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Full Length Research Paper

In human primary lung carcinomas, there is a link between clinicopathology and HSP72 expression.

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Heat shock protein 72 (HSP72) is highly expressed in cancer tissues. Recent studies indicate the possible roles of HSP72 in the development and progression of different carcinomas, but detailed information is still ambiguous. The aim of the study is to investigate the correlation between clinicopathology and immunolocalization of HSP72 in human primary lung carcinoma. The expression of HSP72 was studied in 96 human primary lung carcinomas with or without metastasis, as well as in tissues adjacent to cancer by way of immunohistochemistry. The distribution of HSP72 immunoreactive was examined by continuous cell counting in restricted fields by pathology photograph analysis. HSP72 immunoreactivities were detected in 90 of 96 primary tumors (93.7%). The expression of HSP72 has a correlation with the differentiation of primary lung carcinoma. HSP72 expression in lung carcinomas with lymph node and organ metastasis was significantly higher than those with non-metastasis. The results indicate that there exists a significant correlation between the expression of HSP72 and the progression of primary lung carcinomas. HSP72 expression were significantly associated with the presence of tumor infiltration, lymph node and remote metastasis. The expression characters of HSP72 in primary lung carcinoma may contribute to study the pathogenesis and progression of lung carcinoma.

Key words: Heat shock protein 72, primary lung carcinoma, clinicopathology, prognosis.

INTRODUCTION

The heat shock protein (HSP) family is a highly conserved group of cellular proteins and is up-regulated under stress conditions, such as heat, hypoxia, serum deprivation, neoplasia and virus infection (Argon and Simen, 1999; Morimoto, 1993; Schlesinger, 1990). It functions as molecular chaperone and biochemical regulator to mediate cell growth, apoptosis, protein homeostasis and cellular targets of peptides (Morimoto, 1993). Aside from their response to heat shock and chemical or physical stress stimuli, HSPs have been reported to be over expressed in a wide range of human tumors including breast, endometrial, ovarian, colon, lung and prostate (Ciocca and Calderwood, 2005). Studies have also shown that HSP expression have a close relationship with carcinoma prognosis (Ciocca and Calderwood, 2005; Lebret et al., 2003). They may combine with oncogene products to form complexes and transport them into intracellular special sites and promote cancer cell proliferation and heterogeneous differentiation (Dorsey and Tchounwou, 2003; Villaseca et al., 1997). Recent studies have also shown that heat shock protein 72 (HSP72) is highly expressed in cancer tissues and have been used as prognostic markers in some tumors (Bausero et al., 2004; Gabai et al., 2005; Wang et al., 2002; Wang et al., 2007; Wang et al., 2008).

Study indicates the possible roles of HSP72 in the

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Abbreviations: HSP72, Heat shock protein72; **HSP70,** heat shock protein70; **HSP,** heat shock protein; **gps,** glycoproteins; **PBS,** phosphate buffered saline; **H_2O_2,** hydrogen peroxide; **DAB,** 3,3'-diaminobenzedine solution.

development and progression of lung carcinomas but detailed information is still ambiguous (Maehara et al., 2000). Primary lung carcinoma is one of the most malignant cancers, and there may be a correlation between the progression of lung carcinoma and over-expression of HSP72. The present study aimed to estimate the extent of the expression of HSP72 proteins in tumoral specimens obtained from lung cancer patients. We also aimed to evaluate the association between the extent of expression of HSP72 and various clinicopathological parameters, tumor proliferative capacity. The results showed that there exists a significant correlation between the expression of HSP72 and the progression in primary lung carcinoma.

MATERIALS AND METHODS

Immunochemistry reagents

Mouse anti -human HSP72 monoclonal antibody was obtained from StressGen Biotechnologies (Victoria, British Columbia, Canada). EnVisionTM kits were purchased from Dako Corp (Carpinteria, CA, USA).

Tissue samples

This investigation was approved by the ethics committee on human study at Shaanxi university of Chinese medicine (2004-4B). Paraffin specimens of primary lung carcinoma from 96 patients undergoing lung resection were collected from the affiliated hospital, Shaanxi university of Chinese medicine, Xianyang, China from 2002 to 2008. None of the patients received any kind of anti-cancer treatment or other therapies prior to surgery. The patients consisted of 46 male and 26 female, with a mean age of 58.2 ± 4.6 years, ranging from 42 to 79 years. Routine pathological diagnosis showed that all cases were primary lung carcinoma. Tumors were categorized as: squamous carcinoma type in 61 (63.5%), adenocarcinoma type in 23 (24.0%), and small cell carcinoma type in 12 (12.5%) out of 96 cases. Among the cases, 49 cases had regional lymph node metastases, and 38 cases had remote metastases. The specimens were fixed in 10% buffered formalin and embedded in paraffin. Serial sections, 5-µm-thick, were cut and placed on poly-lysine coated glass slides.

Immunohistochemical staining methods

All sections were deparaffinized and rehydrated with graded alcohols. Endogenous peroxidase was then blocked with 3 mL/L hydrogen peroxide (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 HSP72 mouse monoclonal antibody (1:100) at 4°C overnight. After a complete wash in phosphate buffered saline (PBS), the slides were incubated with horseradish peroxidase labeled goat anti- mouse antibody (1:100) for 45 min at 37°C. After a complete wash in PBS, the slides were developed in 0.5 g/L freshly prepared 3,3'-diaminobenzedine solution (DAB) (DAB, Sigma Co, St.Louis, Mo, USA) for 8 min, and then counterstained with hematoxylin, dehydrated, air dried, and mounted. Normal mouse IgG was used to substitute for the primary antibody as a negative control. No specific immunoreactivity was detected in these tissue sections. Two of the authors initially determined the fields simultaneously using a double-headed light microscope. The evaluation of HSP72 positive cells was performed on high-power fields (×400) using a standard light microscope. Only distinctive intranuclear or intra-cytoplasm immunoreactivity was considered positive. In each case, more than 1000 cells were counted and the percentage of immunoreactivity was independently determined. When interobserver differences were greater than 5%, the immunostained slides were re-examined simultaneously using a double-headed light microscope and the percentage of positive cells were determined. When interobserver differences were less than 5%, the mean value was obtained as the positive rate.

Statistical analysis

HSP72 expression differences between lung carcinomas and tissues adjacent to cancer were analyzed statistically using u test. The relationship between expression of HSP72 in primary carcinoma tissue with or without metastasis was analyzed statistically using ^{2} test. $P < 0.05$ was considered statistically significant.

RESULTS

Immunolocalization of HSP72 in primary lung carcinomas and adjacent tissues to cancer

The results of immunohistochemistry of HSP72 were summarized in Table 1. HSP72 immunoreactivities were detected in 90 of 96 primary tumors (93.7%) and in 16 of 96 mucous membranes adjacent to cancers (16.7%). HSP72 was mainly stained in the nuclei of cancer cells. HSP72 positive rates in lung carcinoma group were significantly higher than that in adjacent tissues to cancer ($P < 0.05$, Table 1).

Relationship between clinicopathology and expression of HSP72 in primary lung carcinomas

Results showed that HSP72 expressed higher in low differentiation of lung carcinomas than that in tissues adjacent to cancers ($P < 0.05$). There were significant differences of HSP72 expression between metastasis groups and non-metastasis groups ($P < 0.05$). To assess if HSP72 bear a pronounced prognostic effect in patient subgroups, we conducted an extensive analysis of HSP72 protein expressions. We stratified by nodal status (absence vs presence of lymph node metastases), presence of organ metastases and histopathological type (squamous and adenocarcinoma vs small cell carcinoma). In cross-tables, HSP72 expression was significantly associated with the presence of organ metastases and lymph node positivity (Table 1). These results suggest that there exists a significant correlation between expression of HSP72 and progression of lung carcinomas.

DISCUSSION

In this study, we examined the expressions of HSP72 in

Table 1. Relationship between clinicopathology and immunoreactivity of HSP72 in lung carcinomas.

Pathologic type	HSP72		
	n	- (%)	+ (%)
Tissues adjacent to cancers	96	80 (83.3)	16 (16.7)
Lung carcinomas[a]	96	6(6.3)	90 (93.7)
Tumor pathologic type[b]:			
Squamous carcinoma	61	4 (14.0)	57 (86.0)
Adenocarcinoma	23	2(8.7)	21(91.3)
Small cell carcinoma	12	0 (0)	12 (100)
Lymph node metastasis[c]:			
Yes	49	0 (0)	49 (100)
No	41	5(12.2)	36 (87.8)
Remote metastasis[d]:			
Yes	38	0 (0)	39 (100)
No	52	6(11.5)	46(88.5)

[a] $P < 0.05$, [b] $P < 0.05$, vs tissues adjacent to cancers; [c] $P < 0.05$, [d] $P < 0.05$, vs non-metastasis groups.

72 lung carcinoma samples by immunohistochemistry. The results showed that almost all of the detected lung carcinomas expressed HSP72, which had significant differences compared with that in tissues adjacent to cancers. By way of immunohistochemistry, we found that there was a definite correlation between expression of HSP72 and development of lung carcinomas.

The HSP family is group of highly conserved proteins synthesized after heat induction or other stressors (Argon and Simen, 1999; Morimoto, 1993; Schlesinger, 1990). In mammalian cells, this system is divided into two predominant categories, which appear to be structurally and functionally related: the HSPs and the glycoproteins (gps) (Schlesinger, 1990). During the growth and development of normal cells, heat shock protein70 (HSP70) is constitutively expressed at low levels but the expression was dramatically enhanced by stressful conditions (Morimoto, 1993). HSP72, belonging to the family of HSP70, is a highly conserved protein synthesized under various stresses (Mohanty et al., 2011). In non-transformed cells at normal conditions, HSP72 is expressed at very low levels. It is, however, present at elevated levels in the major fraction of tumors and in many transformed cell lines (López-Cotarelo et al., 2000; Kato et al., 2000; Volloch and Sherman, 1999). It is commonly assumed that in tumor cells the expression of HSP72 at elevated levels is the consequence of oncogenic transformation and enhanced expression of HSP72 has a close relationship with epithelial carcinoma cells growth (Hwang et al., 2003; López-Cotarelo et al., 2000; Volloch and Sherman, 1999). Up-regulated expression of the HSP70 family in tumor cells may be a requirement to serve as molecular chaperones in regulating and stabilizing oncofetal protein and mutant oncogene products during tumor growth process (Hwang et al., 2003; Lee, 2001; Volloch and Sherman, 1999). Recent studies have shown that HSP72 is highly expressed in cancer tissues and have been used as prognostic markers in some tumors, such as gastric cancers, colonic tumors, breast cancers, lung cancers and so on, which have also been verified to be associated with the development and progression of the above-mentioned carcinomas (Bausero et al., 2004; Gabai et al., 2005; Wang et al., 2002; Wang et al., 2007; Wang et al., 2008) . In this experiment, we found that HSP72 was highly expressed when lung carcinomas progressed, but their roles in lung carcinoma are not clear. It is reasonable to propose that HSP72 up-regulation in these tumor cells are closely related with tumor cell survival and proliferation. Recent studies have suggested that HSPs take part in cell growth and proliferation in several ways such as signal transduction and cell cycle regulation through combining certain proto-oncogene products. This indicates that these proliferating cells need higher level of HSPs to maintain the stability of tumor proteome (Fisher et al., 2000; Liu et al., 1999). It is believed that tumor cells are a group of highly proliferative heterogeneous cells which progress gradually through mutant oncogene products (King et al., 2001; Renan, 1990). Continuous expression of HSP in tumor cells may be required to serve as molecular chaperones in regulating and stabilizing these products during tumor growth process. At the same time, the existence of mutant or oncogene products may stimulate HSP synthesis (Dorsey and Tchounwou, 2003; Villaseca et al., 1997).

The present study further supports the clinical signify-cance of HSP72 expression in the progression of lung

carcinoma. In the study, HSP72 expression was found to be associated with important clinicopathological characteristics for patients' management. Consistently, HSP72 expression was significantly associated with the presence of tumor size, lymph node and organ metastasis. Our results showed that not only the expres-sion of both HSP72 in lung carcinoma was higher than that in tissues adjacent to cancer, but also the expression of HSP72 in lung carcinomas with metastasis was definitely higher than that of lung carcinomas without metastasis. The expression of HSP72 in lung carcinoma was related to the pathologic type of lung cancer. The results indicate that up-regulation of HSP72 is likely to have some relationship with progression, invasion and metastasis of lung carcinomas.

Studies revealed that considerable expression of HSPs was found in tumor cells, showing that HSPs may be induced by other stresses and participate in broader array of defenses during cell growth and cell differentiation of tumors (Dorsey and Tchounwou, 2003; Lebret et al., 2003; Villaseca et al., 1997) . Thus, it may be presumed that under various stimuli and stressful conditions, in order to avoid the damage caused by deleterious factors such as nitrosamines, 3,4-Benzopyrene-oncogenesis evocator, bronchopulmonary epithelial cells have to transcribe and translate high levels of HSPs in order to sustain normal metabolism and functions of cells. Under these conditions, bronchopulmonary epithelial cells should synthesize HSPs rapidly to exert a protective role for themselves. The progression of lung carcinoma is a gradual process under the long-term influence of various stimuli. During the process, inducible HSP synthesis increases gradually (Wang et al, 2010). This viewpoint was confirmed by our results, in that HSP72 was expressed at a higher level in lung carcinoma than that in tissues adjacent to cancer. The expression levels of HSP72 may be useful prognostic markers for lung carcinoma.

Conclusion

In this study, we examined the expressions of HSP72 in primary lung carcinoma samples by way of immune-histochemistry. Human lung carcinomas existed with high level of expression of HSP72. HSP72 expression was significantly associated with the presence of tumor lymph node and remote metastasis. There is a close correlation between the overexpression of HSP72 and progression of lung carcinomas. The expression characters of HSP72 in lung carcinoma may be useful to study the pathogenesis and progression of lung carcinoma.

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