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Heat shock protein 27 is over-expressed in tumor tissues and increased in sera of patients with gastric adenocarcinoma

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Abstract

Background: In a previous study, we found that heat shock protein 27 (HSP27) was over-expressed in gastric adenocarcinoma (GA) tissue. In this study, our goal was to further verify the expression profile of HSP27 in patients with GA.
Methods: Western blot and immunohistochemistry were employed to determine HSP27 expression in 50 paired tumor and adjacent normal tissue. ELISA was used to quantify serum HSP27 concentrations in the same 50 GA patients and 50 healthy individuals.
Results: Compared to adjacent normal tissues, HSP27 was over-expressed in 25 (50%, $p=0.000$) and 24 (48%, $p=0.000$) cases of GA tissue by Western blot and immunohistochemistry, respectively. ELISA revealed significantly higher serum concentrations of HSP27 in patients with GA patients (mean = 986 pg/mL) compared to healthy individuals (mean = 573 pg/mL) ($p=0.003$). In addition, infection with *Helicobacter pylori* (HP) in healthy individuals was associated with increased expression of HSP27 in both gastric mucosa and serum.
Conclusions: These data suggest that HSP27 is over-expressed in GA tissue and serum concentrations of HSP27 are increased in patients with GA. Over-expression of HSP27 may indicate a gastric malignant/infectious process. The detection of serum HSP27 concentrations by ELISA may be useful for screening for GA.
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Keywords: ELISA; gastric adenocarcinomas; heat shock protein 27; immunohistochemistry; Western blot.

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Introduction

Heat shock protein 27 (HSP27) is a member of the small molecular weight HSP family and is a major molecular chaperone with a role in regulating normal cell physiology and the cellular stress response (1–3). HSP27 prevents protein aggregation, helps in the correct folding of proteins, and assists with the formation of multi-protein complexes, and recovers the protein structure for proteins that denature during cell stress (4, 5). Besides these main chaperone functions, HSP27 has been associated with apoptosis and cell proliferation and differentiation (6, 7). Additionally, HSP27 is over-expressed in many kinds of cancers, although its role in cancer remains unclear (8–10).

Gastric cancer is a common and serious malignancy. More than 870,000 new cases are diagnosed worldwide each year, and it has the second highest death rate among all cancers. Previously, we analyzed protein expression patterns in paired gastric adenocarcinoma (GA) and non-neoplastic mucosa tissues using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF/TOF MS) (11). This analysis identified 42 proteins differentially expressed two-fold or greater between tumor and adjacent normal tissues. HSP27 was one of the proteins shown to be over-expressed in GA tissue (11). The present study was performed to further verify its expression profile in patients with GA. To accomplish this objective, expression of HSP27 in GA and matched adjacent normal tissue were detected by Western blot and immunohistochemistry. Also, we measured the concentration of HSP27 in sera of patients with GA and healthy individuals using ELISA.

Materials and methods

Tissue samples and serum samples

Fifty GA and matched normal gastric mucosa tissue samples were obtained. Diagnosis of GA was confirmed by analysis of tissue (Table 1), and matched normal gastric mucosa tissue samples were a minimum of 10 cm from the edge of the cancer tissue. The samples were obtained from fresh surgical material of GA patients from Dongfang Hospital (Fuzhou, Fujian, P.R. China) within 30 min of resection. The samples were stored immediately at –80°C until analysis. In addition, regular paraffin embedded blocks were prepared. For ELISA, we collected serum samples from the 50 patients with GA prior to surgery. We also collected 50 serum samples from healthy individuals at Dongfang Hospital. The age range of the patients with GA was 30–87 years (median 59 years), and the age range for healthy individuals was 28–76 years (median 57 years).

Table 1 HSP27 expression in tumor tissues and sera from patients with gastric adenocarcinomas.

Patient number	Sex	Age, years	HSP27 expression detection		Pathological changes of the paired non-neoplastic mucosa	HSP27 serum level, pg/mL	Histological type	Differentiation grade	TNM stage
			Western blot[a]	IHC[b]					
1	F	64	+1.6	+2.4	Mild AtroG	1866	Intestinal	High	T2N0MX
2	M	75	1.0	1.0	Severe ActiG	881	Intestinal	Poor	T3N2MX
3	M	55	ND	ND	Unchanged	56	Intestinal	Moderate	T4N2MX
4	M	58	+4.6	+3.9	Unchanged	1072	Intestinal	Poor	T2N0MX
5	M	58	+2.1	+5.7	Unchanged	1025	Diffuse	Signet-ring	T3N1MX
6	M	61	1.0	1.0	Severe ActiG	1307	Intestinal	Poor	T2N1MX
7	M	32	+2.5	+3.6	Unchanged	1133	Intestinal	Moderate	T2N0MX
8	M	48	+9.0	+8.3	Unchanged	991	Intestinal	Moderate	T1N0MX
9	F	47	–0.8	–0.6	Severe IM	370	Intestinal	High	T2N0MX
10	F	53	1.0	1.0	Moderate AtroG with mild ActiG	328	Diffuse	Signet-ring	T2N1MX
11	M	49	+3.6	+7.2	Unchanged	2500	Diffuse	Mucinous	T4N2Mx
12	F	71	ND	1.0	Severe AtroG	75	Intestinal	Poor	T2N1MX
13	M	51	–0.6	–0.7	Severe ActiG	400	Intestinal	Moderate	T1N0MX
14	F	72	+4.1	+1.7	Mild AtroG	1131	Intestinal	Moderate	T2N0Mx
15	F	58	1.0	1.0	Severe ActiG	725	Intestinal	Moderate	T2N1Mx
16	M	59	+7.2	+11.6	Unchanged	2500	Intestinal	Poor	T4N1Mx
17	M	87	+5.4	+4.1	Unchanged	1902	Intestinal	Moderate	T3N1Mx
18	F	48	+3.2	1.0	Severe ActiG	980	Intestinal	Poor	T3N1Mx
19	M	54	1.0	1.0	Moderate ActiG	426	Intestinal	Moderate	T3N2Mx
20	M	74	+1.4	+1.7	Mild AtroG	1083	Intestinal	Moderate	T1N0Mx
21	M	48	–0.7	–0.8	Moderate IM	218	Intestinal	Poor	T2N0Mx
22	M	47	+8.3	+8.0	Unchanged	2500	Diffuse	Signet-ring	T3N0Mx
23	M	43	1.0	1.0	Moderate IM	568	Intestinal	Poor	T3N2Mx
24	M	46	ND	1.0	Severe AtroG	67	Diffuse	Mucinous	T3N0Mx
25	M	69	1.0	1.0	Moderate IM	801	Intestinal	Moderate	T2N2Mx
26	M	65	+10.4	+6.5	Unchanged	2500	Intestinal	Poor	T3N1Mx
27	F	50	+1.9	+1.6	Mild AtroG	1018	Intestinal	Early stage GA	T1N0Mx
28	M	72	+2.5	+3.4	Mild AtroG	1212	Intestinal	Moderate	T2N0Mx
29	M	41	ND	ND	Unchanged	51	Intestinal	High	T2N1Mx
30	F	30	1.0	1.0	Severe IM	512	Intestinal	Poor	T3N1Mx
31	M	69	+6.6	+4.3	Unchanged	1574	Intestinal	Poor	T3N0Mx
32	F	38	1.0	1.0	Severe Actig	846	Diffuse	Signet-ring	T3N1Mx
33	M	51	+4.9	+2.9	Mild AtroG	2481	Intestinal	High	T4N2Mx
34	F	36	1.0	1.0	Moderate ActiG	631	Intestinal	Poor	T3N0Mx
35	F	67	1.0	–0.8	Severe IM	466	Intestinal	Early stage GA	T1N0Mx
36	M	75	–0.6	1.0	Moderate ActiG	307	Diffuse	Mucinous	T3N1Mx
37	F	74	+5.2	+9.9	Unchanged	2301	Intestinal	Poor	T3N2Mx
38	F	61	+3.3	+7.4	Unchanged	2094	Intestinal	Moderate	T3N1Mx
39	M	48	ND	ND	Unchanged	177	Intestinal	Moderate	T1N0Mx
40	M	50	+4.4	+2.1	Mild AtroG	1234	Intestinal	Poor	T3N1Mx
41	M	43	1.0	1.0	Moderate ActiG	258	Intestinal	Moderate	T2N0MX
42	F	76	+2.1	+1.8	Mild AtroG	1005	Diffuse	Signet-ring	T2N2MX
43	M	68	1.0	1.0	Mild IM	203	Intestinal	Moderate	T2N1MX
44	M	81	1.0	1.0	Mile IM	341	Intestinal	Moderate	T3N1MX
45	M	74	+2.9	+3.0	Unchanged	1157	Intestinal	Poor	T2N1MX
46	M	53	+3.1	+3.3	Unchanged	1348	Diffuse	Mucinous	T3N2MX
47	F	75	1.0	1.0	Mild IM	162	Intestinal	Moderate	T1N0MX
48	F	69	+3.6	+2.4	Mild AtroG	1292	Intestinal	Poor	T3N1MX
49	F	79	+1.4	+1.6	Mild AtroG	997	Intestinal	Moderate	T2N1MX
50	M	77	1.0	1.0	Moderate Atrog with mild Actig	298	Intestinal	Poor	T2N0MX

ND, none detected; M, male; F, female; IHC, immunohistochemistry; GA, gastric adenocarcinomas. [a]Values indicate the ratios of relative intensity (nomalized to β-tubulin) measured in GA vs. paired non-neoplastic tissue; ''+'' indicates over-expression; ''–'' indicates down-expression, and 1.0 is considered no changes. [b]Values indicate the ratios of GA vs. paired non-neoplastic tissue; ''+'' indicates over-expression; ''–'' indicates down-expression, and 1.0 is considered no changes. ActiG indicates chronic active gastritis; AtroG indicates atrophy gastritis; IM indicates intestinal metaplasia.

Ethics

Use of the serum samples, and GA tissue and paired non-neoplastic mucosa tissue for this study was approved by the Dongfang Hospital Ethical Committees.

Western blots for HSP27

Tissues lysates were prepared according to the method described previously (11). The protein concentrations in the GA and matched normal tissue samples were measured using a BCA Protein Quant Kit (Bio-Rad, Hercules, CA, USA). Equal amounts of protein (35 μg) were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (12%). The blots were transferred onto PVDF membranes (Amersham Bioscience, Piscataway, NJ, USA) and incubated with goat anti-human HSP27 (Santa Cruz Biotechnology, San Diego, CA, USA) at a dilution of 1:1000. The primary antibodies were detected with horseradish peroxidase-conjugated mouse anti-goat antibodies (Santa Cruz Biotechnology) at a dilution of 1:1000. The immunoreactive protein bands were visualized using enhanced chemiluminscent reagents (Amersham Bioscience). Western blotting of the same sample with anti-β-tubulin (Santa Cruz Biotechnology) was used as the control. The signal intensity was measured with ImageQuant TL v2003.03 analysis software (Amersham Bioscience). The relative intensity (RI) of HSP27 was normalized to β-tubulin, and the ratios of RI of GA vs. paired non-neoplastic tissue were calculated.

Immunohistochemistry for human HSP27

The blocks of paraffin-embedded human GA and matched normal tissue sections were sectioned, placed on glass slides, and baked for 2 h at 60°C. Tissues were deparaffinized with three changes of xylene, then with a series of ethanol (100%, 95%, 95% and 80%), followed by rinsing with distilled water for 5 min. Antigen retrieval was performed by putting the slides into boiling citrate buffer (100°C) for 90 s, and 3% H_2O_2 was used to quench endogenous peroxidase interference. After washing with phosphate buffered saline (PBS), the slides were covered with preimmune rabbit serum for 10 min and washed again in PBS. Primary goat anti-human HSP27 antibody (Santa Cruz Biotechnology), at a dilution of 1:200, was added to each sample, followed by incubation for 3 h at room temperature (RT). After three washes in PBS, the slides were incubated with a biotin-labeled rabbit anti-goat IgG secondary antibody (Santa Cruz Biotechnology) for 10 min at RT. The slides were washed in PBS again, and incubated with streptavidin-HRP (Santa Cruz Biotechnology) for an additional 10 min at RT. Finally, the slides were incubated with 0.5% 3,3′-diaminobenzidine tetrahydrochloride (DAB) in PBS containing 0.03% hydrogen peroxide for 10 min and counterstained with hematoxylin for 30 s. The slides were viewed with a microscope and the staining results were quantified using Motic Med 6.0A Material Image Analysis System (Motic, Beijing, China). The grade was expressed using a gray scale value, and the ratios of GA vs. paired non-neoplastic tissue were calculated. Samples incubated with PBS rather instead of the primary antibody were used as the negative control, and paraffin-embedded liver sections with known positive immunoreactivity for HSP27 were used as the positive control.

ELISA for human HSP27

Sera from the same 50 GA patients used for immunohistochemistry and 50 healthy individuals were collected, and the concentration of HSP27 was quantitated by ELISA (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The ELISA plates were measured using the Labsystems microtiter plate reader (Thermo, Waltham, MA, USA) at 450 nm. p-Values were obtained using the Wilcoxon two-sample-test.

ELISA for detection of human anti-*Helicobacter pylori* (HP) IgG in control group

Human anti-HP IgG was detected in sera from the 50 healthy individuals using ELISA (Biocup, Shenzen, Guangdong, China) according to the manufacturer's instructions (12). Briefly, 100 μL of serum diluted 1:100 in PBS was added to ELISA plates that were precoated with purified HP antigens. After incubation and washing, peroxidase-conjugated anti-human IgG diluted 1:5000 was added and the plates were incubated and washed again. The color was developed by adding tetramethylbenzidine (TMB) and the reaction was stopped by the addition of H_2SO_4. In the final step, the ELISA plates were measured with the Labsystems microtiter plate reader (Thermo) at 450 nm. Samples with absorbance values greater than the cut-off threshold (0.21) were considered positive.

Results

The over-expression of HSP27 in GA tissues identified by Western blot

To verify the expression profile of HSP27 in GA tissue, 50 cases of GA and paired non-neoplastic tissues were examined by Western blot with anti-HSP27. In 25 of the 50 GA cases, HSP27 had increased expression compared to the other tissue (50%, Table 1, $p=0.000$, Wilcoxon signed-ranks test). Over-expression of HSP27 was not correlated with age (>50 vs. ≤ 50, $p=0.667$, χ^2-test), gender (female vs. male, $p=0.061$, χ^2-test), histological type (intestinal vs. diffuse, $p=0.654$, χ^2-test), TNM stage ($p=0.763$, χ^2-test), and differentiation grade (high vs. poor, $p=0.512$, χ^2-test).

For the remaining 25 cases, no significant difference in the expression of HSP27 was identified in 16 cases (32%). No signal was observed from either tissue in five cases (10%), and four samples from patients with cancer had weaker expression compared with their paired non-cancerous tissue (8%, Table 1, Figure 1).

Representative results from eight patients are shown in Figure 1. A reactive band of molecular weight 27 kDa was found in GA tissue from seven of eight cases. HSP27 was over-expressed in samples 1, 4, 5, 7 and 8 of the GA tissue, and the intensities of HSP27 expression between GA and paired non-neoplastic tissues were nearly identical in samples 2 and 6, while no signal was detected in either the GA or non-neoplastic tissue from sample 3.

Over-expression of HSP27 in GA tissues identified by immunohistochemistry

To identify the localized expression patterns of HSP27 in cancer tissue, samples were examined by immunohistochemistry with anti-HSP27. To elucidate whether the pathological changes in mucosa affected HSP27 expression, we examined the 50 paired non-neoplastic mucosa for the chronic active gastritis (ActiG) (monocyte infiltration or neutrophil infiltration), gastric atrophy, and intestinal metaplasia (IM).

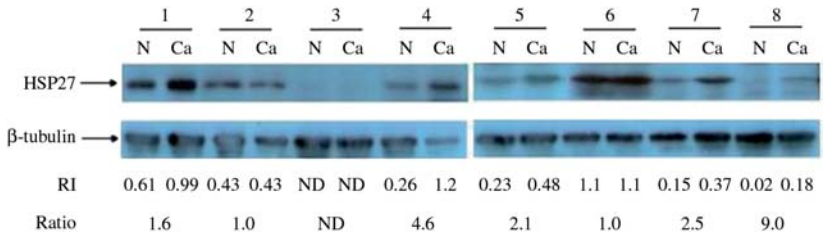

Figure 1 Western blot analysis of HSP27 expression.
Over-expression of HSP27 in GA tissue (Ca) compared to paired non-neoplastic tissue (N) confirmed by Western blotting. Equal amounts of protein from each sample were probed with anti-β-tubulin as control. The signal intensity measured by ImageQuant TL v2003.03 analysis software, the relative intensity (RI) of HSP27 normalized to β-tubulin, and the ratios of RI of GA vs. paired non-neoplastic tissue are at the bottom. HSP27 was over-expressed in samples 1, 4, 5, 7 and 8 from tissues with cancer. The intensity of HSP27 expression in cancer and non-cancerous tissues was nearly identical in samples 2 and 6. No signal was detected (ND) in either the cancerous or non-cancerous tissue samples from sample 3.

The degree of inflammation was graded as normal, mild, moderate, or severe according to the updated Sydney system using visual analog scales applied to microscopic examination results (13). Immunohistochemistry staining revealed that HSP27 was localized to the cytoplasm of cancer tissue (Figure 2C and D) in 24 of the GA samples (24/50, 48%). In contrast, only weak signals were found in the paired non-neoplastic mucosa (Figure 2A and B). Based on the immunohistochemistry, HSP27 expression was higher in patients with GA compared to the paired normal controls ($p=0.000$, Wilcoxon signed-ranks test). Similar to the results from the Western blots, over-expression of HSP27 was not correlated with increased age (>50 vs. ≤ 50 years, $p=0.432$, χ^2-test), gender (female vs. male, $p=0.056$, χ^2-test), histological type (intestinal vs. diffuse, $p=0.820$, χ^2-test), TNM stage ($p=0.759$, χ^2-test), or differentiation grade (high vs. poor, $p=0.951$, χ^2-test).

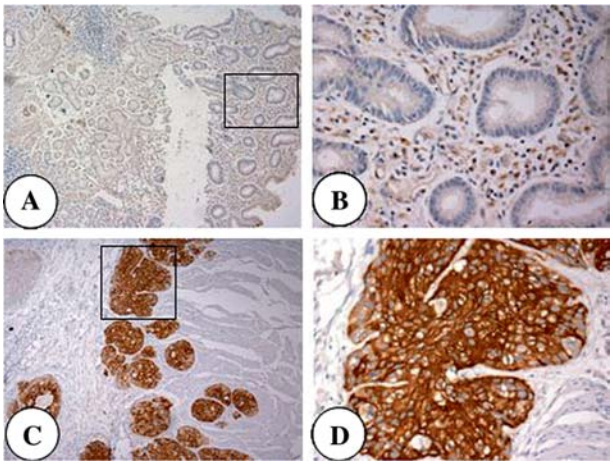

Figure 2 Immunohistochemical analysis of HSP27 expression in gastric adenocarcinoma.
HSP27 was expressed more strongly in the cytoplasm of GA tissue than in the non-neoplastic tissue. The expression of HSP27 in normal stomach (A–B) and GA (C–D). Images on the left panel were made at ×100 magnification; the images on the right were magnified images (×400) of the boxed sections depicted at left.

In the immunohistochemistry analysis, no significant difference between cancer and paired non-neoplastic mucosa cells was seen in 19 pairs (38%). The paired non-neoplastic mucosa in these cases included one, two, and three cases with severe, moderate, and mild IM, respectively, two cases with severe gastric atrophy, two cases with moderate gastric atrophy with mild active gastritis, and five and four cases with severe and moderate active gastritis, respectively. In three (6%) other cases, we detected no HSP27 signal from either tissue. Finally, in the remaining four pairs (8%), the GA samples (cases 9, 13, 21 and 35) had weaker HSP27 signals than their paired non-cancerous tissues. The non-cancerous tissues of three of these four cases had IM (two severe and one moderate). The fourth of these cases had severe active gastritis. In the 24 GA samples with over expression of HSP27, the paired non-neoplastic mucosa had either mild gastric atrophy (10 cases), or were without any significant change (14 cases, Table 1).

The results of histological analysis were consistent with Western blot results showing that HSP27 was over-expressed in the majority of cancer tissues. These results indicate that certain pathological changes in the non-neoplastic mucosa, such as ActiG, gastric atrophy, and IM can show increased HSP27 expression which may mask increased expression by the tumor.

HSP27 concentrations in human serum samples

To investigate whether there is a link between intracellular and extracellular HSP27 concentrations in gastric neoplasia and to investigate whether HSP27 secreted in patients with GA could be of diagnostic significance, we used ELISA to measure HSP27 concentrations in serum samples from 50 patients with GA and 50 healthy individuals. All 50 GA patients had detectable HSP27 in their serum (Figure 3). The mean serum HSP27 concentrations were 986.40 ± 742.51 pg/mL and 572.54 ± 506.16 pg/mL for GA patients and healthy persons, respectively. In most cases, HSP27 concentration was significantly higher in the GA patients ($p=0.003$, Wilcoxon two-sample test). Our results suggest that a possible screening threshold for GA is 573 pg/mL. In addition,

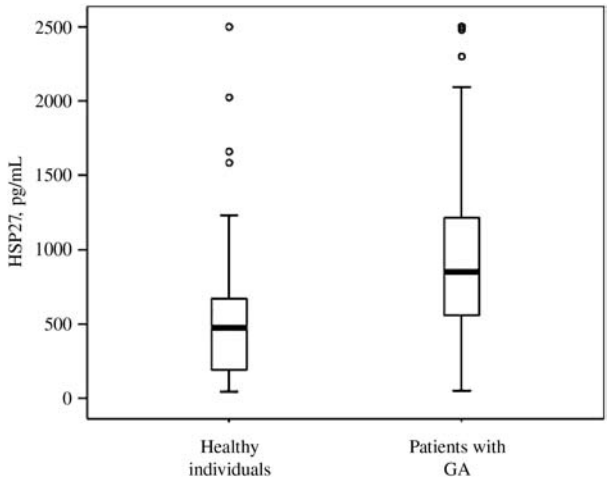

Figure 3 Serum HSP27 concentration by ELISA assay.
HSP27 serum concentrations were significantly higher in patients with GA compared to healthy individuals ($p = 0.003$). The mean concentration of HSP27 was 986 pg/mL (range 51–2500 pg/mL) for GA patients and 573 pg/mL (range, 43–2500 pg/mL) for healthy individuals. Box plots show serum concentrations of HSP27. The lines inside the boxes are the mean. The box represents the interval between the 25th and 75th percentiles except for data >1500 pg/mL for healthy individuals and >2000 pg/mL for GA patients, which are shown as circles. Seven out of 50 serum samples from patients with GA were >2000 pg/mL, and 4 out of 50 serum samples from healthy individuals were >1500 pg/mL.

all GA patients with tissues over-expressing HSP27 had increased serum HSP27 concentrations (shown in Table 1). The HSP27 serum concentrations were correlated positively with HSP27 expression in GA tissues as detected by Western blot and immunohistochemistry.

ELISA for detection of human anti-HP IgG in control group

Anti-HP IgG seropositivity was detected in 46 of 50 healthy individuals. The results indicated that HP infection may be related with increased HSP27 expression in the serum of controls.

Discussion

When a cell becomes cancerous, its stress level can increase resulting in changes in pH, cell metabolism, and other cellular events. These changes can disrupt the cell cycle and DNA synthesis, leading to gene mutations and neoplastic progression (14–16). Some cancerous cells over-express HSP27 which may increase cell proliferation and metastasis. Over expression of HSP27 has been reported for a wide variety of tumors, and is associated with poor prognosis of some tumors, including liver and prostate carcinoma (17–24).

The over-expression of HSP27 in gastric cancer has been observed by several studies (8, 25, 26). In gastric cancer, over-expression of HSP27 may be associated with metastasis to the lymph nodes, advanced stage (III and IV) cancer, and shorter overall survival of patients (27, 28), indicating that HSP27 has prognostic significance for gastric cancer. Additionally, HSP27 expression was closely related to gastric tumor size, and the presence of organ metastases (29). However, another study found that HSP27 was not present in metastatic gastric tumors, and was only detectable in samples from non-metastatic gastric cancer tissue (26). None of these studies measured HSP27 serum concentrations to investigate if there is a link between intracellular and extracellular HSP27 concentrations in gastric neoplasia. Previously, we found HSP27 over-expression in 5 of 10 GA tissues using a proteomics technique. Here, we extended that study to more GA patients and investigated the link between intracellular and extracellular HSP27 concentration.

Both Western blotting and immunohistochemistry revealed over-expression of HSP27 in most tumor tissues. Additionally, the concentration of HSP27 in serum samples from patients with GA was significantly higher than that from healthy individuals. Also, serum HSP27 concentrations were positively correlated with HSP27 expression in GA tissue detected by Western blot and immunohistochemistry. Almost all of the patients with HSP27 over-expression in GA tissues also had increased serum HSP27 concentrations.

Although HSP27 expression in cancer tissue and paired non-neoplastic mucosa was nearly identical in case 6 (Figure 1), this non-neoplastic mucosa had strong positive expression of HSP27. Infection with HP is also associated with mucosal damage and increased stress production, leading to increased expression of HSP27 (30, 31). Using the rapid urease test (RUT) (32), we determined that nine of the non-neoplastic mucosa tissues had HP infection, among these nine samples with HP infection were case 6 and five other cases (cases 9, 13, 21, 35, and 36), in which the paired non-neoplastic mucosa had higher HSP27 expression than the GA tissue. Although HP infection may have resulted in an apparent lack of over-expression in tissue from case 6 GA tissue, the serum concentration of HSP27 (1307 pg/mL) was higher than the mean for patients with GA.

In addition to HP infection, paired non-neoplastic mucosa with ActiG, gastric atrophy, and IM also had increased HSP27 expression, leading to a decreased ratio of HSP27 expression in cancer tissue to normal tissue (Table 1). These pathological changes in non-neoplastic mucosa may mask the increased expression by the tumor and explain why only 50% of GA tissues had an increase in HSP27 expression. In contrast, these pathological changes were not associated with HSP27 serum concentrations. In almost all cases, HSP27 serum concentrations were closely associated with the over-expression of HSP27 in GA tissue. Future studies should focus on the link between HSP27 serum concentration and over-expression in GA tissue.

HP infection increases HSP27 expression (30, 31). Because 46 of the 50 healthy individuals were infected with HP, the control group might also have had increased HSP expression in their sera due to HP infection. These increased HSP27 concentrations in the control individuals may lead to

overlapping distributions of HSP27 in serum in GA patients and healthy controls.

HSP27 is constitutively expressed at low levels in the cytosol of most human cells, including cells located in gastric mucosa. These low levels of expression likely indicate an important role for HSP27 in cellular homeostasis and gastric mucosa defense (33–36). In accordance with previous results, we also found HSP27 expressed in majority of gastric mucosa. Although it is accepted that HSP27 is expressed in normal epithelium (37), HSP27-positive healthy individuals may have experienced microbial or viral infection, resulting in stressed mucosa (38). We identified some cases with no HSP27 expression in either the non-neoplastic gastric mucosa or GA tissue. The lack of HSP27 expression was due to limited sensitivity of current methods, individual differences, or other reasons (37, 38). These results should be confirmed in future studies.

The data from this study also indicated that over-expression of HSP27 was not related to patient's age, gender, TNM stage, histological type and differentiation grade. Due to the over-expression of HSP27 being found in GA from early through late stages, we thus speculate that HSP27 might be associated with early stage carcinogenesis.

In conclusion, our results demonstrate that HSP27 is over-expressed in GA tissues, and increased in sera from patients with GA. Over-expression of HSP27 may indicate a malignant or infectious process, and the measurement of serum HSP27 concentrations by ELISA may be useful for screening for GA.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article. Research funding played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.
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