 (NCL-HSP70, 8B11; Novocastra, Germany) at a dilution of 1:100. Immune complexes were subsequently treated with post primary Block and then detected by Novolink polymer (Novocastra, Germany) for 30min, both incubated for 30 min at room temperature. After rinsing with PBS, the immunoreactivity was visualized by diaminobenzidine (DABO, DAKO, Denmark). The prepared specimens were stained with hematoxyline (Harris), mounted with P.V mount and evaluated via light microscopy (Olympus BX41TF, Tokyo, Japon) by two independent observers.

Positive controls consisted of tissue specimen sections of breast carcinoma with known antigenic reactivity. A negative control was stained by omitting the primary antibody.

Specimen evaluation

A scale of 0 to 4 was used to score relative intensity with 0 corresponding to non detectable immunoreactivity and 1, 2, 3, 4 for very low, low, moderate and high staining, respectively. After staining, the mean percentage of positive cells in 10 high power field of microscope was determined at a scale of 1 to 4 was used. 1 showed staining of 0-25% of cells and 2, 3, 4 showed 25-50%, 50-75%, 75-100% staining respectively.

When inter-observer dissimilarity was seen between scores, the slides were re examined concurrently using a double headed light microscope. Finally staining-intensity distribution (SID) index was determined by multiplying these two scores for each specimen.

Statistical analysis

Statistical analysis was performed using Kruskal –

Wallis, Mann – Whitney and Spearman Rho correlation tests. Significant level was set at P=0.05 and r=0.01.

RESULTS

Cytoplasmic staining for HSP27 was observed in suprabasal keratinocytes in normal mucosa. In SCC diffuse HSP27 staining was seen with higher intensity in differentiated areas [Figure 1]. Percentage of stained cells is shown in Table 1.

Figure 2 shows the mean expression levels of HSP27 in different grades of SCC.

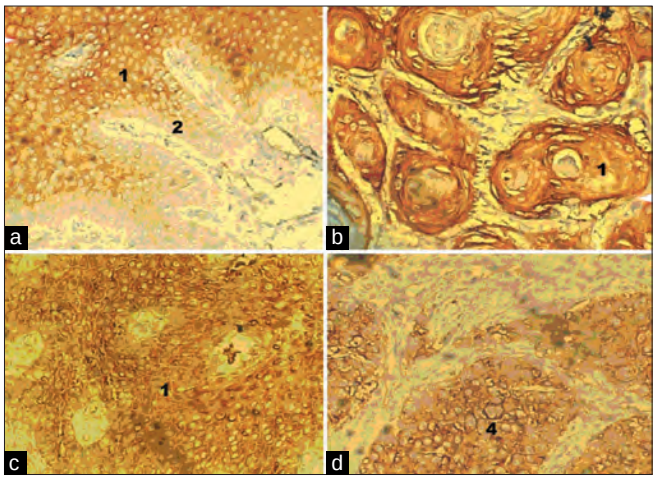

Figure 1: Immunohistochemistry analysis of HSP27 expression: (×100) (a) normal mucosa (1-cytoplasmic staining of parabasal layers, 2-basal layer without staining) (b) well differentiated SCC (1- cytoplasmic staining of epithelial pearls) (c) moderately differentiated SCC (1-cytoplasmic staining of epithelial nests) (d) poorly differentiated SCC (1- cytoplasmic staining of epithelial nests)

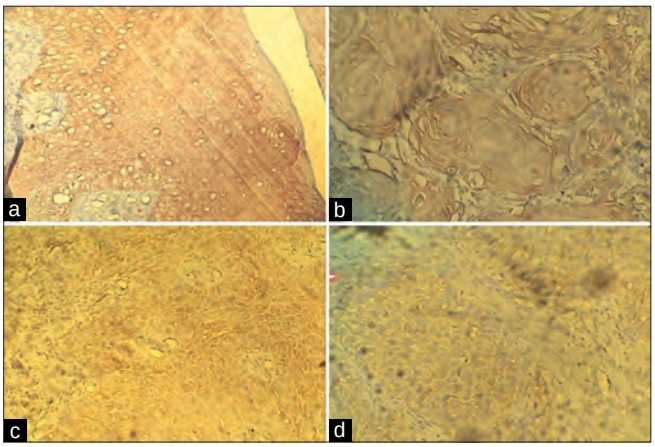

Figure 3: Immunohistochemistry analysis of HSP70 expression (×100): (a) normal mucosa (poor cytoplasmic staining) (b) well differentiated SCC (poor cytoplasmic staining) (c) moderately differentiated SCC (poor cytoplasmic staining) (d) poorly differentiated SCC (poor cytoplasmic staining)

The expression of HSP27 in well-differentiated SCC was significantly higher than normal epithelium (P=0.007) and in moderately differentiated SCC higher than poorly differentiated SCC (P=0.023). Statistical analysis showed inverse correlation between HSP27 expression and SCC's histopathological grade (P=0.001, r=–0.448) [Table 2].

Cytoplasmic staining for HSP70 was observed diffusely in normal mucosa and SCC [Figure 3]. Percentage of stained cells is shown in Table 3. Figure 4 shows the mean expression levels of HSP70 in different grades of SCC.

There was no significant difference between HSP70 staining of specimens (P=0.38).

DISCUSSION

HSPs are unique cytoprotective proteins that are thought to be the most ancient defense system in all living microorganisms of the earth. They were principally named because of their expression after heat exposure, but now it is known that variety of environmental and metabolic stresses may stimulate their expression. HSPs are requisite for folding of newly formed proteins and repair of denatured proteins after stress or injury. In addition, HSPs can

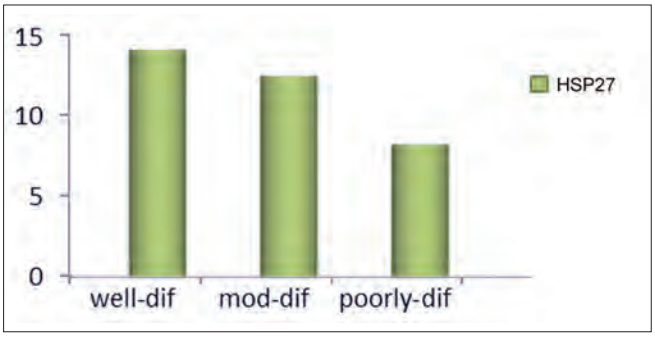

Figure 2: Mean SID index for HSP27 in different grades of SCC

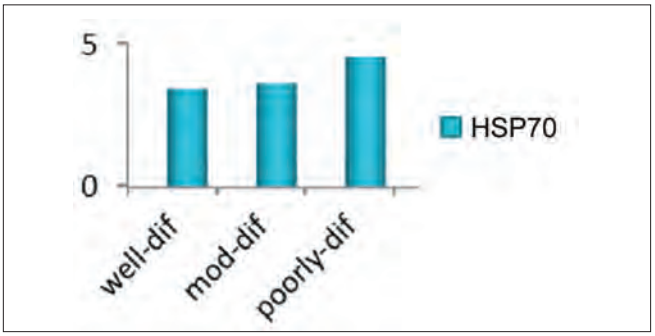

Figure 4: Mean SID index for HSP70 in different grades of SCC

Table 1: Percentage of stained cells with HSP27

Specimen	Percentage			
	0-25%	25-50%	50-75%	75-100%
Normal	0	0	30	70
Well-differentiated SCC	6	6	0	88
Moderately differentiated SCC	6	6	23	65
Poorly differentiated SCC	18	18	41	23

SCC: squamous cell carcinoma

Table 2: Spearman's rho correlation of HSP27 with differentiation grade

		Grade	HSP-27
grade	Correlation coefficient	1	-.448
	Sig.(2-tailed)	0	0.001
	N	51	51
HSP-27	Correlation coefficient	-.448	1
	Sig.(2-tailed)	0.001	0
	N	51	51

Table 3: Percentage of stained cells with HSP70

Specimen	Percentage			
	0-25%	25-50%	50-75%	75-100%
Normal	20	0	30	50
Well-differentiated SCC	29	0	12	59
Moderately differentiated SCC	59	6	12	24
Poorly differentiated SCC	48	18	0	34

SCC: squamous cell carcinoma

induce tumorogenesis via inhibition of apoptosis. Unlike this, some HSPs have significant role in immune response against cancer.[5,12]

Atypical levels of HSPs have been reported in numerous diseases such as autoimmune diseases, Alzheimer's disease and malignancies.[5,6]

Among HSPs, HSP70 and HSP27 seem to have a strong association with cancer and many studies have found alteration of their expression level in different cancers.[2]

In this study, increased expression of HSP27 was observed in oral cancer compared with normal tissue. The reasons for this increased expression could be as follows:

HSP27 prevents function of cytochrome C and procaspase and could prevent mitochondrial apoptosis via this mechanism.[12] Because defective apoptosis is one of the known mechanisms of cancer development,[13] so HSP27 can cause increased risk of cancer progression.

HSP27 expression is increased by the production of free radicals and increases cell resistance to oxidative damage.[8] Based on previous studies that show free radicals can lead cells to become malignant, it can be suggested that the increase in free radicals in cancer cells may be at least somewhat responsible for higher expression of HSP27 in SCC.

Our findings were consistent with the results of Lo Muzio,[4] Anxun Wang[8] and Wan-Yu Lo[2] Romanucci[14] stated that HSP27 plays an important role in differentiation; this theory was also evident in this study because the expression of HSP27 was inversely related to histopathologic grade of SCC so that the lowest expression of HSP27 was observed in poorly differentiated SCC. Moreover, in most specimens of the normal epithelium, staining for HSP27 was limited to parabasal layers with higher intensity in superficial layers which are more differentiated. These observations were consistent with Anxun Wang's findings in 2009.

Despite these findings, prognostic value of HSP27 in oral SCC is not clear. Although in some studies, relationship between HSP27 expression and survival rate was found, inverse correlation was observed in another study. These conflicting results may be due to heterogeneity of oncogenic pathways; so, some oncogenic pathways may remain normal despite cancer development.[8]

Among HSPs, most studies have been done on HSP70. But there is little information about its exact function in the cell.[5] Many studies have been done on the role of HSP70 in cell survival and its probable influence on cancer development and apoptosis. For example, Dudeja and colleagues[15] expressed that HSP70 can prevent apoptosis of cultured pancreatic cancer cells.

Schett[16] announced that increased expression of HSP70 can reduce apoptosis induced by Fas. But unlike these two, Mosser[17] had reached the conclusion that increased HSP70 cannot protect cells against apoptosis.

In another study, Liossis[18] announced that higher expression of HSP70 facilitates Fas-mediated apoptosis in jurkat cells.

Joo[19] showed that HSP70 has a polyotropic function in cell survival and can prevent or cause apoptosis under various conditions.

This study examined the relationship between the expression of HSP70 with oral SCC, and no significant differences between the expression of this marker in normal tissue and oral cancer was observed.

Lee[9] showed increased expression levels of HSP70 in SCC associated with chewing areca quid; it is

possible that the increased expression of HSP70 was due to specific condition of his study (chewing areca) as he also mentioned that high copper content in areca is the probable reason of increase in HSP70 expression.

Moreover, Xiaoping Wang[10] in 2010 found significant correlation between HSP72 (a subgroup of HSP70) and progression of esophageal SCC.

These different results may be due to various roles of HSP70 and also structural variations of its subgroups that can have distinct biological behaviors.

Additional studies may be warranted to evaluate the correlation between HSP27 and HSP70 expression and clinical stages of SCC and it is also proposed to concentrate on only oral samples in further researches for elimination of confounder variable.

CONCLUSION

The current study revealed that the expression level of HSP27 was inversely related to histopathological grade of SCC and it may offer prognostic value for patients with SCC. However there was no significant relationship between the expression of HSP70 and histopathological grades of SCC.

ACKNOWLEDGEMENT

This work was financially supported by a grant (number 389220) from Vice Chancellery of research of Isfahan University of Medical Sciences. Also the authors would like to thank Mrs. Mahmoodi and Dr. Mohajeri's laboratory for technical support.

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How to cite this article: Deyhimi P, Azmoudeh F. HSP27 and HSP70 expression in squamous cell carcinoma: An immunohistochemical study. Dent Res J 2012;9:162-6.

Source of Support: This report is based on a thesis which was submitted to the School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran, in partial fulfillment of the requirements for the M.Sc degree in Oral pathology (#389220). The study was approved by the Medical Ethics and Research Office at the Isfahan University of Medical Sciences and financially supported by this University., **Conflict of Interest:** None declared.