BAG-4/SODD and Associated Antiapoptotic Proteins Are Linked to Aggressiveness of Epithelial Ovarian Cancer

Christina M. Annunziata,[1] Lilach Kleinberg,[5] Ben Davidson,[5] Aasmund Berner,[5] David Gius,[3] Nana Tchabo,[2] Seth M. Steinberg,[4] and Elise C. Kohn[1,2]

Abstract

Purpose: We hypothesized that elevated expression in ovarian cancer of the BAG family of prosurvival proteins and associated partners would be associated with clinical features of aggressiveness in ovarian cancer.

Experimental Design: Expression patterns of BAG-1, BAG-3, BAG-4, and Bcl-xL were determined by immunohistochemical analysis of tissue samples obtained at diagnosis from 28 women with stage III or stage IV ovarian cancer treated with cisplatin, paclitaxel, and cyclophosphamide after initial cytoreduction. Association of these proteins, BAG-6, heat shock protein 70 (Hsp70), Hsp27, and Bcl-2, with clinical variables was tested in ovarian cancer tissue arrays from Gynecologic Oncology Group tissue bank.

Results: A statistically significant relationship was found between elevated cytoplasmic expression of BAG-4 and improved overall ($P = 0.0002$) and progression-free survival ($P = 0.003$) in the prospectively collected samples. Bcl-2 staining was significantly more frequent on the tissue array in lower stage ($P = 0.005$) and grade ($P = 0.0009$) tumors, whereas Hsp70 was prominent in higher grade cases ($P = 0.002$). Furthermore, Bcl-xL was more closely associated with serous compared with endometrioid ovarian cancers ($P = 0.004$).

Conclusion: Unexpectedly, cytoplasmic expression of BAG-4 and Bcl-2 marked less aggressive ovarian cancer, whereas nuclear Hsp70 suggested more aggressive behavior. Bcl-xL may play a more prominent function in the pathology of serous histology ovarian cancers compared with the endometrioid subtype. The findings presented here support involvement of these proteins in the propagation of ovarian cancer and provide a basis for the development of molecular therapeutics modulating these survival pathways.

Ovarian cancer affects over 22,000 women annually, and >70% of these women eventually die of their disease (1). The poor survival of affected women is due in part to three quarters of all patients presenting with advanced stage disease at the time of initial diagnosis (2). Complete response to standard chemotherapy with a taxane and a platinum agent varies from 50% to 85% in advanced disease, depending on the volume of tumor left after cytoreductive surgery (2). Despite this promising initial outcome, these patients have a high cancer-related mortality. New molecular prognostics and therapeutics may identify patients for novel therapies and potentially improve the durability of response. We observed increased expression of the prosurvival protein BAG-3 in platinum-resistant ovarian cancer cells.[6] We therefore sought to determine the expression pattern of the BAG family of proteins and associated partners to discern relationships of these proteins with clinical features of aggressiveness in ovarian cancer.

The Bcl-2, BAG, and heat shock protein (Hsp) families of proteins directly interact with each other to regulate cell survival (see diagram in Fig. 1). The Bcl-2 family and associated proteins have been implicated in the pathogenesis of ovarian cancer (3–5). Bcl-xL and Bcl-2 may have prognostic importance in ovarian cancer (6, 7). The BAG proteins act as cochaperones for Bcl-2 and Hsp70 (8, 9). BAG proteins have been shown to bind directly to Bcl-2, but not to Bcl-xL (9). BAG-1, BAG-3, and BAG-4 [also known as silencer of death domain (SODD)] are involved in the survival of cancer cells and have been linked with aggressiveness of breast, gastric, and pancreatic cancer (8–11). Loss of BAG-4, conversely, has been observed in resistance to platinum chemotherapy (7, 12). The BAG proteins have been shown as both cytoplasmic and nuclear in location, although the functional importance of this has not been elucidated (9). Hsp27 and Hsp70 are cochaperones for the Bcl and BAG proteins and modulate induction

Authors' Affiliations: [1]Medical Oncology Branch, [2]Molecular Signaling Section, Laboratory of Pathology, [3]Radiation Oncology Branch, and [4]Biostatistics and Data Management Section, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland and [5]Pathology Clinic, Rikshospitalet-Radiumhospitalet Medical Center, Oslo, Norway

Received 2/7/07; revised 7/19/07; accepted 8/17/07.

Grant support: Intramural Research Program of NIH, National Cancer Institute, Center for Cancer Research.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Christina M. Annunziata, Medical Oncology Branch, National Cancer Institute, NIH, Building 10, Room 4N115, 10 Center Drive, Bethesda, MD 20892-1374. Phone: 301-402-7259; Fax: 301-496-9956; E-mail: annunzic@mail.nih.gov.

doi:10.1158/1078-0432.CCR-07-0327

[6] N. Rasool and E.C. Kohn, unpublished observations.

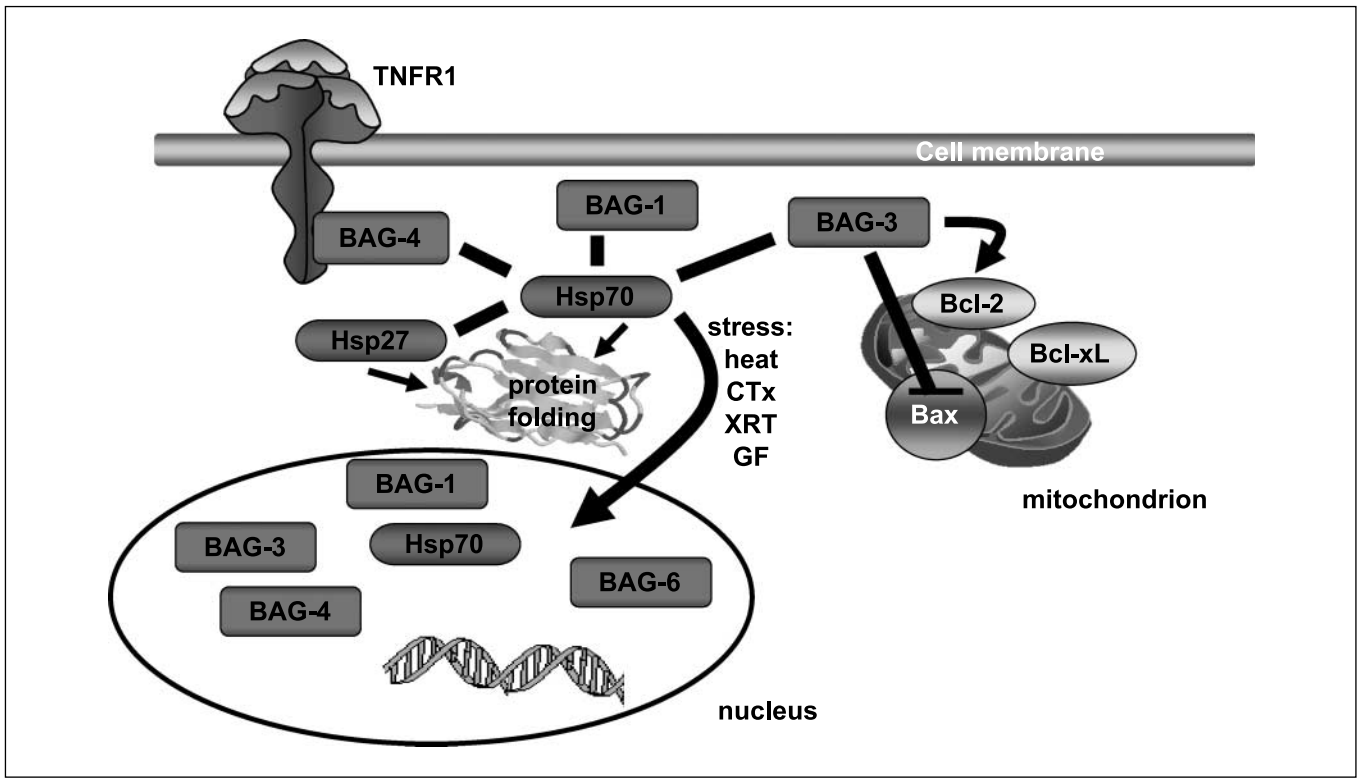

Fig. 1. Schematic diagram of relationships between Bcl-2, BAG, and Hsp protein families. Solid lines, physical interactions; arrows, positive regulation; perpendicular bars, negative regulation. CTx, chemotherapy; XRT, radiation therapy; GF, growth factors.

of apoptosis in response to cellular stresses (13). Both Hsp27 and Hsp70 have been negatively correlated with survival in solid tumors (13, 14). Hsp27 was found to be elevated in tissue and serum from pancreatic cancer patients (14). We hypothesized, based on existing evidence, that elevated expression of the antiapoptotic BAG, Bcl, and Hsp proteins would correlate with increased aggressiveness of ovarian cancer.

In the current study, we examined members of the Bcl-2, BAG, and Hsp protein families by immunohistochemical analysis of tumor tissue. We quantified frequency of expression and subcellular localization. Expression patterns were analyzed in tissue specimens obtained at diagnosis from 28 women treated with the triple therapy regimen, from which we have up to 10-year clinical follow-up information. Details of patient outcome are reported elsewhere (15, 16).[7] We also evaluated expression of these proteins in a tissue microarray of 170 ovarian cancers generated by the Gynecologic Oncology Group tissue bank. Here, we present our findings and their relationship with clinical features of histologic subtype, grade, stage, and patient survival.

Patients and Methods

Patients

Sixty-two women with advanced stage newly diagnosed ovarian cancer were treated between 1995 and 2001 using a triple-drug regimen of cisplatin, high-dose paclitaxel, and cyclophosphamide (15–19).[7] Briefly, patients received cyclophosphamide (750 mg/m^2 i.v.) on day 1, paclitaxel (250 mg/m^2, 24-h infusion) beginning on day 1, and cisplatin (75 mg/m^2) on day 2. Cycles were repeated every 21 days. Tissue blocks of primary and/or metastatic disease from the initial staging and cytoreductive surgery were collected for study of prognostic factors according to the National Cancer Institute Institutional Review Board–approved protocol and consent. Primary cytoreductive surgery was not done at our institution, and patients were referred for treatment; therefore, in some cases, we did not receive tissue blocks from the referring institution or the tissue received was of inadequate quality or quantity for replicate analysis of the multiple proteins included in the analysis presented here. The criterion used to select the 28 cases included in the current analysis from the 62 enrolled patients was simply the availability of tumor blocks that contained tissue adequate for this immunohistochemical staining analysis.

Tissue array

Replicate copies of the "stage and grade" ovarian cancer tissue array were obtained from the Gynecologic Oncology Group (GOG) tissue bank (Children's Hospital) under NIH Office of Human Subjects Protection approval. These arrays contained replicate tissue samples from 170 ovarian cancer patients with endometrioid or serous epithelial histology spanning stages I to IV and grades I to III. Each array contained samples in duplicate within the slide. No clinical outcome data are available for these anonymously presented clinical samples.

Immunohistochemistry

Formalin-fixed paraffin-embedded sections were analyzed for protein expression of BAG-1, BAG-3/CAIR-1, BAG-4/SODD, BAG-6, Bcl-2, Bcl-xL, Hsp27, and Hsp70 using immunohistochemistry. Staining was done manually. Antibody specifications and staining conditions are detailed

[7] Hussain et al., submitted for publication.

Table 1. Antibodies used in the study

Antibody	M/P	Clone	Source	Dilution	Pretreatment	Conditions
Hsp27	M	G3.1	NeoMarkers	1/200	MWO, citrate; pH = 6.0	30′, RT
Hsp70	M	W27	NeoMarkers	1/50	MWO, citrate; pH = 6.0	30′, RT
Bcl-xL	M	2H12	Zymed	1/200	MWO, EDTA; pH = 8.0	30′, RT
Bcl-2α	M	8C8	NeoMarkers	1/25	MWO, citrate; pH = 6.0	30′, RT
BAG-1	P		Santa Cruz Biotechnology	1/400	MWO, EDTA; pH = 8.0	30′, RT
BAG-3/CAIR1	P		(3, 22)	1/800	MWO, EDTA; pH = 8.0	30′, RT
BAG-4/SODD	P		Santa Cruz Biotechnology	1/100	MWO, EDTA; pH = 8.0	30′, RT
BAG-6	P		*	1/400	No pretreatment	30′, RT

Abbreviations: M/P, monoclonal/polyclonal; MWO, microwave oven; RT, room temperature.
*A polyclonal rabbit antibody was generated against amino acids 1118 to 1132 of the COOH terminus of BAG-6/BAT3 isoform α. The peptide affinity-purified antibody was shown to have a single protein band by immunoblot that fully competes with cognate peptide (data not shown).

(BAG-6 antiserum was generously provided by Dr. D. Gius, National Cancer Institute, and purified and validated as described; Table 1). Negative controls consisted of sections that underwent similar staining procedures with a nonrelevant antibody of the corresponding isotype: mouse IgG1-κ (Sigma-Aldrich) as control for Bcl-2 and Hsp27, mouse IgG2-α (DAKO) as control for Bcl-xL and Hsp70, and rabbit IgG (DAKO) for the four polyclonal antibodies. Positive controls consisted of solid ovarian carcinomas that showed immunoreactivity for the studied antigens in a pilot study. Visualization was achieved using the EnVision + peroxidase system (DAKO). Staining was considered positive when cytoplasmic or nuclear for BAG-1, BAG-3/CAIR-1, BAG-4/SODD, BAG-6, and Hsp70. Bcl-2, Bcl-xL, and Hsp27 showed only cytoplasmic expression and were therefore scored only at this subcellular localization. Staining extent was scored, on a scale of 0 to 4, as follows: 0, no staining; 1, 1% to 5%; 2, 6% to 25%; 3, 26% to 75%; 4, 76% to 100% of tumor cells. Positive cells were defined by the presence of any staining and were not graded on the intensity of staining to reduce bias introduced by interobserver variability, as well as by factors related to tissue handling (e.g., fixation time). All sections from the National Cancer Institute cohort contained a large number of viable tumor cells. Nonrepresentative or necrotic tumors in the GOG arrays were excluded from the study, as were all punches containing <100 tumor cells. Cases were scored individually by two experienced surgical pathologists (B.D. and A.B.) who were blinded to clinical outcome. Interobserver agreement was good, with 70% of specimens scored identically by both observers using the 0 to 4 scale detailed in the manuscript. Different scoring in all discrepant cases was of one scoring level and was easily settled in consensus sessions.

Statistical analysis

Prospective triple-therapy trial. Data from the 28 patients were available for analysis. Categorical data from immunohistochemical staining of seven variables were added to information on age, response, survival, and progression. Patients were classified according to response as complete (clinical complete response, pathologic complete response) or less than complete (partial response, pathologic partial response, stable disease). An exact Cochran-Armitage trend test was used to evaluate the difference in marker levels between patients classified according to response (20). Survival was computed from the on-study date until the date of death or last known follow-up. Progression was computed from the on-study date until the date of progression or the last date the patient was known to be progression-free for the patients who had not progressed or for whom recent information on progression was not available. The immunohistochemical data were grouped into categories to allow for a minimum of four patients per category, and the resulting groupings were evaluated for their prognostic association with survival and progression-free survival using the Kaplan-Meier method (21). The log-rank test was used to determine the statistical significance of the difference between Kaplan-Meier curves (22). All P values are two-tailed.

Tissue arrays. Cases from the tissue array (n = 170) were divided into three diagnostic categories [endometrioid, serous, or other (mixed or unspecified)]. A Wilcoxon rank sum test was used to determine the statistical significance of the marker values between patients with endometrioid and serous diagnoses. Tumors were classified into four ordered stages. A Jonckheere-Terpstra test for trend was used to determine the statistical association between increasing marker value and increasing stage (23). Similarly, tumors were classified into three ordered grades, and a Jonckheere-Terpstra test was used to determine the statistical association between increasing marker value and increasing grade. All P values are two-tailed and have not been adjusted for multiple comparisons. However, in view of the large number of statistical tests done in this exploratory analysis, only $P < 0.005$ should be interpreted as indicating statistically significant results, whereas those analyses with $0.005 < P < 0.05$ would reflect trends.

Table 2. Patient characteristics

	Prospective cohort (n = 28)	GOG tissue array (n = 170)
Age at diagnosis (y)		
21-40	0	2
41-50	6	28
51-60	14	32
61-70	5	35
71-80	3	23
>80	0	1
Stage		
I	0	43
II	0	32
III	17	56
IV	11	39
Grade		
1	0	27
2	6	66
3	22	67
NR		10
Histologic subtype		
Endometrioid	7	75
Serous	19	89
Other	2	6*

*Only included in stage and grade analyses.

Results

Patients and samples. Two groups of patients were studied. The first set of tissue samples was collected prospectively from patients receiving initial therapy with cyclophosphamide, high-dose paclitaxel, and cisplatin for epithelial ovarian cancer (15, 16).[7] The regimen resulted in surgical complete remission or microscopic residual disease in 84%; the median overall survival for all women enrolled on the clinical trial is 67.8 months, and the median progression-free survival is 19.8 months. For the subset of 28 patients with tumor tissue available and adequate for the current immunohistochemistry analysis, the respective medians are 65.6 and 16.7 months, which indicate that these patients are approximately representative of the larger group from which they were selected. To further understand associations between the survival protein expression and clinical variables, a second set of samples, the GOG stage and grade tissue array, was also studied. This includes replicate samples of 170 patients with epithelial ovarian cancer of serous or endometrioid histology. Table 2 summarizes the patient characteristics in each group.

Elevated BAG-4 is associated with longer progression-free survival and overall survival in newly diagnosed patients. Seven events were tabulated on the prospective cohort of 28 evaluable cases: cytoplasmic BAG-1, nuclear BAG-1, cytoplasmic BAG-3, nuclear BAG-3, cytoplasmic BAG-4, nuclear BAG-4, and cytoplasmic Bcl-xL (representative staining in Fig. 2). The data were grouped into categories to allow for a minimum of four patients per category (see Materials and Methods). When evaluating cytoplasmic BAG-4, no case was scored 0 to 1 (<6% of cells staining with this marker). Therefore, cases were grouped into 2 to 3+ (n = 4) or 4+ (n = 24). Because of the small numbers of patients in each category, only a P value of <0.005 was considered to be statistically significant. Elevated (4+) expression of cytoplasmic BAG-4 was significantly associated with overall survival (P = 0.0002) and progression-free survival (P = 0.0026; Fig. 3).

Additional analyses were done to determine whether any marker had a relationship to treatment outcome or response. No other marker studied in this cohort of cases showed a statistically significant relationship with overall or progression-free survival. Moreover, no statistically significant associations were identified between the marker levels and response to treatment (data not shown).

Cytoplasmic Bcl-2 and nuclear Hsp70 are expressed inversely in ovarian cancer. The prospective cohort of 28 patients was relatively homogenous in stage (all stage III/stage IV), grade (78% grade 3), and histology (69% serous). Thus, we evaluated BAG proteins and other survival protein expression using the 170 ovarian cancer cases presented on the GOG stage and grade tissue array. This allowed analysis of relationships between expression and other descriptive variables of ovarian

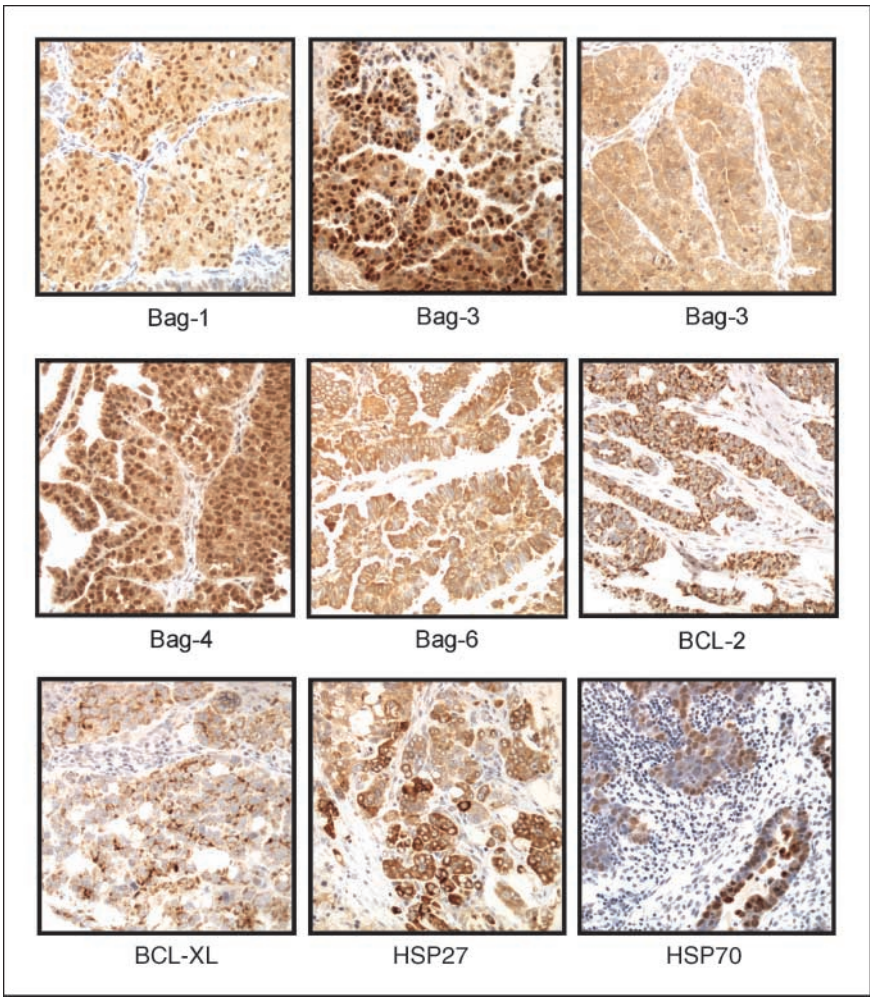

Fig. 2. Expression of Bag proteins, Bcl-2, Bcl-xl, Hsp-27, and Hsp-70 in ovarian carcinoma. Examples of the staining patterns observed with each antibody. The represented antibody is noted below each case. Some antibodies showed both nuclear and cytoplasmic expressions of the proteins, whereas others showed expression localized to either nucleus or cytoplasm in specific cases.

cancer aggressiveness. This analysis covered the expression of 13 markers: both cytoplasmic and nuclear BAG-1, BAG-3, BAG-4, BAG-6, and Hsp70, and cytoplasmic Hsp27, Bcl-2, and Bcl-xL (Fig. 2). An inverse relationship was found between expression of cytoplasmic Bcl-2 and both grade (P = 0.009; Fig. 4A) and stage (P = 0.005; Fig. 4B) of ovarian cancer. Hsp70 staining followed the opposite pattern. A statistically significant relationship was found between quantity of cells expressing nuclear Hsp70 and increasing grade of tumor (P = 0.002). This nuclear Hsp70 staining showed a similar increase with stage of disease but did not reach statistical significance (P = 0.09).

Cytoplasmic Bcl-2 and nuclear Hsp70 association with stage and grade is more pronounced in tumors of endometrioid histology. We next queried whether these protein patterns were a general feature of epithelial ovarian cancer or if the protein expression differed by histologic subtype. The relationship of Bcl-2 with stage held true within the endometrioid ovarian tumors (n = 71), but was not observed within the serous tumors (n = 78; Fig. 4C and D). Two other markers were identified with trends toward statistically significant relationships in stage or grade when the endometrioid subtype of epithelial ovarian cancer was examined independently. Cytoplasmic Hsp27 followed a pattern similar to Bcl-2 in endometrioid tumors, decreasing in expression frequency with increasing stage of disease (P = 0.02). Cytoplasmic Hsp70 was more often expressed in higher grade endometrioid tumors (P = 0.02). No associations were observed with the serous subtype.

Bcl-xL is differentially expressed between ovarian cancer subtypes. The observed differences prompted us to interrogate the samples for markers that might differentiate the endometrial from the serous histology as a whole, without separating the cases by stage or grade of ovarian cancer. The staining of Bcl-xL was significantly different between the endometrial and serous subtypes of ovarian cancer specimens in the GOG tissue array (data not shown). Bcl-xL protein was present in 38% of serous tumors (mean staining ± SE, 0.38 ± 0.10), and was expressed more sparsely in only 18% of cases of the endometrioid subtype (0.18 ± 0.05; P = 0.0035). None of the other analyzed markers were differentially expressed across the two subtypes of ovarian tumor specimens.

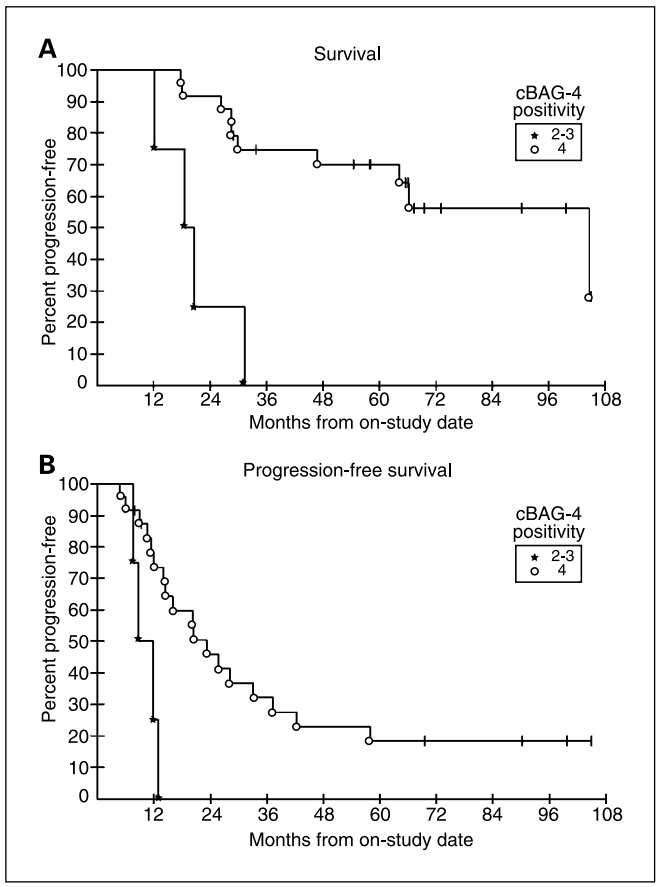

Fig. 3. BAG-4/SODD is associated with improved overall and progression-free survival. *A,* Kaplan-Meier plot of overall survival in ovarian cancer patients treated with triple therapy. Median survival for patients with 2 to 3+ BAG-4 staining was 18.5 mo; median survival for patients with 4+ expression of BAG-4 was 74.7 mo (P = 0.0002). Four of four patients with 2 to 3+ BAG-4 staining died, whereas 10 of 24 patients with 4+ expression of BAG-4 died. *B,* Kaplan-Meier curve for progression-free survival in the same patients. Median progression-free survival in patients with 2 to 3+ BAG-4 signal was 8.7 mo; median progression-free survival in patients with 4+ BAG-4 was 20.8 mo (P = 0.0026). Four of four patients with 2 to 3+ BAG-4 signal progressed, whereas 18 of 24 with 4+ BAG-4 progressed.

Discussion

The role of survival proteins in ovarian cancer etiology, progression, and clinical behavior can lead to new directions in molecular therapy. Overexpression of some BAG and Hsp proteins may provide mechanisms of resistance to platinum- and taxane-based chemotherapy (24, 25) or to the geldanamycin family of agents (26). We therefore hypothesized that elevated expression of these proteins would be associated with poor survival in patients with advanced stage ovarian cancer and would be differentially related to clinical markers of cancer aggressiveness, such as stage of disease or grade of tumor. These proteins were explored in specimens collected prospectively from women with advanced stage ovarian cancer receiving initial therapy in a National Cancer Institute trial of cisplatin, paclitaxel, and cyclophosphamide, from which we have up to 10-year follow-up information. The expression of these proteins was further analyzed using a tissue array of specimens of serous and endometrioid ovarian cancer of varied grades and stages. We found, unexpectedly, that high BAG-4 protein in the cytoplasm of tumor cells was linked to better overall survival. This line of investigation also detected an unanticipated pattern of high Bcl-2 and low nuclear Hsp70 in tumors of lower grade and stage. These findings create a pattern for prognosis of women with epithelial ovarian cancer and implicate BAG-4, Bcl-2, and Hsp70 in the pathogenesis of this disease.

We found a strong positive correlation of cytoplasmic BAG-4 with both progression-free and overall survival after triple chemotherapy with cisplatin, paclitaxel, and cyclophosphamide. BAG-4 blocks apoptosis in some instances, such as that signaled by tumor necrosis factor receptor-1 (27–29). BAG-4/SODD protein associates with the cytoplasmic tail of tumor necrosis factor-α receptors and blocks signaling by preventing multimerization for subsequent activation of apoptosis (27–29). In other situations, however, the role of BAG-4 may not be antiapoptotic. Although tumor necrosis factor-α is toxic to some ovarian cancer cells, in others, this cytokine may promote survival and invasion in part by up-regulating the expression of CD44, a membrane protein involved in cell adhesion and metastasis (30–32). One could then hypothesize that BAG-4

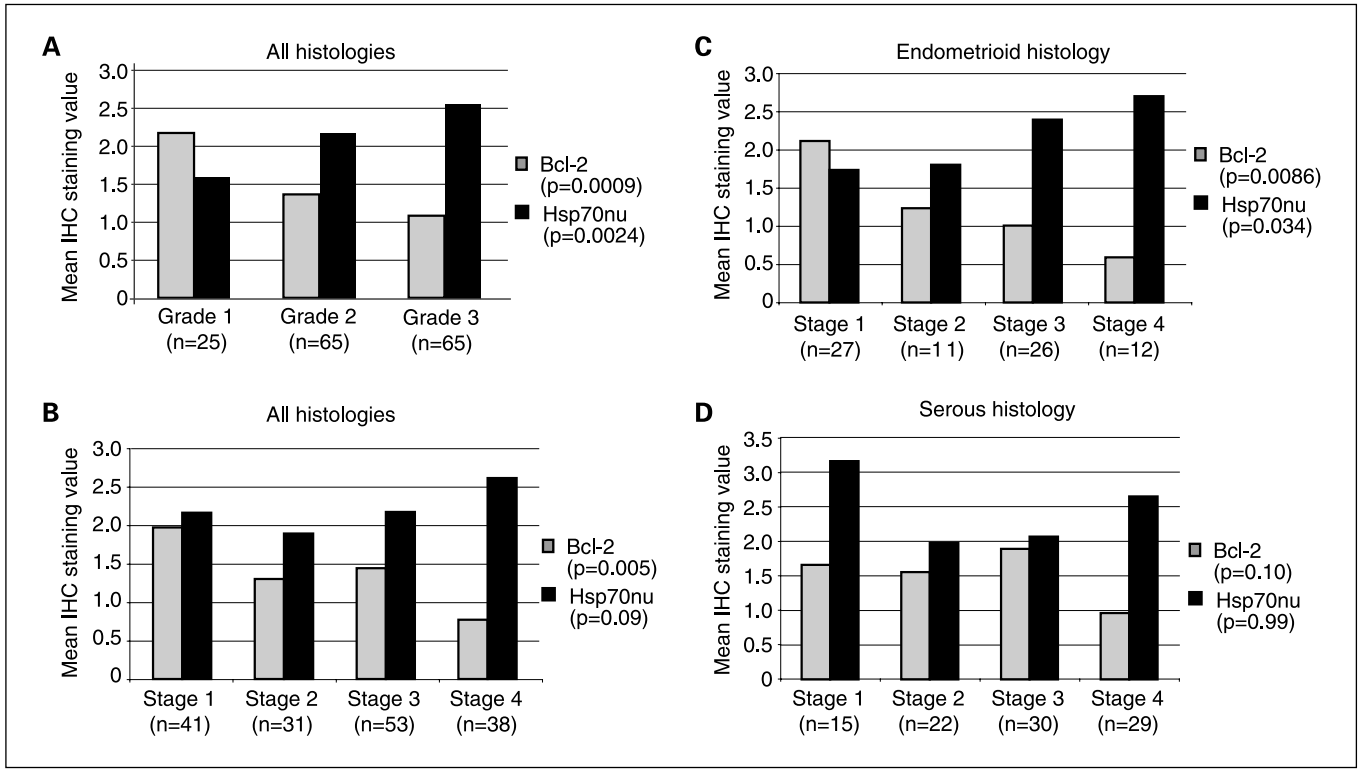

Fig. 4. Bcl-2 and Hsp70 expression across stage and grade of ovarian cancer specimens. *A,* Bcl-2 staining decreases as grade of tumor increases, whereas nuclear Hsp70 staining increases with increasing grade. *B,* Bcl-2 is less frequent in advanced stage tumors; nuclear Hsp70 again follows the opposite pattern, but the trend did not reach statistical significance. *C,* specimens were classified by histologic subtype, and the same two markers were analyzed. The pattern of Bcl-2 decreasing with increasing grade and Hsp70 increasing was present in endometrioid histology of ovarian cancers (*D*), but not in serous tumors.

may be antiproliferative if it blocks growth and invasion signals triggered by tumor necrosis factor-α. BAG-4 may also maintain chemosensitivity, especially to platinum agents, because loss of BAG-4 was observed with the development of platinum resistance (12). BAG-4 protein quantity has been shown to decrease upon development of resistance to cisplatin chemotherapy (12). The reason for the disappearance of BAG-4 was unclear, but it coincided with the appearance of an unidentified protein proposed to promote nuclear factor-κB prosurvival signals emanating from tumor necrosis factor receptor-1. Increased nuclear factor-κB signaling is present in platinum-resistant ovarian cancer, and proteasome inhibitors blocking nuclear factor-κB can restore sensitivity to platinum chemotherapy (33). These antisurvival/proliferative functions of BAG-4 may predominate in epithelial ovarian cancer, resulting in our finding that higher cytoplasmic BAG-4 portends a better prognosis and thus suggesting BAG-4 as a marker of lower invasive potential and/or increased sensitivity of the cancer cell to death upon treatment with platinum and taxane chemotherapy for the set of patients in the current study.

The BAG domain of BAG-4 can bind to either Bcl-2 or Hsp70, but not both simultaneously, and may thus serve as a molecular switch between downstream pathways triggered by these two molecules (28, 34). Overexpression of Bcl-2 in lymphomas promotes cancer cell survival and associates with increased aggressiveness (35). Paradoxically, however, expression of Bcl-2 in both breast and ovarian cancer has correlated with longer survival (36, 37) and was higher in normal ovarian tissue compared with ovarian carcinoma (5). Bcl-2 protein has been observed to decrease upon development of cisplatin resistance (12) similar to BAG-4. We found a statistically significant inverse relationship of Bcl-2 expression with both stage and grade of original tumor specimen. Our finding, in conjunction with others, supports the hypothesis that Bcl-2 is lost as ovarian tumors acquire more aggressive behavior.

Lower Bcl-2 occurred in our samples with higher nuclear accumulation of Hsp70. Hsp70 can protect cancer cells from death after stresses, such as heat shock and chemotherapy (38, 39), and has been proposed to cause resistance of ovarian cancer cells to farnesyltransferase inhibitors (4). Hsp70 is known to shuttle between the cytoplasm and the nucleus and remains in the nucleus during times of cellular stress to protect cells from death (40). We show that the level of Hsp70 in the nucleus of ovarian cancer cells was significantly higher with increasing grade of ovarian tumors in our present analysis. This finding could be interpreted as an underlying aggressive nature of tumors with elevated nuclear Hsp70 at baseline, implicating the function of this protein in protecting damaged cells from death (41).

We found a similar trend suggesting the transition of BAG-3 protein from the cytoplasm to the nucleus in more aggressive tumors, although this trend did not reach statistical significance in our samples (data not shown). This finding is consistent with a prior study of BAG-1, which inhibited apoptosis when located in the nucleus, and was lost from the nucleus upon induction of apoptosis with γ irradiation (42). We and others have shown BAG-3 to have prosurvival function, although not correlated with subcellular localization

(26, 43, 44). Cytoplasmic BAG-3 is a chaperone for Bcl-2 and Hsp proteins; in the nucleus, however, the function of BAG-3 has not been elucidated. Similarly, BAG-4 shuttles to the nucleus in conjunction with Hsp70 during times of cellular stress (45); one might expect then a similar pattern as we found with Hsp70, but this was not observed. We did not find a significant relationship between the nuclear and cytoplasmic patterns of BAG-4 nor did we observe an association of nuclear-localized BAG-4 with patient outcome. We did not find a significant association between the nuclear and cytoplasmic staining of any marker. Therefore, we cannot infer biological significance of their movement between cellular compartments.

Our data show cytoplasmic Hsp27 expression decreases in endometrioid ovarian cancer as stage increases. Because increased stage is a harbinger of worse outcome, this would suggest that Hsp27 may be a marker of better survival. Our findings are opposite to the trend previously published which associated the presence of Hsp27 with poor survival of ovarian cancer patients (38). Our findings were done in a background wherein survival could not be ascertained, and the second study did not reach statistical significance for the poor outcome finding. This provocative question needs to be investigated further.

The antiapoptotic protein Bcl-xL has been associated with chemoresistance and shorter-time progression in ovarian cancer (7). We found a differential distribution of Bcl-xL protein across the different subtypes of ovarian cancer. The highest expression occurred in serous tumors, especially those with advanced stage at diagnosis, a subset of patients for whom long-term outcome is still limited. The strong subtype association of this marker suggests that the serous variant of epithelial ovarian cancer may depend on Bcl-xL for growth and survival or that Bcl-xL may influence the development of the serous histology from an undifferentiated precursor cell. This mechanism has not been experimentally shown, but previous work has shown that exposure of endometrial cells to progestins promotes survival by preferential splicing of Bcl-x to the long form (46), thus suggesting a role for progestins in Bcl-xL production.

In summary, we have surveyed the expression of Bcl-2, BAG, and Hsp families of proteins to search for relationships with clinical features across 198 cases of ovarian carcinoma. We discovered significant associations of Bcl-2 and Hsp70 with aggressiveness of disease on the basis of tumor grade and stage at diagnosis. Importantly, cytoplasmic BAG-4 was strongly associated with long-term survival after platinum-based chemotherapy. These data provide additional insight to the potential protein interactions providing signals for proliferation and chemoresistance to ovarian cancer cells. This model points toward elevation of BAG-4 and Bcl-2 in the cytoplasm as markers of either less aggressive or more chemosensitive ovarian cancer cells, whereas nuclear Hsp70 suggests more aggressive behavior. The findings presented here support the involvement of these proteins in the propagation of ovarian cancer and provide a basis for the development of novel therapeutic agents modulating these survival pathways.

References

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2006. CA Cancer J Clin 2006;56:106–30.
2. Ozols RF. Systemic therapy for ovarian cancer: current status and new treatments. Semin Oncol 2006; 33:S3–11.
3. Beale PJ, Rogers P, Boxall F, Sharp SY, Kelland LR. BCL-2 family protein expression and platinum drug resistance in ovarian carcinoma. Br J Cancer 2000;82: 436–40.
4. Hu W, Wu W, Verschraegen CF, et al. Proteomic identification of heat shock protein 70 as a candidate target for enhancing apoptosis induced by farnesyl transferase inhibitor. Proteomics 2003;3: 1904–11.
5. Marone M, Scambia G, Mozzetti S, et al. bcl-2, bax, bcl-XL, and bcl-XS expression in normal and neoplastic ovarian tissues. Clin Cancer Res 1998;4:517–24.
6. Geisler JP, Geisler HE, Miller GA, Wiemann MC, Zhou Z, Crabtree W. p53 and bcl-2 in epithelial ovarian carcinoma: their value as prognostic indicators at a median follow-up of 60 months. Gynecol Oncol 2000;77: 278–82.
7. Williams J, Lucas PC, Griffith KA, et al. Expression of Bcl-xL in ovarian carcinoma is associated with chemoresistance and recurrent disease. Gynecol Oncol 2005;96:287–95.
8. Doong H, Price J, Kim YS, et al. CAIR-1/BAG-3 forms an EGF-regulated ternary complex with phospholipase C-γ and Hsp70/Hsc70. Oncogene 2000;19: 4385–95.
9. Doong H, Vrailas A, Kohn EC. What's in the 'BAG'? — a functional domain analysis of the BAG-family proteins. Cancer Lett 2002;188:25–32.
10. Cutress RI, Townsend PA, Brimmell M, Bateman AC, Hague A, Packham G. BAG-1 expression and function in human cancer. Br J Cancer 2002;87:834–9.
11. Ozawa F, Friess H, Zimmermann A, Kleeff J, Buchler MW. Enhanced expression of Silencer of death domains (SODD/BAG-4) in pancreatic cancer. Biochem Biophys Res Commun 2000;271:409–13.
12. Eichholtz-Wirth H, Sagan D. IκB/NF-κB mediated cisplatin resistance in HeLa cells after low-dose γ-irradiation is associated with altered SODD expression. Apoptosis 2000;5:255–63.
13. Ciocca DR, Calderwood SK. Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. Cell Stress Chaperones 2005;10: 86–103.
14. Melle C, Ernst G, Escher N, et al. Protein profiling of microdissected pancreas carcinoma and identification of HSP27 as a potential serum marker. Clin Chem 2007;53:629–35.
15. Hussain MM, Seiden MV, Fuller A, et al. A two institution phase II study of paclitaxel (T), cyclophosphamide (C), cisplatin (P) with G-CSF (G) for patients with newly diagnosed advanced stage ovarian cancer [abstract 813]. Proc Am Soc Clin Oncol 2002;21.
16. Kohn EC, Sarosy GA, Davis P, et al. A phase I/II study of dose-intense paclitaxel with cisplatin and cyclophosphamide as initial therapy of poor-prognosis advanced-stage epithelial ovarian cancer. Gynecol Oncol 1996;62:181–91.
17. Engblom P, Rantanen V, Kulmala J, Helenius H, Grenman S. Additive and supra-additive cytotoxicity of cisplatin-taxane combinations in ovarian carcinoma cell lines. Br J Cancer 1999;79:286–92.
18. Parker RJ, Dabholkar MD, Lee KB, Bostick-Bruton F, Reed E. Taxol effect on cisplatin sensitivity and cisplatin cellular accumulation in human ovarian cancer cells. J Natl Cancer Inst Monogr 1993;15:83–8.
19. Reed E, Kohn EC, Sarosy G, et al. Paclitaxel, cisplatin, and cyclophosphamide in human ovarian cancer: molecular rationale and early clinical results. Semin Oncol 1995;22:90–6.
20. Agresti A. In: Categorical Data Analysis. New York: John Wiley and Sons, Inc.; 1990. p. 79–129.
21. Kaplan E, Meier P. Non-parametric estimation from incomplete observations. J Am Stat Assoc 1958;53: 457–81.
22. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chem Rep 1966;50:163–70.
23. Hollander M, Wolfe DA. In: Nonparametric Statistical Methods. 2nd ed. New York: John Wiley and Sons, Inc; 1999. p. 189–269.
24. Chen J, Xiong J, Liu H, Chernenko G, Tang SC. Distinct BAG-1 isoforms have different anti-apoptotic functions in BAG-1-transfected C33A human cervical carcinoma cell line. Oncogene 2002;21: 7050–9.
25. Sugimura M, Sagae S, Ishioka S, Nishioka Y, Tsukada K, Kudo R. Mechanisms of paclitaxel-induced apoptosis in an ovarian cancer cell line and its paclitaxel-resistant clone. Oncology 2004; 66:53–61.
26. Doong H, Rizzo K, Fang S, Kulpa V, Weissman AM, Kohn EC. CAIR-1/BAG-3 abrogates heat shock protein-70 chaperone complex-mediated protein degradation: accumulation of poly-ubiquitinated Hsp90 client proteins. J Biol Chem 2003;278: 28490–500.
27. Jiang Y, Woronicz JD, Liu W, Goeddel DV. Prevention of constitutive TNF receptor 1 signaling by silencer of death domains. Science 1999;283:543–6.
28. Miki K, Eddy EM. Tumor necrosis factor receptor 1 is an ATPase regulated by silencer of death domain. Mol Cell Biol 2002;22:2536–43.
29. Takada H, Chen NJ, Mirtsos C, et al. Role of SODD in regulation of tumor necrosis factor responses. Mol Cell Biol 2003;23:4026–33.
30. Muthukumaran N, Miletti-Gonzalez KE, Ravindranath AK, Rodriguez-Rodriguez L. Tumor necrosis factor-α differentially modulates CD44 expression in ovarian cancer cells. Mol Cancer Res 2006;4:511–20.
31. Naor D, Sionov RV, Ish-Shalom D. CD44: structure, function, and association with the malignant process. Adv Cancer Res 1997;71:241–319.
32. Wu S, Boyer CM, Whitaker RS, et al. Tumor necrosis factor α as an autocrine and paracrine growth factor for ovarian cancer: monokine induction of tumor cell

proliferation and tumor necrosis factor α expression. Cancer Res 1993;53:1939–44.

33. Aghajanian C. Clinical update: novel targets in gynecologic malignancies. Semin Oncol 2004;31:22–6; discussion 33.

34. Antoku K, Maser RS, Scully WJ, Jr., Delach SM, Johnson DE. Isolation of Bcl-2 binding proteins that exhibit homology with BAG-1 and suppressor of death domains protein. Biochem Biophys Res Commun 2001;286:1003–10.

35. Hermine O, Haioun C, Lepage E, et al. Prognostic significance of bcl-2 protein expression in aggressive non-Hodgkin's lymphoma. Groupe d'Etude des Lymphomes de l'Adulte (GELA). Blood 1996;87:265–72.

36. Ceccarelli C, Santini D, Chieco P, Taffurelli M, Marrano D, Mancini AM. Multiple expression patterns of biopathological markers in primary invasive breast carcinoma: a useful tool for elucidating its biological behaviour. Ann Oncol 1995;6:275–82.

37. Herod JJ, Eliopoulos AG, Warwick J, Niedobitek G, Young LS, Kerr DJ. The prognostic significance of Bcl-2 and p53 expression in ovarian carcinoma. Cancer Res 1996;56:2178–84.

38. Elpek GO, Karaveli S, Simsek T, Keles N, Aksoy NH. Expression of heat-shock proteins hsp27, hsp70 and hsp90 in malignant epithelial tumour of the ovaries. APMIS 2003;111:523–30.

39. Witkin SS. Heat shock protein expression and immunity: relevance to gynecologic oncology. Eur J Gynaecol Oncol 2001;22:249–56.

40. Kodiha M, Chu A, Lazrak O, Stochaj U. Stress inhibits nucleocytoplasmic shuttling of heat shock protein hsc70. Am J Physiol Cell Physiol 2005;289: C1034–41.

41. Krynetski EY, Krynetskaia NF, Bianchi ME, Evans WE. A nuclear protein complex containing high mobility group proteins B1 and B2, heat shock cognate protein 70, ERp60, and glyceraldehyde-3-phosphate dehydrogenase is involved in the cytotoxic response to DNA modified by incorporation of anticancer nucleoside analogues. Cancer Res 2003;63:100–6.

42. Barnes JD, Arhel NJ, Lee SS, et al. Nuclear BAG-1 expression inhibits apoptosis in colorectal adenoma-derived epithelial cells. Apoptosis 2005;10:301–11.

43. Seo YJ, Jeon MH, Lee JH, et al. Bis induces growth inhibition and differentiation of HL-60 cells via up-regulation of p27. Exp Mol Med 2005;37: 624–30.

44. Takayama S, Reed JC. Molecular chaperone targeting and regulation by BAG family proteins. Nat Cell Biol 2001;3:E237–41.

45. Lau PP, Chan L. Involvement of a chaperone regulator, Bcl2-associated athanogene-4, in apolipoprotein B mRNA editing. J Biol Chem 2003;278: 52988–96.

46. Pecci A, Scholz A, Pelster D, Beato M. Progestins prevent apoptosis in a rat endometrial cell line and increase the ratio of bcl-XL to bcl-XS. J Biol Chem 1997; 272:11791–8.