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• *BRIEF REPORTS* •

Correlation between clinicopathology and expression of heat shock protein 70 and glucose-regulated protein 94 in human colonic adenocarcinoma

Xiao-Ping Wang, Fan-Rong Qiu, Guo-Zhen Liu, Rui-Fen Chen

Xiao-Ping Wang, Guo-Zhen Liu, Rui-Fen Chen, Department of Pathology, Capital University of Medical Sciences, Beijing 100054, China
Fan-Rong Qiu, Department of Gastroenterology, Beijing Chaoyang Hospital, Capital University of Medical Sciences, Beijing 100054, China
Supported by the Research Fund for Young Scholars of Beijing, No. 02120031
Correspondence to: Dr. Xiao-Ping Wang, Department of Pathology, Capital University of Medical Sciences, Beijing 100054, China. wxpphd@yahoo.com.cn
Telephone: +86-10-63051455
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Abstract

AIM: To investigate the correlation between clinicopathology and expression of heat shock protein 70 (HSP70) and glucose-regulated protein 94 (grp94) in human colonic carcinoma.

METHODS: The expression of HSP70 and grp94 was studied in 80 human colonic cancers with or without metastasis as well as in their adjacent mucous membrane by way of immunohistochemistry and pathology photograph analysis.

RESULTS: The expression of HSP70 and grp94 was significantly higher in cancer than that in adjacent mucous membrane (92.5%, 85.0% *vs* 56.3%, 42.5%, $P<0.01$). HSP70 and grp94 expressed higher in moderately- and poorly-differentiated colonic cancers than that in their adjacent tissues (93.7%, 87.5%; 100%, 90% *vs* 56.3%, 42.5%; $P<0.01$). Dukes C and D stages of colonic cancers showed higher positive rates than Dukes A and B stage groups (97.1%, 91.2%; 100%, 90.9%; *vs* 80%, 70%; 78.6%, 71.4%; $P<0.05$). There were definite differences in HSP70 and grp94 expression between metastasis groups and non-metastasis groups (100% *vs* 75%, 100% *vs* 50%, $P<0.05$).

CONCLUSION: The HSP70 and grp94 expression rates in colonic cancer groups are significantly higher than that in their adjacent mucous membrane. The HSP70 and grp94 expression in poorly-differentiated colonic cancers with metastasis is significantly higher than well-differentiated cancers without metastasis. The overexpression of HSP70 and grp94 can be used as diagnostic or prognostic markers for colonic cancer.

Key words: Heat shock protein 70; Glucose-regulated protein 94; Colonic adenocarcinoma

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INTRODUCTION

Heat shock protein (HSP) is a highly conserved group of cellular proteins and is up-expressed under stress conditions, such as heat, hypoxia, serum deprivation, neoplasia and virus infection[1-3]. It functions as a molecular chaperone and a biochemical regulator to mediate cell growth, apoptosis, protein homeostasis and cellular targets of peptides[2]. Studies showed that HSPs have a close relationship with carcinoma[4,5]. They may combine with oncogene products to form complexes and transport them into intracellular special sites and promote cancer cell proliferation and heterogeneous differentiation[6,7]. Recent studies showed that heat shock protein 70 (HSP70) and glucose-regulated protein 94 (grp94) are highly expressed in cancer tissues and have been used as prognostic markers in some cases[8-10]. Colonic cancer is one of the most malignant cancers and there may be a close relationship between the occurrence of colonic cancer and the overexpression of HSPs[11]. However, limited information is available on the HSP70 and grp94 molecules in colonic cancer tissues. In this study, we used immunochemical staining methods to detect the expression of HSP70, grp94 in colonic cancer and their adjacent mucous membrane and colonic cancer tissue with or without metastasis in order to explore the relationship among them and their significances. The results show that there exists a significant correlation between the expression of HSP70, grp94 and the progression of colonic cancer.

MATERIALS AND METHODS

Immunochemistry reagents

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

Tissue samples

Paraffin specimens from 80 patients with primary colonic cancer undergoing colonic resection were collected from Beijing Chaoyang Hospital, Capital University of Medical Sciences, Beijing, China, from 2000 to 2003.The patients consisted of 54 males and 26 females, with a mean age of 59.5 years, ranging from 42 to 80 years. All cases were diagnosed as adenocarcinoma by routine pathological examination. Well-differentiated type (grade I) was found in 28 cases, moderately-differentiated type (grade II) in 32 cases, and poorly-differentiated type (grade III) in 20 cases. According to the revised Dukes stages[12], stage A included 10 cases, stage B 14 cases, stage C 34 cases, and stage D 22 cases. Among the cases, 56 had regional lymph node metastasis, and 22 had remote metastasis.

Methods

All specimens were deparaffined and dehydrated with graded alcohol. Endogenous peroxidase was then blocked with 3 mL/L 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 oven 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, respectively. 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 0.5 g/L freshly prepared diaminobenzedine 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 colonic cancers and their adjacent mucous membrane were analyzed using *U* test. The relationship between expression of HSP70 and grp94 in colonic cancer tissue with or without metastasis was analyzed using χ^2 test. $P<0.05$ was considered statistically significant.

RESULTS

Expression of HSP70 and grp94 in colonic cancers and their adjacent mucous membrane

Immunohistochemical staining showed that HSP70 was expressed in 74 of 80 primary tumors (92.5%) and 45 of 80 adjacent mucous membranes (56.3%). The grp94-positive rate in colonic cancer and adjacent mucous membrane was 85.0 and 42.5%, respectively. HSP70 was mainly stained in cell nuclei, whereas grp94 was mainly stained in cytoplasm. The HSP70 and grp94-positive rates in colonic cancer were significantly higher than that in adjacent mucous membrane ($P<0.01$) (Table 1).

Relationship between clinicopathology and expression of HSP70, grp94 in colonic cancer

HSP70 and grp94 expressed higher in moderately- and poorly-differentiated colonic cancers than that in adjacent tissues (93.7%, 87.5%; 100%, 90% *vs* 56.3%, 42.5%; $P<0.01$). Dukes C and D stage groups showed distinct higher positive rates than Dukes A and B stage groups (97.1%, 91.2%; 100%, 90.9%; *vs* 80%, 70%; 78.6%, 71.4%; $P<0.05$). HSP70 and grp94-positive rates were 100% in lymph node metastasis and remote metastasis groups. There were significant differences in HSP70 and grp94 expression between metastasis groups and non-metastasis groups ($P<0.05$) (Table 1).

Table 1 Relationship between clinicopathology and expression of HSP70 and grp94 in colonic cancers

Pathologic types	*n*	HSP70		grp94	
		- (%)	+ (%)	- (%)	+ (%)
Tissues adjacent to cancers	80	35 (43.7)	45 (56.3)	46 (57.5)	34 (42.5)
Colonic cancers[b]	80	6 (7.5)	74 (92.5)	12 (15.0)	68 (85.0)
Tumor differentiation[d]					
High	28	4 (14.3)	24 (85.7)	6 (21.4)	22 (78.6)
Moderate	32	2 (6.3)	30 (93.7)	4 (12.5)	28 (87.5)
Low	20	0 (0)	20 (100)	2 (10)	18 (90)
Dukes stages					
A	10	2 (20)	8 (80)	3 (30)	7 (70)
B	14	3 (21.4)	11 (78.6)	4 (28.6)	10 (71.4)
C[a]	34	1 (2.9)	33 (97.1)	3 (8.8)	31 (91.2)
D[a]	22	0 (0)	22 (100)	2 (9.1)	20 (90.9)
Lymph node metastasis					
Yes[c]	56	0 (0)	56 (100)	0 (0)	56 (100)
No	24	6 (25)	18 (75)	12 (50)	12 (50)
Remote metastasis					
Yes[c]	22	0 (0)	22 (100)	0 (0)	22 (100)
No	58	6 (10.3)	52 (89.4)	12 (20.7)	46 (79.3)

[b]$P<0.01$, [d]$P<0.01$, *vs* adjacent tissues; [a]$P<0.05$, *vs* Dukes A and B groups: [c]$P<0.05$, *vs* non-metastasis groups.

DISCUSSION

In this study, we examined the expressions of HSP70 and grp94 in 80 colonic cancer samples by immunohistochemistry. The results showed that almost all the detected colonic cancers expressed HSP70. By way of immunohistochemistry and microscope analysis, we found that there was a definite correlation between expression of HSP70 and grp94 and differentiation, development and metastasis of colonic cancers. The lower the differentiation, the higher the level of HSP70 and grp94 expression. The worse the colonic cancer progresses, the higher the level of HSP70 and grp94 expressions.

There is evidence that heat shock protein (HSP) is a group of highly-conserved proteins synthesized after heat induction[1-3]. In mammalian cells, this system is divided into HSPs and glucose-regulated proteins (grps)[1], which appear to be structurally and functionally related. During the growth and development of normal cells, HSP70 is constitutively expressed at low levels but the expression is dramatically enhanced by stressful conditions[2]. Studies suggested that HSP70 continuously expressed at high level in tumor cells without any stimulation[4,5], and that there is a possible correlation between the expression of HSP70 and the growth and progression of tumor cells[9,10]. In normal cells, grp94 could also be induced by various stresses to function as molecular chaperones[13,14]. Enhanced expression of grp94 has a close relationship with cancer cell growth[11,15]. High-

level expression of grp94 contributes to tumorigenicity of certain tumors[15,16]. However, few reports have studied the expression of grp94 in colonic cancers especially during the course of tumor growth and differentiation in comparison with HSP70. In these experiments, HSP70 and grp94 express high level when colonic cancers differentiate and progress. The results are consistent with some researches[17,18]. It is reasonable to propose that HSP70 and grp94 up-expression in these tumor cells is closely related with tumor cell survival and proliferation. Recent studies have suggested that HSPs take part in cell growth and proliferation by several ways such as signal transduction and cell cycle regulation through combining certain proto-oncogene products, indicating that these proliferating cells need much more HSPs to maintain protein activities[19,20]. Tumor cells are a group of high-proliferation heterogeneous cells which progress gradually through mutant oncogene products[21]. Continuous expression of HSP proteins in tumor cells may be required to serve as molecular chaperones in regulating and stabilizing these products during tumor growth[6-8]. The existence of mutant or oncogene products may stimulate HSP synthesis[8]. It has been verified that HSP70 interacts with mutant p53 to stabilize its function, conversely wild-type p53 may down-regulate HSP70 expression[22]. Our results showed that the expression of HSP70 and grp94 in colonic cancer was higher than that in adjacent tissues, and the expression of HSP70 and grp94 in poorly-differentiated colonic cancer with metastasis was definitely higher than that in well-differentiated colonic cancer without metastasis, indicating that up-expression of HSP70 and grp94 is likely to have some relationship with progression, invasion and metastasis of colonic cancer.

Studies revealed that considerable expression of HSPs is found in tumor cells, showing that HSPs may be induced by other stresses and participate in broader body defense during tumor cell growth and differentiation[7-9]. It may be presumed that under various stimulations and stressful conditions, colonic mucous membrane has to transcript and translate high level of HSPs which may sustain normal metabolism and functions of cells in order to avoid the damage caused by deleterious factors such as methylcholanthrene-oncogenesis evocator. Under these conditions, colonic mucous membrane should synthesize HSPs rapidly to exert the protecting role for colonic mucous cells. Progression of colonic cancer is a gradual process under the long-term influence of various stimulations. During the process, inducible HSP synthesis increases gradually[23], which is confirmed by our results that HSP70 and grp94 were expressed higher in colonic cancer than that in adjacent tissues. The lower the differentiation, the higher the level of HSP70 and grp94 expression. The worse the colonic cancer progresses, the higher the level of HSP70 and grp94 expressions. The expression level of HSP70 and grp94 can be used as diagnostic or prognostic marker for colonic cancer.

HSP70 and grp94 have been identified as murine tumor rejection antigens[24,25]. The classical mechanisms of HSP70 and grp94 against tumors are that they may act as chaperones to facilitate MHC-I peptide loading and therefore increasing the tumor peptides presented by MHC-I[26,27]. HSP70-associated peptides may directly activate $\gamma\delta T^+$ lymphocytes or natural killer cells as superantigens independently of the stimulation of MHC-I class molecules[28,29]. Through this way CTL response can be induced and anti-tumor immunity is activated. Our data show that there is high-level expression of HSP70 and grp94 in colonic cancer, and their expression has a significant correlation with differentiation and metastasis of tumors.

In conclusion, there is a close correlation between the overexpression of HSP70, grp94 and progression, metastasis of colonic cancer. The high-level expression of HSP70 and grp94 can be used as diagnostic or prognostic markers for colonic cancer.

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