P.O.Box 2345, Beijing 100023,China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

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• BRIEF REPORTS •

Significance of expression of heat shock protein90α in human gastric cancer

Dong-Sheng Zuo, Jie Dai, Ai-Hua Bo, Jie Fan, Xiu-Ying Xiao

Dong-Sheng Zuo, Ai-Hua Bo, Jie Fan, Xiu-Ying Xiao, Department of Pathology, Zhangjiakou Medical College, Zhangjiakou 075029, Hebei Province, China
Jie Dai, Capital University of Medical Sciences, Beijing 100054, China
Supported by the Natural Science Fundation of Hebei Province, No. 301427
Correspondence to: Dong-Sheng Zuo, Department of Pathology, Zhangjiakou Medical College, Zhangjiakou 075029, Hebei Province, China. dshz2003@yahoo.com.cn
Telephone: +86-313-8041652 **Fax:** +86-313-8041652
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Abstract

AIM: To evaluate the significance of hsp90α expression in human gastric cancer tissues.

METHODS: Immunohistochemical staining was used in clinical specimens from 33 cases of gastric cancer and 33 cases of gastritis with rabbit anti-human hsp90α multi-clonal antibody in order to explore the relationship between the expression of hsp90α in gastric carcinoma tissue and gastritis tissue as well as in mucous membrane adjacent to cancer and lymph node metastasis.

RESULTS: Hsp90α was detected in 88 % of gastric carcinoma cases and 55 % of gastritis cases. The hsp90α positive rate in gastric cancer group was significantly higher than that in gastritis group ($P<0.01$, $P=0.005$). The hsp90α positive rate in gastric cancer and in mucous membrane adjacent to cancer was 88 % and 55 % respectively ($P<0.01$,$P=0.005$). The hsp90α positive rate in lymph node metastasis group and non-lymph node metastasis group was 100 % and 60 % respectively, and a significant correlation between hsp90α expression and lymph node metastasis was shown ($P<0.01$, $P=0.005$).

CONCLUSION: The hsp90α expression rate in gastric cancer group was significantly higher than that in gastritis group as well as that in the group of mucous membrane adjacent to cancer. The hsp90α expression in lymphatic node metastasis group was higher than that in non-lymphatic node metastasis group. The results indicate that increased hsp90α expression has a close relationship with occurrence and lymph node metastasis of gastric cancer.

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INTRODUCTION

Heat shock protein90α, commonly known as heat stress protein, is a highly conserved cytosolic protein during the evolution of living things. It extensively exists in the living organisms and has many important biological functions such as enhancing cellular tolerance to stress and maintaining cellular homeostasis, etc. There are two forms of hsp90 in the advanced organisms, i.e. α form and β form. Under stress, the synthesis of hsp90 increases. For example, high temperature and infection can induce increase of hsp90 synthesis. However, different inducers play different roles in inducing hsp90 synthesis. hsp90α is more sensitive to heat induction, hsp90β is more sensitive to mitosis induction[1]. Recent studies showed that hsp90 had a close relationship with carcinoma. It was highly expressed in cancer tissue[2]. hsp90 combines with many transformed proteins to form complexes that are transported into intracellular special sites and correlated with cancer cell proliferation and differentiation[3,4]. There is a close relationship between the occurrence of gastric cancer and the synthesis of heat shock protein. At present there have been few reports on the study of hsp90 expression during the genesis of gastric cancer. In our study, we used immunohistochemical staining SP method to detect the expression of hsp90α in gastric cancer, gastritis, mucous membrane adjacent to cancer and gastric cancer tissue with or without lymph node metastasis in order to explore the relationships among them and their clinical significances,as well as the roles of hsp90α expression in the genesis and development of gastric cancer. Our study showed that the hsp90α expression rate in gastric cancer group was significantly higher than that in gastritis group, and in group of mucous membrance adjacent to cancer, hsp90α expression in the lymphatic node metastasis group was higher than that in the non-lymphatic node metastasis group.

MATERIALS AND METHODS

Samples collection

A total of 66 samples were collected from our hospital which were cut off from the stomach after operation, including 33 cases of gastric cancer and 33 cases of gastritis. Twenty-three of the 33 cases of gastric cancer had lymph nodes metastases and 10 cases had no lymph node metastasis. The samples were fixed in 10 % formaldehyde, dehydrated and embedded in paraffin. Five μm-thick sections were sliced. All samples were confirmed by pathological diagnosis.

Immunohistochemistry reagents

Rabbit anti-human hsp90α multi-clonal antibody (650-871-1919 CA, U.S.A), immunohistochemical staining S-P kit and DAB were purchased from Maixin LTD.

Methods

Immunohistochemical SP staining method was used in our experiment. The conventional staining procedures were carried out. The main procedures were as follows. The tissue sections were routinely dewaxed and hydrated, then treated with 3 % peroxide for 10 minutes. Antigen restoration was carried out by heating in citrate buffer, blocked with normal goat serum, incubated overnight with anti-human hsp90α multi-clonal antibody at 4 ℃, washed three times with PBS, treated with antibody II for 30 minutes at 37 ℃ and then with antibody III

for 30 minutes at 37 ℃. Color was displayed with DAB. Negative control was designed with PBS instead of antibody I. The known positive tissue sections were used as positive control.

Statistic analysis

SPSS10.0 software was used for statistical analysis.

RESULTS

Evaluation standard

Under light-microscope the hsp90α immunoreactive products showed as granules with brown color. These granules were mainly located in cytoplasm, only a few in nuclei. According to the amount and color density of granules, the staining was divided into three grades: +: few granules, canary color; ++: lots of granules filled cytoplasm, brown color ; +++: cytoplasm was filled with brown-black granules. The granules were also found in nucleoli. Detailed expressions of hsp90α in tissues of gastric cancer, gastritis and lymph nodes are shown in Tables 1, 2 and 3.

Table 1 Hsp90α expression in tissues of gastric cancer and gastritis

Pathologic types	*n*	Grade of hsp90α expression				Positive rate (%)
		-	+	++	+++	
Gastric cancer	33	4	23	4	2	88
Gastritis	33	15	13	4	1	55

[b]$P<0.01$ *vs* group of Gastritis, $\chi^2=8.943$.

Table 2 Hsp90α expression in gastric cancer tissues and tissues adjacent to cancer

Pathologic types	*n*	Grade of hsp90α expression				Positive rate (%)
		-	+	++	+++	
Gastric cancer	33	4	23	4	2	88
Tissues adjacent to cancer	33	15	17	1	0	55

[b]$P<0.01$ *vs* group of tissue adjacent to cancer, $\chi^2=8.943$.

Table 3 Hsp90α expression in tissues of gastric cancer with and without lymph node metastasis

Pathologic types	*n*	Grade of hsp90α expression				Positive rate (%)
		-	+	++	+++	
With lymph node metastasis	23	0	17	4	2	100
Without lymph node metastasis	10	4	5	1	0	60

[b]$P<0.01$ *vs* group without ymph node metastasis, $\chi^2=10.469$.

The hsp90α immunoreactive signals in gastric cancer were mostly strong or very strong. The positive rate was 88 %. However, in gastritis samples, the positive rate of hsp90α immunoreactive signals was 55 %, most of which were weakly positive. There was a significant difference between gastric cancer and gastritis ($P<0.01$). The hsp90α immunoreactive positive rates in gastric cancer or in mucous membrane adjacent to cancer were 88 % and 55 % respectively. There was also a significant difference between them ($P<0.01$). A significant difference also existed between gastric cancer with lymph node metastasis (100 %) and that without lymph node metastasis (62.5 %) ($P<0.01$).

DISCUSSION

The expression of hsp90α in normal cells is controlled by cell cycle[5], but it can continuously express at high level in tumor cells without heat stimulation. The existence of mutant or abnormal proteins also stimulates HSPs synthesis[6-9]. Heat shock proteins can maintain oncogene products in inactive state[10]. On the other hand, it has the functions of transportation and transfer. In tumor cells, the expression of hsp90α is higher than that in normal cells. An increasing trend of hsp90α expression was seen in virus-transformed and chemical-induced tumor cells[11,12]. In pancreatic cancer, hsp90α showed a selective expression at high level. Jamell[13] found that hsp90α expressed in all human breast cancers and hsp90α expression was higher in malignant breast tissue[14]. An increased expression of hsp90α mRNA was also found in ovary cancer and the more serious the disease was, the higher the expression of hsp90α mRNA was[1]. It was also found that hsp90α showed a high expression in 29 % of endometrium cancer. Yano' s research[1] showed that the hsp90α mRNA level in breast cancer was higher than that in non-cancer tissues[14,15]. The expression of hsp90α mRNA has a close correlation with proliferating cell nuclear antigen labeling index (PCNA LI), indicating that high expression of hsp90α mRNA should have an important role in cell proliferation. It was identified in our study that the expression of hsp90α in gastric cancer was obviously higher than that in gastritis and mucous membrane adjacent to cancer.

HSPs take part in cell growth and proliferation by several means such as signal transduction and cell cycle regulation. HSPs express highly in germ cells and embryonic cells, but express lowly in aging cells. This suggests that the increase of protein synthesis in proliferating cells needs much more HSPs to take part in the formation of protein activities. Because tumor cells are a group of high proliferation heteromorphic cells, they may need much more HSPs to sustain their proliferation[16,17]. Our results also showed that the expression of hsp90α in gastric cancer with lymph node metastasis was higher than that without lymph node metastasis. All of these were consistent with the results from Jamell' s report that the higher the breast cancer malignancy is, the higher the hsp90α expresses. This indicats increase of hsp90α expression probably has some relationship with genesis, development, invasion and lymph node metastasis of gastric cancer.

Under various stimulations, gastric mucous membrane can transcript and translate high levels of HSPs that can change the metabolism and functions of cells in order to alleviate the damage caused by deleterious factors including exogenous stimulants such as heat, chemicals and ethanol, and endogenous stimulants such as acid, local ischemia, hypoxia. In this case, gastric mucous membrane should synthesize HSP rapidly to exert the protecting role for gastric mucous cells[1]. The genesis of gastric cancer is a gradual process under the long-term influence of various stimulants as well as other factors. During the process, HSPs synthesis increases gradually[16]. This viewpoint was confirmed by our results that hsp90α positive rate in gastric cancer was higher than that in gastritis and gastric tissues adjacent to cancer. The discovery of our study may provide some useful clues for early detection and clinical diagnosis of gastric cancer.

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