Original Articles

HSP27 in patients with ovarian carcinoma: still an independent prognostic indicator at 60 months follow-up

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Summary

Objective: Heat shock protein 27 (HSP27) is produced in response to pathophysiologic stress in animal cells. The authors have previously shown that HSP27 is an independent prognostic indicator in patients with ovarian carcinoma. The present study was performed to see whether HSP27 remained an independent prognostic indicator with longer follow-up.

Methods: One hundred and three consecutive patients with epithelial ovarian carcinoma were studied. Slides were prepared from fresh tissue. HPS27 staining was performed as previously described. Patient records were examined for FIGO stage, grade, histology, level of cytoreduction and survival.

Results: One hundred and three patients were followed for a mean of 60 months. Twenty patients had FIGO Stage I disease, four Stage II, 59 Stage III, and 20 Stage IV. Immunohistochemical (IHC) staining for HSP27 was not related to histologic grade, level of cytoreduction or histologic subtype. A statistically significant decrease in HSP27 staining was found to correlate with increased FIGO stage ($p = 0.008$). Using cox-regression analysis, HSP27 staining ($p = 0.025$), stage ($p = 0.0012$), and level of cytoreduction ($p < 0.0001$) were independent predictors of survival in these patients.

Conclusion: Cox-regression analysis found HSP27 to be an independent indicator of prognosis and survival in patients with ovarian carcinoma who had longer follow-up. Decreased HSP27 staining was related to decreased survival. This study confirms the authors' earlier report on the importance of HSP27 as a prognostic indicator in ovarian carcinoma.

Key words: Ovarian cancer; Heat shock protein 27; HSP27; Survival.

Introduction

In the United States, ovarian cancer is the most deadly of all gynecologic malignancies accounting for only 4% of newly diagnosed cancers annually, but 5% of deaths from cancer in women. In 2003 alone, an estimated 25,400 women will be diagnosed with ovarian cancer and 14,300 will die from their disease [1]. SEER data reveals an average 5-year survival in the United States of 46% compared to 32% for the countries involved in the EUROCARE cancer registry [2]. Researchers continue to devote much effort into discovering the cause of this devastating disease.

In 1974, the discovery of a high level expression of stress response or heat shock proteins (HSPs) was found to accompany chromosomal puffing previously described as the heat shock response by Drosophilia after an applied heat stress [3, 4]. Further studies, during the 1970's and 1980's demonstrated that the same rapid synthesis of a small group of highly conserved HSPs occurs in most organisms in response to heat shock and various other stressors including exposure to heavy metals, oxidants, tissue trauma or ischemia, inflammation, and antineoplastic drugs [5, 6]. Less stressful conditions such as the normal cell cycle, cell differentiation, hormonal stimulation, and stimulation of proto-oncogenes also elicit an HSP response. HSPs apparently assist the cell in surviving or resisting stressful conditions by an incompletely understood mechanism. There are suggestions that HSPs are important in protein assembly, immunity, and autoimmunity [7]. The major functions of stress-induced HSPs seem to be prevention from protein aggregation, misfolding of denatured cellular proteins, and renaturation of cellular proteins [8]. The role of HSPs in cellular proliferation and drug resistance makes these proteins particularly intriguing for cancer research.

This manuscript represents a follow-up on our previously published work [9]. The authors found that HSP27 was a prognostic indicator of survival. The aim of this study was to determine prospectively whether HSP27 staining, as shown by immunohistochemical (IHC) methods, was still an independent prognostic indicator of survival in patients with epithelial ovarian carcinoma when a longer follow-up period was analyzed.

Patients and Methods

One hundred and three consecutive patients with epithelial ovarian carcinoma had their tumor's HSP27 staining studied by image analysis. The hospital, office, and tumor registry records were examined for FIGO stage, grade, histology, level of cytoreduction, and survival. No tumors of low malignant poten-

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tial wee included. A further criterion was that the initial surgical procedure, prior to chemotherapy, had to be performed by one gynecologic oncologist (H.E.G.). Since only one surgeon's patients were studied, the level of optimal cytoreduction achieved during surgery remained the same throughout the years of the study and was determined by the amount of unresectable tumor left after primary surgery. Optimal cytoreduction was achieved when no gross residual tumor mass greater than 1 cm in diameter remained following surgery. All patients, other than those with FIGO Stage IA or IB grade 1 tumors, were treated with either six courses of cisplatin/carboplatin and paclitaxel or cisplatin/carboplatin and cyclophosphamide. The standard doses per course used were: cisplatin 75-100 mg/m^2, carboplatin 300-350 mg/m^2, cyclophosphamide 750-1000 Mg/m^2, or paclitaxel 135-175 mg/m^2. With the platinum-based agents, the lower doses were the starting doses. If a patient's hematologic system tolerated the lower dose, the dose was increased.

Fresh frozen tissues from ovarian carcinomas were used for IHC detection of HSP27 protein. Five-micrometer sections were cut with a Cryostat (Reichert-Jung Cryocut 1800) and immediately fixed in neutral buffered formalin for 15-30 min. After fixation, the slides were rinsed with PBS and endogenous peroxidases were blocked with 1% hydrogen peroxide in methanol for 10 min. The anti-HSP27 monoclonal antibody NCL-HSP27 (Novocastra Lab, U.K.) at a 1:30 working dilution was added to the slides and incubated 90 minutes at room temperature. After being rinsed with PBS twice, biotinylated anti-mouse IgG and avidin-biotin complex (Vector Laboratory, Burlingame, CA, ABC kit 6102) were used for immunohistochemical staining. The slides were developed with 3, 3 diaminobenzidine tetrahydro-chloride (Polyscience Inc., Warinton, PA) and counter-stained with 0.5% methyl green in 0.1 M sodium citrate buffer pH 4.0 for one minute. Sections were dehydrated with 100% isopropanol and mounted with Permount. HSP27 IHC staining was determined based on the percentage of carcinoma cells stained in the section (0 ≤ 10%, 1 = 10-25%, 2 = 26-50%, 3 ≥ 50%), intensity (1 = weak; 2 = moderate; 3 = strong), and heterogeneity (-1 = marked; 0 = moderate; +1 = mild). Heterogeneity was defined as non-uniform immunostaining in a tumor section. The final score was calculated by multiplying the percentage of cells stained by the intensity and then adding the heterogeneity score. The highest score possible was ten and lowest 0.

Statistics were performed utilizing SPSS for Windows version 7.5 (Chicago, IL), namely, the Student's t-test, one-way analysis of variance (one-way ANOVA), or Cox-regression analysis.

Results

One hundred and three patients with epithelial ovarian carcinoma had their tumors analyzed for HSP27 staining. The median age of the patients in the study was 62 years with a range from 41-97 years (mean 62 years). Age was not related to length of survival (p = 0.25). There was no correlation between age and HSP27 staining (p = 0.21).

Clinical and pathologic characteristics are listed in Table 1. There was not a significant difference in the mean HSP27 staining when comparing serous versus non-serous carcinomas. The mean HSP27 staining of serous carcinomas was 4.8 while the mean HSP27 staining of non-serous carcinomas was 3.9 (p = 0.58). There was no correlation between histologic grade and HSP27 staining (p = 0.18). One-way ANOVA revealed increasing stage to be significantly correlated with decreasing HSP27 scores (p = 0.008). The mean HSP27 staining of tumors in patients able to have their tumors optimally cytoreduced (5.1) was not significantly different than the mean HSP27 staining of tumors in patients unable to undergo optimal cytoreduction (3.3) (p = 0.064).

Forty of the 103 patients were alive at a mean of 60 months (median 55 months). The relationship between survival and FIGO stage, grade, histology, cytoreduction and HSP27 staining were analyzed by Cox-regression analysis with survival as an endpoint (Table 2). Optimal cytoreduction (p < 0.0001), FIGO stage (p = 0.0012), and increased HSP27 staining (p = 0.025) were independent prognostic indicators of survival. Figure 1 illustrates the importance of HSP27 staining when the patients are stratified into high staining scores (6-10) and (0-5). Patients with increased HSP27 staining have a much higher chance of survival at 60 months as compared to patients with lower staining.

Table 1. — *Pathologic characteristics.*

Characteristic	Number of Patients
Histology	
Serous	79
Mucinous	4
Endometrioid	4
Clear cell	12
Transitional cell	2
Undifferentiated	2
Histologic grade	
1	2
2	20
3	81
FIGO stage	
I	20
II	4
III	59
IV	20
Level of cytoreduction	
Optimal	74
Non-optimal	29
Patient status at 5 years	
Alive	40
Dead	63

Table 2. — *HSP27 staining and FIGO Stage.*

FIGO Stage	HSP27 stainaing
I	7.5
II	5.0
III	3.4
IV	3.0

Table 3. — *Multivariate analysis of prognostic factors.*

Factors	p value
Age	0.44
Histologic grade	0.084
Histologic type	0.12
Level of cytoreduction	< 0.0001
FIGO stage	0.0012
HSP27 staining	0.025

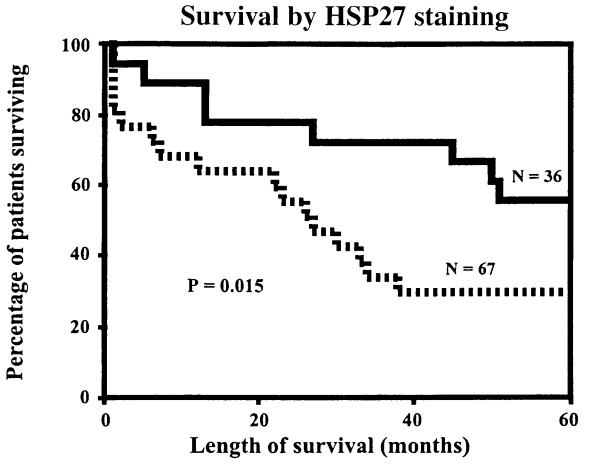

Figure 1. — Kaplan-Meier survival graph demonstrating that increased HSP27 staining is significantly related to increased survival.

Discussion

The role of HSP27 is complex with involvement in many very different processes such as embryogenesis, oncogenesis, cytokine effect, endothelial function, platelet activation, growth arrest in lymphocytes, growth stimulation of some epithelial cells, steroid sensitivity, drug resistance, tumorogenesis, and the signal transduction pathways of several cell regulators [10-14]. HSP27 is present in the normal cells of the fallopian tubes, skin, placenta, vagina, endometrial epithelium and stroma, the cervix, and occasionally in breast tissue [15-20]. The literature continues to describe a link between HSP27 and estrogen as either a protein that reacts with activated non-native estrogen receptors (ER) [21, 22]. However, it has been described that estrogen increases the level of HSP27 in glandular endometrial cells, and that progesterone decreases the amount of HSP27 present. Thus, HSP27 is at its zenith in the late proliferative phase and decrease greatly after ovulation [23]. In patients with endometrial carcinoma, not only has an elevated HSP27 has been correlated with longer survival, but it has been shown to be an independent prognostic indicator of survival in those patients [24]. Interestingly, although HSP27 has been found in other female genital tissues, it has been shown to be absent in normal ovarian tissue [25].

Few have addressed the role of HSP27 in ovarian carcinoma [9, 26-28]. Schneider *et al.* showed a relationship between HSP27 and the multi-drug resistance protein MDR1 [26]. They found that increased MDR1 and HSP27 staining were correlated but not with shorter survival or resistance to chemotherapy in the 95 patients studied [26]. Germain *et al.* also did not find a correlation between HSP27 and resistance to chemotherapy [27]. Using ELISA, HSP27 has been shown to be elevated in advanced as compared to early ovarian cancers [28]. The authors believed that secondary to the increased cellularity found in carcinomas as compared to normal tissue, their method, ELISA, gave falsely elevated levels and thought that immunohistochemical (IHC) methods would give an accurate interpretation [28].

Geisler and colleagues have analyzed the relationship between HSP27 and survival by IHC [9]. Using two-year survival as an endpoint, these authors found that HSP27 staining was elevated in early stage epithelial ovarian carcinomas as compared to later stage carcinomas. Multivariate analysis found that HSP27 staining was an independent prognostic indicator [9].

Arts *et al.* ascertained very different findings than the other previous survival analyses for HSP27 in ovarian carcinoma [29]. They found that a decrease in HSP27 was related to increased median survival. These results were only studied by univariate and not multivariate analysis. Furthermore, recently, Forsdyke described the role of heat shock proteins in acting as danger signals for the cell [30]. Decreased heat shock proteins allow the cancer cell to escape discovery and go undetected secondary to decreased antigenicity. Therefore, not only are Arts *et als.* results contrary to the presented multivariate analysis, they are contrary to the known function of heat shock proteins.

The current study is a follow-up that previously published report and finds HSP27 staining ($p = 0.025$) still to be an independent prognostic indicator at a mean follow-up of 60 months. As in the previous study, FIGO stage ($p = 0.0012$) and level of cytoreduction ($p < 0.0001$) are also found to be independent prognostic indicators. By IHC methods, increased HSP27 staining occurs in patients with early stage disease and staining decreases with advancing stage. Increased HSP27 staining is related to improved survival. Therefore, it appears that loss of part of the individual stress response to disease may occur in those patients with advanced tumors.

Conclusions

These results further support the authors' belief that HSP27 may have utility as a prognostic indicator of survival in patients with epithelial ovarian carcinoma. The fact that the results have remained the same with longer follow-up supports the use of IHC techniques for studying HSP27, as well as the scoring system which Geisler *et al.* have utilized [9, 24].

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