Cancer Therapy: Clinical

Clinical Cancer Research

Functional Proteomic Analysis of Advanced Serous Ovarian Cancer Using Reverse Phase Protein Array: TGF-β Pathway Signaling Indicates Response to Primary Chemotherapy

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Abstract

Purpose: Using reverse phase protein array, we measured protein expression associated with response to primary chemotherapy in patients with advanced-stage, high-grade serous ovarian cancer.

Experimental Design: Tumor samples were obtained from 45 patients with advanced high-grade serous cancers from the Gynecology Tumor Bank at the British Columbia Cancer Agency. Treatment consisted of platinum-based chemotherapy following debulking surgery. Protein lysates were prepared from fresh frozen tumor samples, and 80 validated proteins from signaling pathways implicated in ovarian carcinogenesis were measured by reverse phase protein array. Normalization of Ca-125 by the 3rd cycle of chemotherapy was chosen as the primary outcome measure of chemotherapy response. Logistic regression was used for multivariate analysis to identify protein predictors of Ca-125 normalization and Cox regression to test for the association between protein expression and progression-free survival. A significance level of $P \leq 0.05$ was used.

Results: The mean age at diagnosis was 56.8 years. epidermal growth factor receptor, YKL-40, and several transforming growth factor β (TGF-β) pathway proteins [c-jun–NH_2–kinase (JNK), JNK phosphorylated at residues 183 and 185, plasminogen activator inhibitor 1, Smad3, TAZ] showed significant associations with Ca-125 normalization on univariate testing. On multivariate analysis, epidermal growth factor receptor ($P < 0.02$), JNK ($P < 0.01$), and Smad3 ($P < 0.04$) were significantly associated with normalization of Ca-125. Contingency table analysis of pathway-classified proteins revealed that the selection of TGF-β pathway proteins was unlikely because of false discovery ($P < 0.007$; Bonferroni adjusted).

Conclusion: TGF-β pathway signaling likely plays an important role as a marker or mediator of chemoresistance in advanced serous ovarian cancer. On this basis, future studies to develop and validate a useful predictor of treatment failure are warranted. *Clin Cancer Res; 16(10); 2852–60. ©2010 AACR.*

High-grade serous ovarian cancer is the most common pathologic diagnosis in patients with advanced ovarian cancer. In several randomized trials, most patients presenting with advanced disease have high-grade serous cancers (1, 2). Within this group, the prognosis for patients treated with carboplatin-paclitaxel chemotherapy varies remarkably from a few months to many years, although median times to progression are in the order of 18 months (3).

It is recognized that performance status, age, grade, residuum, histology, and stage influence outcomes to primary chemotherapy in patients with advanced ovarian cancer (4, 5). In addition, Ca-125 levels pretreatment and at nadir, normalization of Ca-125 by the 3rd cycle of chemotherapy, and Ca-125 half-life are also significant predictors of progression-free survival and overall survival (6–9). Despite this knowledge, these factors are only used in a minority of patients to guide primary treatment selection due to limited sensitivity and specificity. Combination platinum/taxane chemotherapy is offered and completed in most patients knowing that up to ≥20%

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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doi: 10.1158/1078-0432.CCR-09-2502

Translational Relevance

We studied functional proteomic aberrations in tumor lysates from patients with advanced serous ovarian cancers to determine predictors of response to primary chemotherapy. Having the ability to determine those patients who benefit from a particular therapy will allow the triage of patients to the most effective approaches. Strikingly, pretreatment assessment of epidermal growth factor receptor, YKL-40, and several transforming growth factor β pathway proteins (Smad3, c-Jun N-terminal kinase, plasminogen activator inhibitor 1, TAZ, and phospho–c-*jun*–NH_2–kinase) was associated with normalization of Ca-125 before cycle 3 of chemotherapy. Contingency table analysis showed that the identification of transforming growth factor β pathway proteins is unlikely because of false discovery. Thus, this information provides a sound basis for future studies on outcome prediction and drug development.

Recognizing that >20% of patients with advanced ovarian cancer do not respond to first-line therapy, the early identification of patients with poor outcomes based on tumor proteomics assessment will also have important implications for future clinical trial design.

of patients will not have a demonstrable response to therapy, and most of these patients are destined to progress and succumb to their disease despite therapy (10).

With the development of modern high-throughput molecular technologies, efforts are being directed to the discovery of biomarkers of treatment response and survival. Markers that function as effective surrogates of these important outcomes have been challenging to identify in ovarian cancer. Ongoing studies in ovarian cancer suggest that there are distinct molecular characteristics that are specific to histologic subtypes such as advanced high-grade serous cancers (11). Molecular classifications may help distinguish various subgroups within high-grade serous cancers that have different outcomes and responses to therapy.

Reverse phase protein array is a new high-throughput technology in which a multiplexed assessment of protein expression on >100 differing proteins can be carried out simultaneously on up to 1,000 tumors samples (12). Reverse phase protein array can be used as a tool to explore cellular signaling pathway activation that relates to the biology of cancer progression and treatment (13), including the assessment of proteins involved in cellular functions such as growth, proliferation, and apoptosis. We have applied this technology to the study on a well-characterized cohort of patients with advanced high-grade serous cancers from the same institution. Our objective was to determine if we could identify patterns of tumor protein expression that were associated with normalization of Ca-125 by the 3rd cycle of chemotherapy (Ca-125 normalization). This endpoint was chosen to characterize proteins able to predict response to chemotherapy. Currently, there are no biomarkers in clinical use that predict response to first-line chemotherapy. Such a biomarker(s) could facilitate the introduction of investigational drugs much earlier in the disease course, particularly in those patients who might be identified as poor responders to conventional treatment, as well as identify potential therapeutic targets for drug development.

Materials and Methods

Between November 2000 and May 2006, the Gynecology Tumour Group at Vancouver General Hospital and the British Columbia Cancer Agency collected 221 ovarian cancer tumor samples. The collection and study of these tissues were approved by the University of British Columbia Ethics Review Board. Tumor samples were collected in the operating room at the time of primary surgery and snap frozen within 30 minutes after collection in liquid nitrogen. Samples were processed in accordance with tissue bank guidelines and stored at -120°C. All patient samples in the ovarian tumor tissue bank have been subjected to pathology review (B. Gilks) to verify the histology and site of origin. Only patients with advanced (stage III or IV) high-grade serous tumors with adequate clinical information were selected for this study. To be included in the analysis, patients must have had Ca-125 measurements taken postoperatively within 2 weeks of starting chemotherapy, and pretreatment Ca-125 levels must have been at least twice normal (>70 U/mL). An abnormal Ca-125 was defined as a level >35 U/mL. In most cases, Ca-125 measures were available before each treatment cycle, and normalization before the 3rd cycle of chemotherapy (Ca-125 normalization) was used as the primary outcome measure. Although we examined other measures of Ca-125 prognostication such as Ca-125 half-life, Ca-125 normalization (before the 3rd cycle of chemotherapy) was a stronger determinant of outcome than Ca-125 half-life and is simpler to measure.

Clinical information on study patients was collected prospectively and retrospectively as part of a formal process to collect and update clinical data on a regular basis. Patients were classified as having macroscopic residuum or no macroscopic residuum because this distinction most clearly separates patients into prognostic risk groups. For the duration of this study, combination chemotherapy using carboplatin and paclitaxel was the standard regimen.

Reverse phase protein array. Tissues were processed by reverse phase protein array in our laboratory as previously described (12). Briefly, 5 to 25 mg of tumor tissue was cut from the frozen banked tumor specimen and immediately homogenized in cold lysis buffer (1% Triton X-100; 50 mm HEPES, pH 7.4; 150 mmol/L NaCl; 1.5 mmol/L $MgCl_2$; 1 mmol/L EGTA; 100 mmol/L NaF; 10 mmol/L sodium pyrophosphate; 1 mmol/L Na_3VO_4; 10% glycerol). After centrifugation, the supernatant was removed, and

total protein concentrations were corrected to a final concentration of 1 μg/μL and boiled in denaturing buffer with SDS (final concentration of 1% SDS).

Samples were serially diluted and spotted on nitrocellulose-coated glass slides (FAST Slides, Schleicher & Schuell BioScience, Inc.) using an Aushon 2470 robotic printer (Aushon Biosystems). Printing and staining quality control was done by reprinting five slides. These slides were printed >8 months after the original 120-slide print run and stained for AKT, cyclin E1 (CCNE1), c-*jun*–NH_2–kinase phosphorylated at residues 183 and 185 (JNKp183_185), PTEN, and GSK3. The Pearson *r*'s for these five slides were 0.90, 0.95, 0.95, 0.97, and 0.97, respectively. Negative and positive controls were printed on each slide only as a means of assessing printing and staining quality across each slide. Slides are examined microscopically, and those with obvious defects in print spot quality or staining consistency are not used. Each arrayed slide was probed with a specific primary antibody followed by a secondary antibody (anti-mouse or anti-rabbit) as a means for signal amplification (Dako; catalyzed system). Each antibody is validated as previously described (12, 13), and a list of the 80 validated antibodies and others used for staining, including the applied antibody dilutions and the source of the antibody, is included as Supplementary Table S1. The stained slides were scanned using an HP flatbed scanner then analyzed and quantified using Microvigene software (VigeneTech, Inc.). Serial dilution-signal intensity curves were generated for each sample. Using the logistic fit model $\ln(y) = a + (b - a) / (1 + \exp(c * (d - \ln(x))))$, logarithmic values were determined for each spot in the serial dilution after background correction. A representative value of the fit of each sample dilution curve relative to a fitted curve generated from all spots on the slide was used to quantify the relative protein expression (Supercurve v.997 Department of Bioinformatics and Computational Biology, M.D. Anderson Cancer Center) of each specific protein or phosphorylated protein counterpart evaluated in each sample (14). The estimated protein concentrations are normalized by a median polish method and corrected for protein loading using the average expression levels of all measured proteins in each sample.

Statistical methods. To obtain a global visualization and assessment of tumor protein expression profiles, supervised cluster analysis was done using Treeview software (University of Glasgow, Scotland), and X-cluster software was used to generate heat maps and cluster groups. Data were analyzed using SPSS software (version 17). Proteins were classified according to their relevant signaling pathway using the Gene Set Enrichment Analysis Database (version 2.05; www.broad.mit.edu/gsea). The curated Molecular Signatures Database (*Homo sapiens* only) was used to classify each protein by pathway status (Supplementary Table S2). The Fisher's exact test was used to assess the association between categorical variables, test for significance between cluster analyses of patient groups by Ca-125 normalization status, and evaluate false discovery rates by assessing differences in the signaling pathway classification of the identified protein predictors. Means were compared using the Student's *t* test, whereas logistic regression was used for assessing the effect of individual protein expression on Ca-125 normalization. The log-rank test was used to compare survival curves using the Kaplan-Meier method. Significant variables on univariate analysis were subsequently examined as predictors of progression-free survival as continuous variables using Cox regression. Median protein expression levels were used as cut points for the regression models. A level of significance of 0.05 was used throughout.

Results

Forty-five cases fit the inclusion criteria from the 221 tumor samples collected for reverse phase protein array. Most patients (87) were excluded based on histologic findings that included, nonserous histology, nonovarian primaries, and low-grade or borderline serous tumor. Another 19 patients had early-stage (I or II) disease. Patients with unclassified tumors and those with mixed tumors were not included. Thirty-three patients were excluded who did not complete or receive chemotherapy or were treated with neoadjuvant chemotherapy. Patients were also excluded if the pretreatment Ca-125 was less than twice normal (10 patients), or they were missing Ca-125 measures at necessary time points (17 patients).

The median age of the population was 56.8 years, with a range of 34 to 84 years. The distribution of stage in the population was as follows: six patients with stage IIIB disease, 31 with stage IIIC, and eight stage IV patients. There were 41 patients with macroscopic residuum and four patients with no macroscopic residuum. All but four patients received paclitaxel/carboplatin chemotherapy following primary debulking surgery. Three patients received carboplatin alone, whereas one patient was treated with carboplatin and gemcitabine. Pretreatment Ca-125 values ranged from 88 to 6,300 U/mL. Thirty-three percent of patients had a pretreatment Ca-125 level of <200 U/mL, whereas 51% of patients had values between 200 and 1,000 U/mL. There were 25 patients who normalized their Ca-125 before the 3rd cycle of chemotherapy, whereas 20 patients retained abnormal levels.

Table 1 shows the proteins associated with Ca-125 normalization on univariate and multivariate analyses. Expression of these proteins was not associated with stage, residuum, or age, with the exception of YKL-40, which correlated with stage ($P < 0.05$; data not shown). None of the clinical variables were significantly associated with normalization of Ca-125, with stage showing a trend ($P = 0.06$; data not shown). Those proteins that were significant determinants of outcome on univariate analyses and known prognostic clinical factors were then tested by multivariate analyses. Box plots showing the distributions of each protein in accordance with Ca-125 normalization are shown in Fig. 1. Proteins showing a significant association with Ca-125 normalization on univariate testing are epidermal growth factor receptor (EGFR), JNK, JNKp183_185, PAI-1 (plasminogen activator inhibitor

Table 1. Univariate and multivariate analyses of clinical variables and proteins on normalization of Ca-125 and progression-free survival

Variables	Ca-125 normalization by cycle 3*			Progression-free survival	
	Univariate	Multivariate†		Univariate	
	P	HR‡ (95% CI)	*P*	HR§ (95% CI)	*P*
Clinical					
Age‖	0.57	—	—	—	—
Stage¶	0.06	—	—	—	—
Residuum**	0.90	—	—	—	—
Pretreatment Ca-125‖	0.13	—	—	—	—
Proteins					
JNK	0.01	6.25 (1.6-25.0)	0.01	1.61 (1.07-2.4)	0.02
EGFR	0.05	6.67 (1.3-33.3)	0.02	1.50 (0.98-2.3)	0.06
Smad3	0.04	0.14 (0.02-0.9)	0.04	—	NS
TAZ	0.04	—	NS	1.58 (0.97-2.6)	0.07
PAI-1	0.02	—	NS	—	NS
JNKp183_185	0.05	—	NS	—	NS
YKL-40	0.04	—	NS	—	NS

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval; NS, not significant.
*Ca-125 normalization defined as ≤35 IU/mL.
†Only the significant variables in univariate analysis for Ca-125 normalization were taken forward into the multivariate analysis.
‡Logistic regression hazard ratio > 1 indicates lower chance of normalization of Ca-125 with higher levels of protein expression.
§Hazard ratio > 1 indicates higher risk for progression with higher levels of protein expression.
‖Variable analyzed as a continuous variable.
¶Stage III versus IV.
**Macroscopic versus not macroscopic.

1), Smad3, TAZ, and YKL-40, with higher levels associated with failure of Ca-125 normalization, except for Smad3. In contrast, higher Smad3 expression was associated with normalization of Ca-125 levels. On multivariate logistic regression analysis (Table 1), only EGFR, JNK, and Smad3 were independent determinants of Ca-125 normalization. Table 2 shows the sensitivity and specificity for predicting Ca-125 normalization using EGFR, JNK, and Smad3 expression. The expression of these three proteins in the combined regression model had a sensitivity of 80%, with a specificity of 75%, and an overall accuracy of 78% for predicting Ca-125 normalization.

A number of methods were used to assess false discovery rates. The expected number of falsely discovered proteins should on average identify four by chance ($\alpha = 0.05$) if testing 80 validated proteins. As a crude measure, the false discovery rate is therefore 57% (four of seven identified proteins). Alternatively, the expected number of significant proteins follows a binomial distribution with a "success rate" of 5%. If the *t* test is applied 80 times, the probability of identifying seven or more significant proteins by chance is 0.11. In terms of further assessing the potential for false discovery, it is statistically relevant that five of the seven proteins selected were in the transforming growth factor β (TGF-β) pathway, whereas there were only six other proteins that were identified as TGF-β signaling members. Using contingency tables, all validated proteins were assessed according to pathway classification (Supplementary Table S2). Fisher's exact testing revealed a highly significant *P* value ($P < 0.007$; Bonferroni adjusted) for the enriched identification of signaling proteins in the TGF-β pathway. All pathways were tested, and this was the only pathway identified of significance in this analysis.

The results of unsupervised clustering of proteins and samples are shown in Fig. 2. It is worth noting that, overall, there are marked differences in protein expression that can be clearly distinguished on cluster analysis in a group of clinically similar high-grade serous cancers. In particular, there are two major groups of advanced high-grade serous cancers identified by changes in protein expression. Group 1 (Fig. 3) identifies a cluster of patients with high cyclin D1 and cyclin E2, pAkt, and stromal markers that include collagen VI, CD31, and vascular endothelial growth factor receptor 2. In addition this group, contains high expression of many of the proteins associated with a persistently elevated Ca-125 by cycle 3. A second large cluster (group 2) has high expression of cyclins B and E1, ER and ER phosphorylated at residue 118, Rb, mTOR, and c-Myc. This latter group may contain markers associated with better outcomes, such as ER and those indicative of increased cell cycle progression that may indicate sensitivity to cytotoxic therapy, such as cyclins Rb and *myc*.

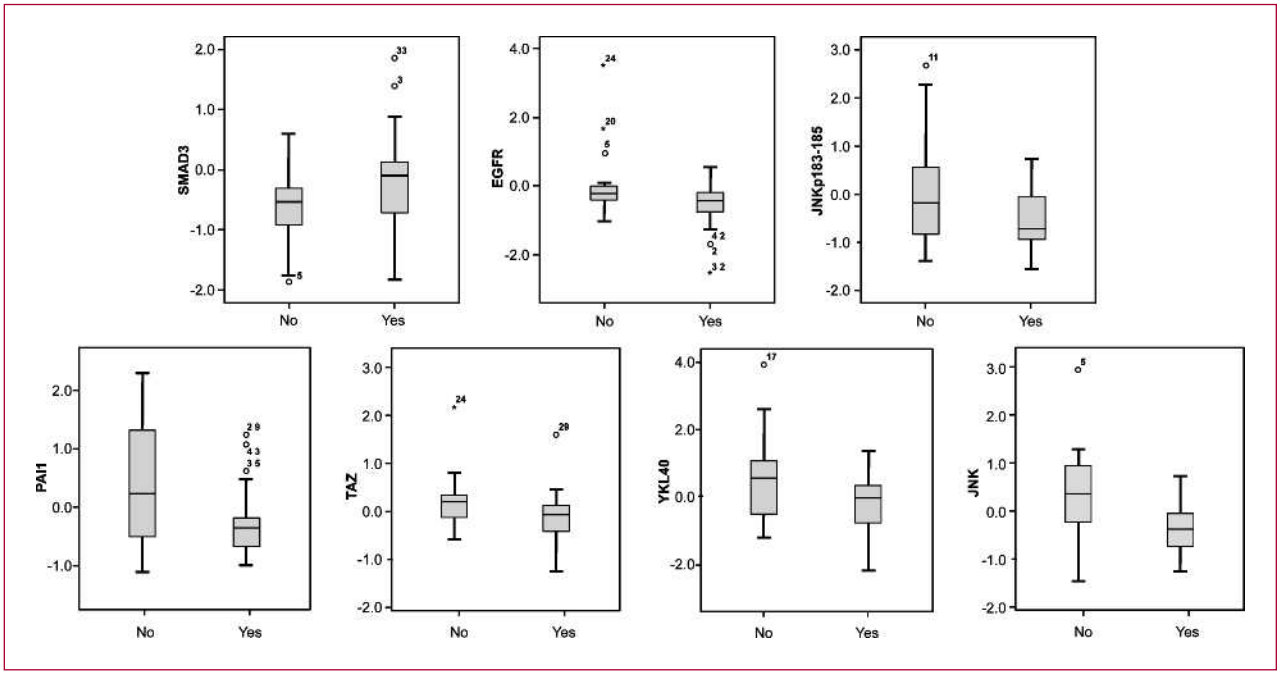

Fig. 1. Box plots of protein expression by reverse phase protein array and Ca-125 normalization status by 3rd cycle (no versus yes). *Y*-axis values are mean centered and represent log 2 protein expression.

Classification of patients into these two groups showed a trend to being associated with their Ca-125 normalization status (group 1, N = 7 and E = 11; group 2, N = 18 and E = 9; $P = 0.1$; Fisher's exact test), with patients that normalized indicated by the prefix "N" and those that remained elevated indicated with an "E" (Fig. 2). Figure 3 shows a supervised clustering analysis using only those proteins identified on univariate testing as predictors of Ca-125 normalization by the 3rd cycle of chemotherapy. There are a number of patients seen with high Smad3 and low JNK and phospho-JNK levels, forming a cluster of patients with a significantly higher rate of Ca125 normalization (group 1, N = 18 and E = 8; group 2, N = 7 and E = 12; $P = 0.04$; Fisher's exact test).

Although our primary interest was to examine the relationship between protein expression and Ca-125 normalization, we also assessed whether these same proteins influenced progression-free survival. As expected, Ca-125 normalization as a clinical predictor significantly influenced progression-free survival times in this cohort ($P < 0.001$; log-rank). Progression-free survival curves by normalization status are shown in Fig. 4. There is a marked difference in progression-free survival between the two groups, with those patients who fail to normalize Ca-125 by the 3rd cycle of chemotherapy showing much higher rates of progression. In terms of determining whether the seven proteins of interest associated with Ca-125 normalization also predicted for progression-free survival, we did univariate Cox regression analyses (Table 1). Higher levels of JNK expression were significantly associated with a poorer progression-free survival ($P = 0.02$), whereas high EGFR and TAZ expression were both of borderline significance ($P = 0.06$ and $P = 0.07$, respectively) for adverse outcomes.

Discussion

This study identifies a number of candidate proteins that may serve as surrogates of Ca-125 normalization in advanced high-grade serous cancers. Expression of Smad3,

Table 2. Sensitivity and specificity of protein expression modeling to predict Ca-125 normalization (using Smad3, JNK, and EGFR)

		Predicted Ca-125 normalization based on protein expression		Test characteristics
		No	Yes	
Observed Ca-125 normalization by 3rd cycle	No	15	5	Sensitivity, 75%
	Yes	5	20	Specificity, 80%

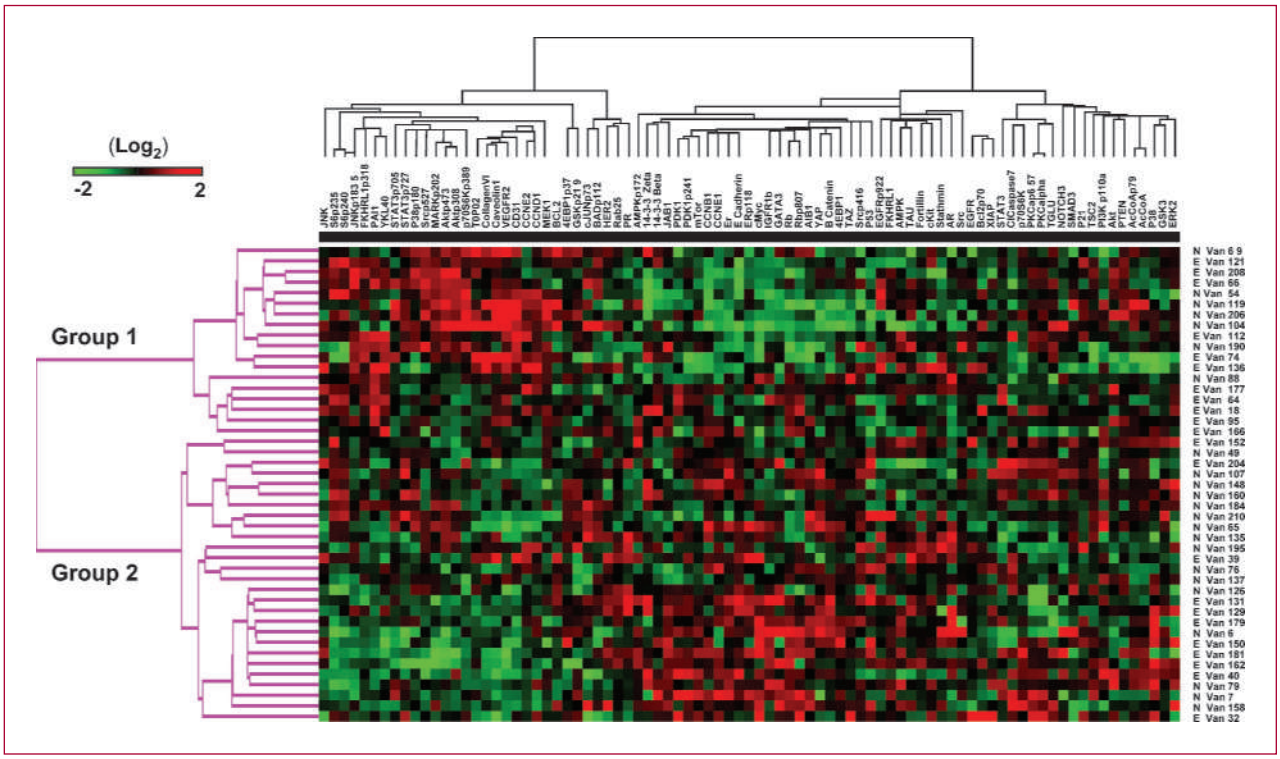

Fig. 2. Heat map of unsupervised cluster analysis of proteins and samples by reverse phase protein array. Proteins tested by reverse phase protein array are listed across the top of the heat map, whereas the patient samples are listed down the right side. E, an elevated Ca-125 by 3rd cycle of chemotherapy; N, Ca-125 normalization. Left, cluster trees of patient groups; top, cluster trees of proteins. Red, increased protein expression; green, low relative to the other samples. Van, Vancouver sample.

JNK, and EGFR by reverse phase protein array are strongly associated with normalization of Ca-125. This analysis was done as a training set, and although the study population is small, it was the result of a deliberate strategy to use a homogenous well-characterized group of patients with verification of pathology from a single institution. An obvious advantage to such an approach is to reduce the confounding effects of other important clinical variables such as stage, grade, histology, and treatment on protein expression patterns. The individual proteins that have been identified as predictors of Ca-125 normalization represent interesting candidate markers. Subsequent validation in independent sample sets is required to ensure that the current findings do not represent false discoveries from multiple parameter testing (15) and that the selected proteins can be modeled in such a way to effectively predict early treatment failure.

This study also shows marked differences in protein expression and signaling pathway activation profiles within this homogeneous population of advanced serous tumors. This is evident by the unsupervised and supervised cluster analysis. The potential to exploit these differences for therapeutic purposes is intriguing. Although less studied than mRNA expression profiling, reverse phase protein array is a useful technology for identifying important overexpressed or functionally activated proteins and their pathways as desirable targets for drug development (13, 16). In this respect, reverse phase protein array may be more advantageous than expression profiling because protein expression/function may not correlate well with mRNA levels, with proteins being the ultimate modulators of cellular function (17).

We have identified a number of proteins of interest using reverse phase protein array that may have relevance from a clinical standpoint. Overexpression of EGFR, particularly as a result of EGFR mutation, is associated with adverse outcomes in lung cancer (18). In ovarian cancer, EGFR mutations are detected in <4% of patients (19). There is conflicting information with respect to the prognostic significance of EGFR overexpression in ovarian cancer. For the most part, studies using immunohistochemistry have failed to show EGFR expression as a determinant of survival (20, 21). The difference between these results and our data may reflect a limitation of immunohistochemistry relative to reverse phase protein array. Reverse phase protein array affords a more objective quantitative measure of protein expression than immunohistochemistry and is not subject to the potential fixation effects of paraffin embedded tissue on antibody binding characteristics. It will be of future interest to examine EGFR expression by reverse phase protein array in relation to outcome and Ca-125 normalization in validation sets.

In our study, we found remarkable associations between Smad/TGF-β signaling proteins and Ca-125 normalization. It is most unlikely that this finding is due to false discovery from multiple parameter testing; if it were, we would not have expected a statistically significant enrichment of TGF-β pathway proteins by contingency table analysis ($P < 0.007$; Bonferroni adjusted). Low levels of Smad3 were associated with a failure to normalize Ca-125 measures by the 3rd cycle of chemotherapy, suggesting that Smad3 may be acting as a tumor suppressor gene. Smad3 is a transcription factor mediating TGF-β signaling. Smad3 plays a role in gonadal tumorigenesis (22) and may function as a positive or negative regulator of carcinogenesis depending on the cell type and phosphorylation status (23, 24). The finding that TAZ is associated with Ca-125 normalization on univariate analysis lends additional support to the role of Smad3. TAZ is involved in Smad3 nucleocytoplasic shuttling (25). Moreover, PAI-1 is transcriptionally regulated by TGF-β (26), overexpressed in ovarian cancer cells (27), and modulates cell invasion and metastasis (27, 28). It is important to note that there is conflicting data with respect to the role of TGF-β/Smad3 signaling and the malignant phenotype. Although there is evidence that high Smad3 levels promote epithelial-to-mesenchymal transition in ovarian cancer cells (29), our data on tumor tissue and other studies indicate that Smad3 may function as a tumor suppressor in more complex interactions that involve important signaling pathways and immune response regulation (24, 30).

We found that increased JNK expression correlated with chemoresistance and poorer progression-free survival. JNK phosphorylates the linker region of Smad3, promoting cell growth and invasion, in contrast to Smad3 phosphorylation by TGF-β, which suppresses the malignant phenotype

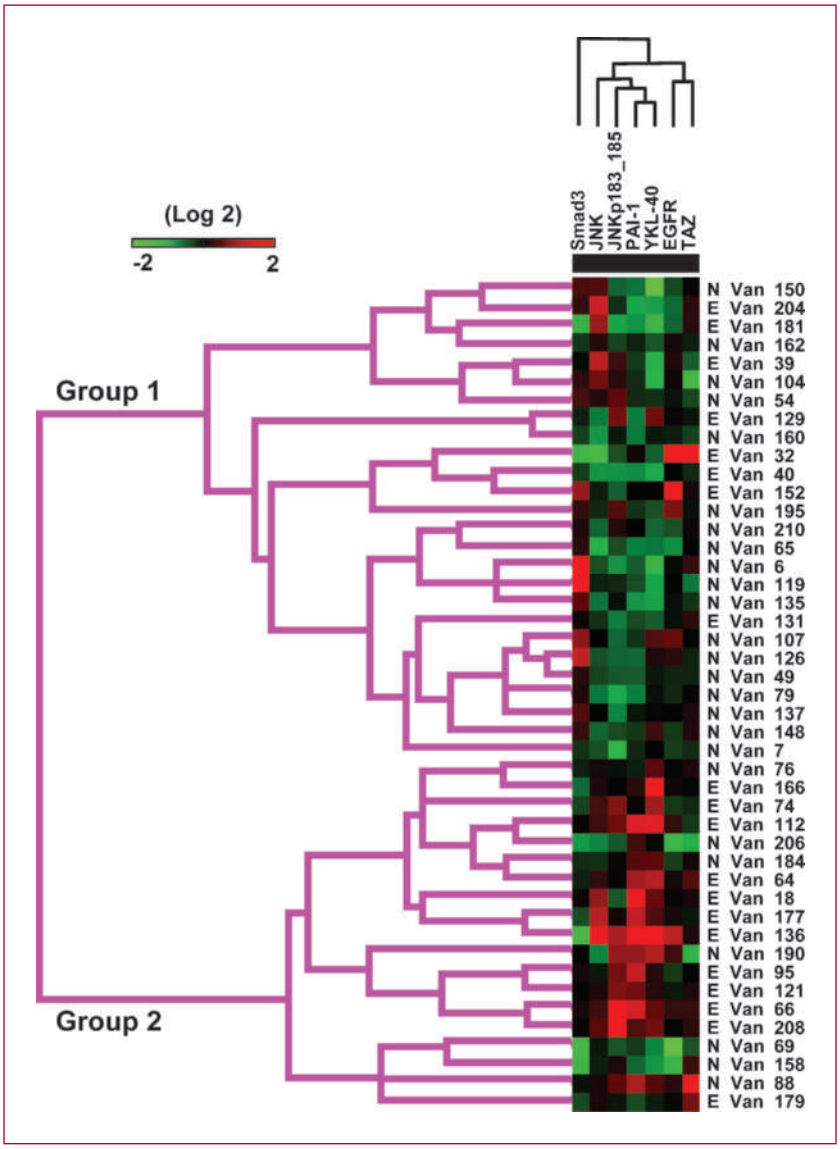

Fig. 3. Heat map of supervised cluster analysis using only proteins associated with Ca-125 normalization by univariate analysis. Proteins tested by reverse phase protein array are listed across the top of the heat map, whereas the patient samples are listed down the right side. Left, cluster trees of patient groups; top, cluster trees of proteins. Van, Vancouver sample.

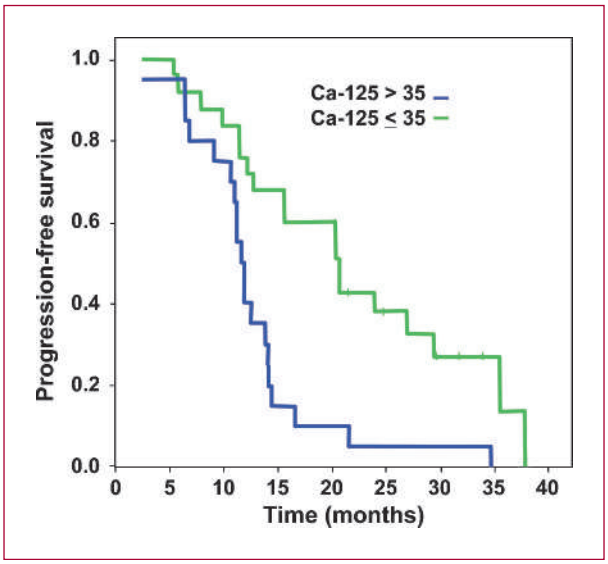

Fig. 4. Progression-free survival and Ca-125 normalization status by the 3rd cycle of chemotherapy.

(23, 31). This mechanism may in part explain the differential oncogenic effects Smad/TGF-β signaling as shown in a rat hepatocellular carcinoma model (32). In keeping with this, it has been recently reported that SP600125, an inhibitor of JNK, sensitizes mouse ovarian cancer cells to paclitaxel (33). Furthermore, in a recent study on a distinct cohort of patients from the M.D. Anderson Cancer Center, reverse phase protein array analysis of tumor tissues found that increased JNKp183_185 expression was associated with shorter progression-free survival and JNK inhibition by small interfering RNA (siRNA) or using a novel JNK inhibitor resulted in increased sensitivity to paclitaxel (34). Thus, JNK seems to play an important role in modulating chemoresistance in ovarian cancer.

This study represents proof-in-principle that reverse phase protein array can be used to develop a predictor of chemoresistance in ovarian cancer. It also further shows the usefulness of reverse phase protein array as a tool for individual tumor-based proteomics assessment and pathway signaling assessment in patients with serous ovarian cancer. Because of the potential for false discovery, we cannot presently determine which individual proteins should be used in models as predictors. Our future validation studies will focus on those proteins involved primarily in TGF-β pathway signaling because it is most likely that this pathway plays an important role as a marker or mediator of chemoresistance in high-grade serous ovarian cancer.

Ideally, the earlier one can predict outcome to primary therapy, the better. In ovarian cancer, a change in serum Ca-125 levels over the course of primary treatment is a validated marker of chemotherapy response and an important determinant of outcome (7–9, 35). If we are to develop a test that is applied at the time of initial diagnosis, then we require a surrogate for Ca-125 response. It is encouraging that a considerable amount of prognostic information relating to Ca-125 normalization can be captured by proteomic testing at the time of diagnosis by measuring three proteins: Smad3, EGFR, and JNK. Future studies should further explore proteomic testing as a predictive tool at the time of diagnosis. On the other hand, if proteomic markers are not robust enough on their own to effectively predict chemoresistance, it is possible that they could be used in combination with Ca-125 assessment. Finally, our findings lead to some important considerations relating to clinical trial design. If a proteomic marker for treatment failure is developed for patients with serous ovarian cancer, there will be an opportunity to incorporate investigational drugs earlier into the primary treatment setting for those patients with very adverse outcomes. Basing this type of design on proteomic assessment of individual tumors would represent an important advance in terms of assessing new targeted therapies or those that are designed to disrupt TGF-β pathway signaling to enhance the effectiveness of chemotherapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Dr. Molli McGahren, Yang Li, and Jane Li for their valuable assistance with this work.

Grant Support

Clinician Scientist Fellowship from Cancer Research UK (CA2757/A5902; R. Agarwal); National Cancer Institute P50CA083639 and CCSG P30 CA16672 (G.B. Mills and B.T. Hennessy); Myer-Levy Fellowship (M.S. Carey); and the Kleberg Center for Molecular Markers (G.B. Mills, B.T. Hennessy, M.S. Carey, and R. Agarwal).

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Received 09/15/2009; revised 03/22/2010; accepted 04/01/2010; published OnlineFirst 05/11/2010.

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