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• BRIEF REPORTS •

Expression and significance of heat shock protein 70 and glucose-regulated protein 94 in human esophageal carcinoma

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Abstract

AIM: To investigate the expression and significance of heat shock protein 70 (HSP70) and glucose-regulated protein 94 (grp94) in human esophageal carcinoma and adjacent normal tissues.

METHODS: The expression of HSP70 and grp94 in 78 human esophageal cancer and adjacent normal tissues was studied by immunohistochemistry and pathology photograph analysis.

RESULTS: Both esophageal cancer and adjacent normal tissues could express HSP70 and grp94. Of the 78 cases of esophageal carcinoma, 95.0%(72/78) showed positive HSP70, mainly stained in nuclei, while grp94 was mainly stained in cell plasma, and the positive rate was 71.8% (56/78).There was a significant difference in the expression of HSP70 and grp94 between esophageal cancer and adjacent normal tissues (P<0.01). Compared with adjacent normal tissues, there was a significant difference between differential types and HSP70 expression (P<0.01).

CONCLUSION: HSP70 and grp94 express differently in cell plasma and nuclei. The expression intensity of HSP70 is related to the differentiation of esophageal carcinoma.

Key words: Esophageal carcinoma; Heat shock protein 70; Glucose-regulated protein 94

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INTRODUCTION

Heat shock proteins (HSPs) are molecular chaperones, which are the biochemical regulators of cell growth, apoptosis, protein homeostasis and cellular targets of peptides. HSP70 or grp94 peptide complexes derived from a tumor can elicit cancer-specific immunity against the same tumor by their ability to bind to tumor-specific peptides[1-5]. Further researches have indicated that tumor-specific immunity induced by HSP70 or grp94 peptide is complex, and its mechanism involves MHC-1 molecule-restricted and non-MHC-1 molecule-restricted responses[6-10]. Esophageal cancer is one of the most malignant cancers. However, only limited information is available on the HSP70 and grp94 molecules in esophageal cancer tissues. Therefore, it is important to investigate the expression and significance of HSP70 and grp94 in esophageal cancer before the construction of tumor peptide vaccines against esophageal cancer.

MATERIALS AND METHODS

Reagents

Mouse anti-human HSP70 and grp94 monoclonal antibodies were purchased from Santa Cruz Company. EnVision™ kits were purchased from Dako Biological Technology Company.

Tissue samples

Paraffin specimens from 78 patients with primary esophageal cancer undergoing esophagus resection were collected at the Beijing Friendship Hospital, Capital University of Medical Sciences, Beijing, China, from 1999 to 2003. The patients consisted of 62 males and 16 females, with a mean age of 58.5 years, ranging from 44 to 76 years. Routine pathological diagnosis showed that all cases were squamous cell carcinoma. Among them, 24 cases were well-differentiated type (grade I) and 28 cases were moderately differentiated type (grade II), while 26 poorly differentiated type (grade III).

Immunohistochemistry

All sections were deparaffinnized and dehydrated with graded alcohol. Endogenous peroxidase was then blocked with 0.3% H_2O_2 diluted in methanol for 30 min at room temperature. Antigen retrieval was performed by treating the slides in citrate buffer in a microwave for 10 min. The slides were incubated in a moist chamber with HSP70 mouse monoclonal antibody (1:100) and grp94 mouse monoclonal antibody (1:100) at 4 ℃ overnight. After a complete wash in PBS, the slides were treated with goat anti-mouse antibody (1:100) for 45 min at 37 ℃. After a complete wash in PBS, the slides were developed in 0.05% freshly prepared diaminobenzidine solution (DAB, Sigma Co.) for 8 min, and then counterstained with hematoxylin, dehydrated, air-dried, and mounted. PBS was used to substitute the primary antibody as a negative control.

Statistical analysis

HSP70 and grp94 expression differences between esophageal cancer and adjacent normal tissues were analyzed statistically using u test. The relationship between the expression of HSP70, grp94 and the differentiation of esophageal cancers was analyzed statistically using χ^2 test. P<0.05 was considered statistically significant.

RESULTS

Expression of HSP70 and grp94 in esophageal cancer and adjacent normal tissues

Positive expression of HSP70 and grp94 showed brown staining

Table 1 Expression of HSP70 and grp94 in primary esophageal cancer and adjacent normal tissues detected by immunohistochemistry

Group	HSP70			grp94		
	Total	Positive	%	Total	Positive	%
Primary esophageal cancer tissue	78	72	95.0[b]	78	56	71.8[d]
Adjacent normal tissue	78	11	14.1[b]	78	8	10.3[d]

[b]$P<0.01$ *vs* group c, [d]$P<0.01$ *vs* group d.

Table 2 Relationship between the expression of HSP70 and differentiation of esophageal cancer

Positive expression (cases)	Differentiation grades of primary esophageal cancers (78)							
	HSP70(72)				grp94(56)			
	I[d]	II[d]	III[d]	Adjacent normal tissues[d]	I	II	III	Adjacent normal tissues
+	14	5	3	5	5	6	5	3
++	4	12	2	3	6	5	4	3
+++	3	6	6	3	8	3	4	2
++++	1	3	13	0	1	4	5	0

[d]$P<0.01$ *vs* group d.

in nuclei or cytoplasm. More than 500 cells calculated in different microscopic fields of each slide, and the percentage of positive cells was evaluated. Staining intensities were scored according to the following scales: -, no cells were stainted; +, less than 25% of cells were stained; ++, 26% to 50% of cells were stained; +++, 51% to 75% of cells were stained; and ++++, more than 75% of cells were stained.

Immunohistochemical staining showed that HSP70 was expressed in 72 of 78 primary tumors (95.0%) and 11 of 78 adjacent normal tissues (14.1%), while grp94 was expressed in 56 of 78 primary tumors (71.8%) and 8 of 78 adjacent normal tissues (10.3%). HSP70 was mainly stained in cell nuclei, whereas grp94 was mainly stained in cytoplasm (Table 1, Figure 1).

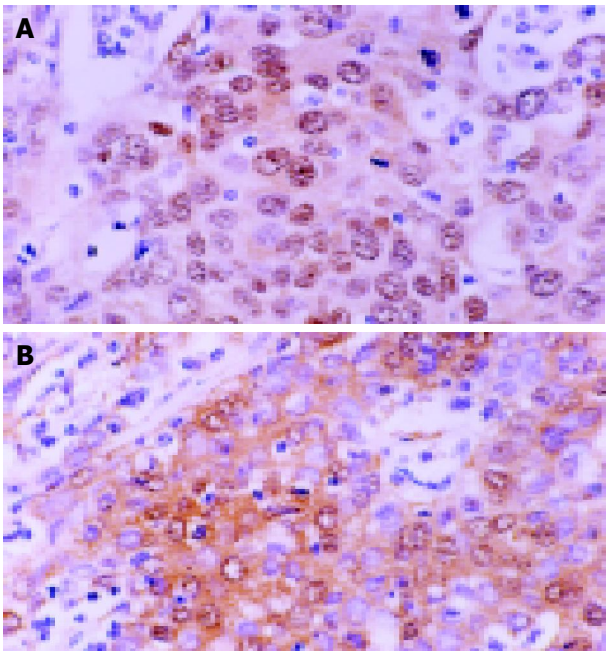

Figure 1 Expressions of HSP 70 (A) and grp94 (B) in esophageal cancer by immunohistochemstry, EnVision ×400.

Relationship between expression of HSP70, grp94 and differentiation of esophageal cancer

The results showed a significant correlation and difference between the expression of HSP70 and the differentiation of esophageal cancer, while no significant relationship and difference were observed between the expression of grp94 and the differentiation of tumors (Table 2).

DISCUSSION

In this study we examined the expressions of HSP70 and grp94 in 78 esophageal cancer samples by immunohistochemistry. The results showed that almost all investigated esophageal cancer samples expressed HSP70, and the majority of tumors expressed grp94, which differed significantly from that in adjacent normal tissues. By immunohistochemistry and microscope analysis, we found that there was a definite correlation between the expression of HSP70 and the differentiation of esophageal cancers The lower the grade of tumor differentiation, the stronger the HSP70 expression, while there was no significant relationship between the expression of grp94 and the differentiation of esophageal cancers. Earlier work suggested that during the growth and development of normal cells, HSP70 and grp94 were shown to accumulate mainly in the cytoplasm under cellular stresses, such as non-lethal heat shock. In the study, HSP70 mainly localized in nuclei, partially anchored in cytoplasm and cell membranes, while grp94 mainly expressed in cytoplasm, partially anchored in nuclei. This is consistent with the results of other groups[11-14]. It is still unclear how HSP70 is transported to cell membranes. Dressel *et al*[15] postulated that some HSPs might be transported to the cell surface via autoregulatory mechanisms like in normal cells. The different localization of HSPs indicated the heterogeneity of esophageal cancer.

Heat shock proteins (HSPs) are a group of highly conserved proteins synthesized after heat induction. In mammalian cells, this system is divided into two predominant categories, which appear to be structurally and functionally related to heat shock proteins (HSPs) and glucose-regulated proteins (grps). The most prominent HSPs of mammalian cells have molecular weights of approximately 90 000 (HSP90) and 70 000 (HSP70). Many studies have suggested a possible correlation between the expression of HSPs and the growth and differentiation of tumor cells[16-22]. Recent evidence reveals that a considerable expression of HSPs can be found in non-heat-shocked cells showing that HSPs may be induced by other stresses and participate in normal growth and development of the body[23].

Several studies have suggested that HSPs play some role during cell growth and differentiation of tumor cells[20,21]. Continuous expression of HSP in tumor cells may be required to serve as molecular chaperones in regulating and stabilizing tumor growth. Several studies have also revealed that some HSP genes are activated by differentiation inducers, suggesting their roles during differentiation of tumor cells[17,18]. For instance, an increase in the mRNA or protein expression of HSP70 gene was shown during the differentiation of human leukemic cells, K562 to erythrocytes, monocytes or macrophages and the inhibition in the production of HSP70 protein could reduce accumulation of hemoglobin, indicating that HSP70 is needed for a full differentiation capability of K562 cells[24]. It is not entirely clear whether a stress response induces differentiation or if differentiation *per se* involves the induction of HSPs.

Similarly, grps sharing a high amino acid identity to HSPs, are thought to act as molecular chaperones helping in transporting, folding and processing of their target proteins. Grp78 and grp94 are members of the HSP70 and HSP90 gene family respectively, and available evidence indicates that members of each family express analogous functions and physical characteristics. However, grps do differ from HSPs, which are localized in cytoplasm and mitochondria, whereas, grps are mostly located in endoplasmic reticulum. Furthermore, grp94 has also been identified as a murine tumor rejection antigen[25-30]. Although the cellular implication of the increased production of these proteins is unknown, it may be expected that each response would enhance the capacity of the pathway (s) in which these proteins function and perhaps protect the associated cellular compartments from damage via abnormal protein interactions. Grps can also be induced by various stresses to function as molecular chaperones[25-27]. Enhanced expression of grps during the growth of cancer cells implies its close relationship with cell growth[26-29]. However, few reports studied the expression of grp especially during the course of tumor growth and differentiation in comparison with those of HSPs. Grp94, an ER molecular chaperone sharing a high identity with HSP90, belongs to HSP90 gene family and participates in many important biological processes as molecular chaperones [18-20]. Our data showed there was an overexpression of grp94 in esophageal cancer, but no significant correlation and difference between its expression and the differentiation of tumors was observed. Nevertheless, the observed fact that differently differentiated esophageal carcinoma could synthesize more grp94, and the result may be useful to study the immunity between grp94 expression and tumor growth.

Numerous investigations have shown that HSP itself has no antigenicity and its immunogenicity is attributed to the chaperoned peptide[1-5]. It has been verified that HSP70 and grp94 have better molecular chaperones, which can process and present weak viral, bacterial or tumor antigens to host APC, gendering specific T-cell response and CTL reaction[16-21], even anchor antigens on the cell membrane and directly present antigen to NK or $\gamma\delta T^+$ cells[22-24]. Several studies have shown that HSP70-associated peptides could directly activate $\gamma\delta T^+$ lymphocytes or natural killer cells as superantigens without being dependent on the stimulation of MHC-I class molecules[6-10]. HSP70 is known to have strong protein-binding capacities, so cell-surface localization of HSP70 may make it possible to increase the immmunogenicity of esophageal cancers. Murine studies have carefully assessed the usage of these chaperone molecules as cancer vaccines, and their immune efficacy have been demonstrated in tumors of different histologies[16-22]. Our data suggest that owing to the heterogeneity in esophageal carcinoma, HSP70 and grp94 could express differently in cell plasma, nuclei and membranes. The expression intensity of HSP70 is related to the differentiation of esophageal carcinoma. The study may be useful in construction of tumor vaccines against esophageal cancers.

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