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Elevated AKAP12 in Paclitaxel-Resistant Serous Ovarian Cancer Cells is Prognostic and Predictive of Poor Survival in Patients

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Abstract

A majority of high-grade (HG) serous ovarian cancer (SOC) patients develop resistant disease despite high initial response rates to platinum/paclitaxel-based chemotherapy. We identified shed/secreted proteins in preclinical models of paclitaxel-resistant human HGSOC models and correlated these candidate proteins with patient outcomes using public data from HGSOC patients. Proteomic analyses of a HGSOC cell line secretome was compared to those from a syngeneic paclitaxel-resistant variant and from a line established from an intrinsically chemorefractory HGSOC patient. Associations between the identified candidate proteins and patient outcome were assessed in a discovery cohort of 545 patients and two validation cohorts totaling 795 independent SOC patients. Among the 81 differentially abundant proteins identified ($q < 0.05$) from paclitaxel-sensitive vs -resistant HGSOC cell secretomes, AKAP12 was verified to be elevated in all models of paclitaxel-resistant HGSOC. Furthermore, elevated AKAP12 transcript expression was associated with worse progression-free and overall survival. Associations with outcome were observed in three independent cohorts and remained significant after adjusted multivariate

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modeling. We further provide evidence to support that differential gene methylation status is associated with elevated expression of AKAP12 in taxol-resistant ovarian cancer cells and ovarian cancer patient subsets. Elevated expression and shedding/secretion of AKAP12 is characteristic of paclitaxel-resistant HGSO cells, and elevated AKAP12 transcript expression is a poor prognostic and predictive marker for progression-free and overall survival in SOC patients.

Keywords

Ovarian cancer; proteomics; secretome; AKAP12; paclitaxel resistance

Introduction

Ovarian cancer is the most lethal gynecologic malignancy and is the fifth leading cause of cancer-related death in women.¹ It is estimated that 21 980 new cases will arise and 14270 women will die from ovarian cancer in 2015.^{2,3} The current standard of care for ovarian cancer includes surgical cytoreduction combined with platinum and taxane-based chemotherapeutics.⁴ Advances in treatment of ovarian cancer patients have improved 5 year survival rates from 33.6% in 1975 to 45.2% in 2010, based on both more aggressive surgical cytoreduction and improved chemotherapy. Nevertheless, these gains are largely due to improved short-term survival. Long-term survival or cure rates are relatively unchanged, as approximately 75% of patients ultimately develop recurrence and progressive chemotherapeutic resistance. Thus, development of drug resistance continues to present a challenge toward improving long-term survival for ovarian cancer patients.

Paclitaxel (Taxol), a member of the taxane family of drugs, has been approved for the treatment of many solid tumor malignancies including ovarian, genitourinary, breast, and non-small-cell lung as well as those of the head and neck.^{6,7} Taxanes bind to beta-tubulin subunits and stabilize the polymerization of cellular microtubules, resulting in mitotic arrest and subsequent cell death.^{8,9} Recent *in vitro* characterization of clinically relevant paclitaxel doses has expanded on this classic mechanism of action, showing that paclitaxel causes the formation of multipolar spindles during cell division, producing chromosomal missegregation and cell death at cellular concentrations achieved during treatment.¹⁰ Cellular resistance to paclitaxel encompasses various mechanisms, including the

overexpression of drug efflux pumps, alterations in cellular drug metabolism, and molecular alterations in beta-tubulin.^{11,12} Differential gene expression analyses of paclitaxel-resistant ovarian cancer cells have identified abundant expression of the canonical drug-efflux pump, multidrug resistance protein 1 (MDR1/ABCB1), as well as the stem cell marker, aldehyde dehydrogenase 1 family, member A1 (ALDH1A1), which further correlates with increased paclitaxel resistance *in vivo*. Proteomic analyses of *in vitro* models of ovarian cancer have confirmed that elevated ABCB1 and ALDH1A1 are common features of paclitaxel-resistant ovarian cancer cells in addition to the differential modulation of several members of the Rho GDP dissociation inhibitor family, i.e., RhoGDI and Rho GDI2, and class III beta-tubulin binding partners, such as the disulfide isomerase ERpS7.^{14–16}

How the extracellular environment may contribute to the development of chemotherapeutic resistance and, more specifically, the ensemble of proteins shed and/or secreted by ovarian cancer cells that may function as biomarker surrogates of paclitaxel-resistant disease is not fully appreciated. A recent investigation demonstrated that microvesicles shed by paclitaxel-resistant ovarian cancer cells harbor appreciable levels of ABCB1 and that transfer of these microvesicles to paclitaxel-sensitive ovarian cancer cells can endow a paclitaxel-resistant phenotype.¹⁷ These findings expand the role of the extracellular proteome in conferring resistance to paclitaxel and further underscore the importance of ongoing investigations to identify additional mechanisms that may confer or perpetuate chemotherapeutic resistance to paclitaxel. Additionally, such analyses may identify biomarker candidates that would greatly aid in identifying *a priori* or emergent paclitaxel resistance.

In this analysis, mass spectrometry-based proteomics identified shed/secreted (secretome) proteins from a cell line model of high-grade serous ovarian cancer (OV90) that were quantitatively compared to secretomic analyses of a syngeneic OV90 model of paclitaxel resistance (OV90-TR1)¹⁸ and a newly established HGSOc cell line derived from a 46 year old with HGSOc who was *de novo* and intrinsically resistant to treatment (E3 cells). Secretome analyses revealed conserved modulation of proteins associated with regulation of abdominal neoplasms and mechanisms underlying malignancy in paclitaxel-resistant vs -sensitive cells. We further assessed the clinical relevance of candidates modulated with paclitaxel resistance by comparison with public microarray and RNA-seq analyses of ovarian cancer patient gene expression levels relative to diverse clinical criteria, including patient outcome. These analyses resulted in prioritization of A kinase (PRKA) anchor protein 12 (AKAP12), as elevated gene expression directly correlated with poor overall and progression-free survival in three cohorts of women with SOC. These findings support further mechanistic studies of AKAP12 to determine its role in paclitaxel resistance and further assessment of the utility of shed/secreted AKAP12 as a putative circulating biomarker of paclitaxel resistance in ovarian cancer patients.

Materials and Methods

Cell Culture and Reagents

CaOV3 and OV90 (ATCC, Manassas, VA) were cultured in complete DMEM or DMEM F12 media, respectively (ATCC), i.e., supplemented with 10% FBS and 1% penicillin/streptomycin (pen/strep). A paclitaxel-resistant serous EOC cell line (E3) was generated

from tumor tissues harvested from a patient-derived xenograft mouse established from a chemo-refractory ovarian cancer patient and maintained in complete DMEM-F12. E3 cells were confirmed to be of human origin by a species-specific, cytochrome c oxidase 1 (COI) PCR assay (ATCC). Serum- and phenol red-free media was obtained from ATCC and supplemented with 1% pen/strep. Paclitaxel and 5-aza-2'-deoxycytidine was obtained from Sigma-Aldrich.

Syngeneic models of paclitaxel resistance were generated in-house by acute exposure to paclitaxel for 72 h followed by a recovery period of 1–2 weeks. Briefly, paclitaxel-resistant OV90 cells were established by an acute treatment/recovery strategy with paclitaxel (1 nM) for a period of 7 months. At this time, the dosing was increased (ISO nM) and continued for an additional 4 months. The paclitaxel dose was further increased (700 nM to 1 μ M) for a period of 1 month. Paclitaxel-resistant CaOV3 cells (CaOV3_TRI) were established over a 10 month period following acute/recovery dose titrations from 2.0 to 10 nM paclitaxel. Paclitaxel treatments were withheld for a minimum of 1 month prior to generating the secretome and total protein/RNA lysates.

Cell Viability Assay

Cells were trypsinized with 0.25% Trypsin-EDTA (ATCC), plated equivalently in 96-well plates, and incubated overnight. Media was removed and replaced with fresh media containing dose titrations of paclitaxel. Cell viability was assessed 72 h later using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) CellTiter 96 aqueous one solution cell proliferation assay (Promega, Madison, WI) according to the manufacturer's instructions; absorbance (490 nm) was measured using a microplate spectrophotometer (xMark, Bio-Rad) following incubation in MTS reagent at 37 °C. Three biological replicates were performed for each cell line, where each paclitaxel dose was assayed in triplicate for each experiment.

Secretome Collection and Processing

Secretome purification was performed as described previously.¹⁹ Briefly, cells were plated in triplicate 75 cm² tissue culture flasks (BD Falcon) and maintained until ~80% confluent. Cultures were then washed thrice with serum-free, phenol red-free medium supplemented with 1% pen/strep (SF-medium), followed by culture in 10 mL of SF medium for 24 h. Conditioned medium was supplemented with protease and phosphatase inhibitors at a 1 $\times$ final concentration (Halt, Thermo Fischer Scientific Inc., Rockford, IL, USA), pooled from replicate flasks, and maintained on ice. Samples were centrifuged at 4 °C for 10 min at 500g to remove cellular debris. Samples were concentrated using 3K MWCO ultra centrifugal filters (Amicon Millipore, Billerica, MA, USA) followed by buffer exchanges into a total volume of 45 mL of 25 mM ammonium bicarbonate (AmBic) supplemented with 0.1 M phenylmethylsulfonyl fluoride (Sigma-Aldrich) during a series of 30 min centrifugations at 3000g and 4 °C until a final volume of 1.0 mL was achieved. Further concentration of samples was performed using 10K MWCO ultra centrifugal filters (Amicon Millipore, Billerica, MA, USA) at 14000g for 6 min at 4 °C until a final volume of 100 μ L was obtained. Protein content was measured using the bicinchoninic assay (Pierce BCA protein

assay kit, Thermo Fisher Scientific Inc., San Jose, CA, USA) as per manufacturer's recommendations. Samples were stored at -80°C .

Proteomic Analyses

Thirty micrograms of total secretome lysate was resolved halfway into a 4–15% ID SDS-PAGE gel (Mini-PROTEAN TGX gel, Bio-Rad), briefly stained with Coomassie blue (SimplyBlue SafeStain; Invitrogen, Carlsbad, CA, USA), and destained in double-distilled water overnight. Five equivalently sized gel bands were excised, and proteins were digested in-gel as previously described.^{19,20} Samples were desalted (Pepclean C18 spin columns, Thermo Fischer Scientific Inc., Rockford, IL, USA) according to the manufacturer's instructions. Peptide digests were resuspended in 25 mM AmBic at a final concentration of $0.15\text{ }\mu\text{g}/\mu\text{L}$ and stored at -80°C . Tryptic peptide digests were analyzed in quadruplicate by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a nanoflow LC (Easy-nLC, ThermoFisher Scientific Inc.) coupled online to an LTQ-Orbitrap Velos MS (Thermo-Fisher Scientific Inc., San Jose, CA, USA) as previously described.^{19,20} Briefly, $6.0\text{ }\mu\text{L}$ of sample was resolved on $100\text{ }\mu\text{m}$ i.d. $\times$ $360\text{ }\mu\text{m}$ o.d. $\times$ 200 mm long fused silica capillary columns (Polymicro Technologies, Phoenix, AZ, USA) slurry-packed in-house with $5\text{ }\mu\text{m}$, $300\text{ }\text{\AA}$ pore size C-18 silica-bonded stationary phase (Jupiter, Phenomenex, Torrance, CA, USA). After sample injection, peptides were eluted from the column using a linear gradient of 0.33% mobile phase B (100% AcN and 0.1% formic acid) over 120 min at a constant flow rate of $200\text{ nL}/\text{min}$ followed by a column wash consisting of 95% B for an additional 30 min at a constant flow rate of $200\text{ nL}/\text{min}$. The LTQ;Orbitrap Velos MS was configured to collect high-resolution ($R = 60\text{ }000$ at m/z 400) broadband mass spectra (m/z 375–1800), from which the 20 most abundant peptide molecular ions dynamically determined from the MS scan were selected for tandem MS using a relative CID energy of 30%. Dynamic exclusion was utilized to minimize redundant selection of peptides for CID.

Peptide Identification and Spectral Counting

Tandem mass spectra were searched against the SwissProt human protein database from UniProt (downloaded Oct 2, 2014; 20193 protein entries, from the Universal Protein Resource (www.uniprot.org) utilizing the Mascot Daemon (Matrix Science Inc., Boston, MA, USA). The data was searched with a precursor mass tolerance of 10 ppm and a fragment ion tolerance of 0.6 Da. Cysteine carbamidomethylation (57.021464 Da) was set as a fixed modification, and methionine oxidation (15.99492 Da), as a dynamic modification; a maximum of two missed tryptic cleavages was allowed. Identified peptides were filtered using an ion score cutoff to result in a false peptide discovery rate of less than 1% for all peptides identified, as determined from a decoy database search. Total peptide spectral matches (PSM) were derived by summing counts for each SwissProt protein accession from the replicate LC-MS/MS analyses for each sample, and normalization was performed using the lowest total PSM value across all samples in the comparison group. A value of 0.5 was added to each spectral count value prior to $\log_2$ transformation to enable ratio values to be calculated for presence/absence events.²¹ Significantly, differentially abundant proteins identified by at least two peptides were determined by z -test statistics. Corresponding p -values were then converted to q -values as per Storey et al.²² for each comparison analyses, and differentially abundant proteins with a q -value < 0.05 were considered to be

significantly different between taxol-resistant and -sensitive cells. Significant ratios were then $\log_2$ transformed for downstream comparative analyses. Enrichment of putatively secreted proteins was assessed using SignalP designations for all SwissProt accessions, with candidates labeled “potentials” removed.

Biostatistical Analyses of Public Patient-Derived High-Throughput and Clinical Outcome Data

High-throughput data including transcriptomic, methylation profiling, copy number alterations, and exomic sequencing data^{23–30} were downloaded with relevant clinical annotation from CuratedOvarianData³¹ (<http://bcb.dfci.harvard.edu/ovariancancer>) using the statistical computing language R with the Bioconductor package, the Cancer Genome Atlas (TCGA) Research Network data on the Memorial Sloan Kettering Cancer Center (MSKCC) Cancer Genomics Data Servers (<http://www.cbioportal.org/>) using the CGDS-R package in the TCGA Data Portal (<https://tcga-data.nci.nih.gov/tcga/findArchives.htm> or <https://tcga-data.nci.nih.gov/tcga/tcgaDownload.jsp>) following user guidelines and instructions, and from GEO (<http://www.ncbi.nlm.nih.gov/geoprofiles/>).^{23–27,29,30} Analyses were performed in women with a diagnosis of SOC with the following mandatory data items: hybridization-based transcriptomic data, site of primary cancer, stage, histologic cell type, vital status, and overall survival (OS) time in months from diagnosis. Subsets of cases also had age at diagnosis, disease status, progression-free survival (PFS) time, RNA sequencing data (RNA-seq), and/or data from methylation profiling, copy number alteration analyses, and/or exomic sequencing. A case cohort designed with a robust and well-vetted discovery cohort from the TCGA Research Network was utilized to evaluate and rank the clinical relevance of 81 candidate biomarkers from secretome analysis based on strength of associations among patient-derived Affymetrix-based mRNA expression (Affy-mRNA) data, overall survival (OS), and progression-free survival (PFS) in eligible serous ovarian cancer patients (described below). PFS data was evaluable only in a subset of SOC patients. Significant candidates (p -value ≤ 0.05) then underwent technical validation to examine the correlation between probeset-level Affy-mRNA expression and transcript-level RNA-seq data. Outcome analyses were also performed in the subset of TCGA cases with Affy-mRNA and transcript-level RNA-seq data. Table 1 provides details regarding the five independent Affy-mRNA data sources that were integrated and combat-corrected to represent Validation Cohort 1^{23–27} and the Operon-transcript data used as Validation Cohort 2.²⁹

OS and PFS analyses were performed using univariate and multivariate Cox regression modeling with Wald testing and by Kaplan–Meier analysis with log-rank testing. The cutoff value that was used to determine high vs low expression of gene candidates of interest was the median. Distributions in 2×2 or $R \times R$ contingency tables were compared using Fisher's exact test. Relationships between different types of high-throughput data were evaluated using Spearman's correlation coefficients, Kappa statistic, and means compared using an analysis of variance (ANOVA) and t -test. Predictive accuracy was assessed from an area under the curve estimate for a receiver operator curve for 5 year survival. Statistical analyses were performed using the statistical computing language R with the Bioconductor package and SPSS version 22.0 (IBM Corp, Armonk, NY, USA).

Immunoblot Analyses

Subconfluent cells were lysed in 1× SDS buffer (1% SDS, 10 mM Tris-HCl, pH 7.4) to generate total cell lysates. Twenty micrograms of secretome or total cell lysate was resolved on a 4–15% mini-PROTEIN TGX gels (Stain-Free gels for secretome blots, Bio-Rad) and transferred to PVDF membranes. Membranes were blocked for 1 h with 5% nonfat dry milk in 1× TBST and incubated with primary antibody overnight at 4 °C. Secondary antibody was incubated for 4 h at ambient temperature followed by incubation in SuperSignal West Dura chemiluminescent substrate (ThermoFisher Scientific) for 5 min. Anti-AKAP12 rabbit polyclonal (HPA006344, Sigma-Aldrich, 1:1000) and anti-GAPDH rabbit polyclonal (ab9485, Abcam, 1:1000) primary antibodies were used, and HRP-linked goat anti-rabbit IgG (Cell Signaling Technologies, 1:1000) secondary antibody was used. Images were acquired using a ChemiDoc XRS+ system (Bio-Rad).

Quantitative-PCR Analyses

Total RNA was extracted from subconfluent cells with TRIzol (Invitrogen), and cDNA was prepared from equivalent amounts of total RNA by reverse-transcription using the Superscript VILO cDNA synthesis kit (Invitrogen). AKAP12 (hCG21985) and 18S rRNA (Hs03003631_g1) TAQMAN assays were obtained from Invitrogen. Quantitative PCR was performed using TAQMAN gene expression master mix of equivalent amounts of total cDNA (ABI GeneAmp 9700 DNA thermal cycler) for 50 cycles. End point data was assembled by comparison of Delta-Ct values for AKAP12 versus corresponding 18S rRNA Delta-Ct values for each cell line. Data reflects duplicate biological replicates and triple technical replicate analyses.

Results

Proteomic Analyses of Secretomes Harvested from a Syngeneic and Patient-Derived Cell Line Model of Paclitaxel-Resistant Ovarian Cancer

Paclitaxel-resistance was confirmed in a syngeneic model of paclitaxel-resistant ovarian cancer cells (OV90 and OV90-TR1) and a primary cell line established from a treatment refractory ovarian cancer patient (E3) (Supporting Information Figures S1 and S2). A global proteomic analysis of total protein harvested from conditioned media collected from OV90 (paclitaxel sensitive), OV90-TR1, and E3 (paclitaxel resistant) cells following overnight incubation in serum-free media identified an average of 1315 (± 213 , RSD = 16%) proteins by at least two peptide spectral matches (PSM) across the sample set (Supporting Information Table S1), with an average 2.4-fold enrichment in proteins bearing an N-terminal signal peptide compared to that predicted in the reference human Swiss-Prot database (Supporting Information Figure S3). Comparative analysis of OV90-TR1 and E3 secretomes with OV90 revealed a total of 164 (± 28) differentially abundant proteins (q -value = 0.05), among which 81 were commonly shared in the OV90-TR1 and E3 secretomes (Supporting Information Table S2). Notably, these 81 proteins exhibited identical abundance deflections, where 15 were increased and 63 were decreased in abundance in paclitaxel-resistant (OV90-TR1 and E3) vs -sensitive cells (OV90) (Figure 1A). Significant enrichment of proteins associated with disease and cellular function categories that included cancer, abdominal neoplasm, and mechanisms underlying malignancy was observed (Figure 1B).

AKAP12 Expression Is Associated with Poor Outcome in SOC Patients

Univariate Cox regression modeling was used to determine whether the expression of any of these 81 candidates correlated with ovarian cancer patient outcome using publicly available transcript expression data from a cohort of 545 SOC patients for which detailed clinical and outcome data were available (Table 1).²⁸ Seventy-two of the 81 candidates mapped to corresponding Affymetrix probesets, of which eight significantly correlated with OS in the full Discovery cohort (Wald p -value = 0.05; Supporting Information Table S3). To confirm this result, which is based on hybridization-based probeset-level transcript expression data, the association between these eight candidates and OS was technically verified in a cohort of 251 SOC patients for which transcript expression data derived from RNA-seq analyses were publicly available (Table 1 and Supporting Information Table S4). This analysis verified that AKAP12 was significantly associated with OS from hybridization-based probeset-level and whole transcript-level data (Table 2). Further confirmation of the reproducibility of hybridization-based probeset level AKAP12 expression with whole transcript-level AKAP12 RNA-seq data was demonstrated in a subset of 155 patients with transcript expression derived from both hybridization-based and RNA-seq methods from different aliquots of frozen primary tumor.²⁸ This analysis verified that the expression measures derived from these two analytical platforms were highly equivalent (Spearman correlation = 0.953; Supporting Information Figure S4) and AKAP12 expression levels categorized at the median were in strong agreement (Kappa = 0.873). Univariate and multivariate Cox regression modeling in two independent cohorts with two different variants of publicly available hybridization-based transcript data composed of 638 (Affy-mRNA in Validation 1) and 157 (Operon mRNA in Validation 2) SOC patients (Table 1) confirmed the association between elevated AKAP12 expression and poor OS and that AKAP12 transcript levels appear to be an independent prognostic factor for OS, respectively (Table 2).

When categorized as high versus low expression in SOC patients, AKAP12 was again significantly associated with worse OS (Figure 2A,B), representing 10.2 and 17.9 month shorter median survival time, and with worse PFS (Figure 2C,D), representing 8.6 and 6.9 month shorter median progression-free survival in the Discovery (Figure 2A,C) and Validation 1 (Figure 2B,D) cohorts, respectively. Cox regression modeling for OS demonstrated that women with high vs low AKAP12 Affy-mRNA levels categorized at the median had a 49 and 39% increased risk of death in univariate analyses and a 39 and 55% increased risk of death in multivariate analyses of the Discovery and Validation 1 cohorts, respectively, all with 95% confidence intervals that excluded 1. High vs low AKAP12 Affy-mRNA transcript expression was also associated with a 34 and 38% vs 59 and 47% increased risk of disease progression in univariate and multivariate Cox modeling for PFS in the Discovery and Validation 1 cohorts, respectively; again, all of these 95% confidence intervals excluded 1. PFS data was not available for Validation cohort 2.

Receiver operator curve prediction of 5 year survival was performed in patients in the Discovery and Validation Cohort 1 to evaluate the predictive accuracy of Affy-mRNA expression of AKAP12 evaluated as a continuous variable or categorized at the median as high vs low relative to that achieved by FIGO stage. This analysis demonstrated that both measures of AKAP12 mRNA expression were predictive of 5 year survival in women with

HGSOC in the Discovery Cohort and in Validation Cohort 1 with an accuracy equivalent to FIGO stage, a well-established standard (Supporting Information Table S5). Validation cohort 2 focused on unspecified late-stage SOC patients, and we were not able to compare the predictive accuracy of AKAP12 mRNA level with stage in this cohort

AKAP12 Protein and Transcript Expression Is Increased in Paclitaxel-Resistant Ovarian Cancer Cells

AKAP12 detection by immunoblot confirmed that this protein is elevated in secretome samples harvested from paclitaxel-resistant (OV90-TR1 and E3) cells as compared to paclitaxel-sensitive cells (Figure 3). This analysis also included secretome samples harvested from another syngeneic model of paclitaxel-resistance established in house from CaOV3 cells, another representative cell line model of HGSOC (Supporting Information Figure S1). This immunoblot result is consistent with previous analyses of AKAP12, a protein that produces multiple immunoreactive bands at ~240 kDa and in the 280–290 kDa range.³² We further confirmed that elevated levels of AKAP12 in the secretome of taxol-resistant ovarian cancer cells was not an artifact of serum-deprivation conditions used for proteomic discovery analyses by immunoblot analyses of AKAP12 levels in conditioned media harvested from OV90 (paclitaxel-sensitive) versus OV90-TR1 (paclitaxel-resistant) cells supplemented with 10% fetal bovine serum (Supporting Information Figure S7). In an extension of these experiments, AKAP12 protein and transcript levels were assessed by immunoblot and qPCR analyses, respectively, from whole-cell lysates from these five cell line models of HGSOC. These data show that AKAP12 is significantly elevated at both the transcript and total protein level in paclitaxel-resistant ovarian cancer cells, with transcript levels ranging from 5- (CaOV3-TR1) to ~200-fold (OV90-TR1) elevated over those observed in paclitaxel-sensitive cell lines (Figure 4A), with similar elevations observed in protein abundance (Figure 4B).

AKAP12 Methylation Status Is Associated with Elevated Expression in Taxol-Resistant Ovarian Cancer Cells and Ovarian Cancer Patient Subsets

A mechanistic basis for elevated AKAP12 expression in HGSOC patients was assessed by examining the somatic mutation, gene copy number, and methylation status for AKAP12 in publicly available data (Table 1). One ovarian cancer patient was identified to harbor a splice junction mutation (V107, patient TCGA57.1584), ruling out extensive, deleterious AKAP12 mutation as a factor underlying differential AKAP12 protein levels. A gene copy number analysis (CNA) of AKAP12 in ovarian cancer patients ($n = 534$) categorized into either three groups or two groups revealed a subset of low AKAP12 expressing ovarian cancer patients that exhibit AKAP12 CNA loss (CNA $\log_2 -0.5$, Supporting Information Figure S5). In addition, an inverse relationship in HGSOC patients was observed between AKAP12 methylation status categorized into tertiles and Affy-based AKAP12 transcript expression, with the lowest expression observed in patients exhibiting hypermethylation of AKAP12 (tertile 3) relative to those in tertiles 1 and 2 (Supporting Information Figure S6). We further evaluated the relationship between the combined impact of hypermethylation of AKAP12 (tertile 3 vs tertiles 1 or 2) and copy number loss (<-0.5 vs not lost) to determine if CNA or methylation may underlie the observed differential AKAP12 transcript expression in paclitaxel-resistant HGSOC cells and in SOC patients with poor outcome. These results

show that SOC patients with low AKAP12 methylation levels and no AKAP12 gene copy number loss exhibited the highest expression levels of AKAP12 (Figure 5A). We therefore evaluated the impact of gene methylation on AKAP12 levels in taxol-resistant HGSOC models. AKAP12 transcript expression levels were measured following treatment of taxol-sensitive (OV90) and -resistant (OV90-TR1) cells with 5-aza-2'-deoxycytidine (5-Aza). AKAP12 transcript expression significantly increased in 5-aza-treated taxol-sensitive OV90 cells; however, no significant change was observed in taxol-resistant OV90-TR1 cells (Figure 5B).

Discussion

A global proteomic analysis of secretome samples harvested from human HGSOC cell line models of paclitaxel-sensitive and -resistant disease identified conserved modulation of extracellular-localized proteins previously implicated in abdominal cancers and metastatic disease. Correlation of proteins modulated in paclitaxel-resistant HGSOC with ovarian cancer patient outcomes using large patient cohorts for which transcript expression and outcomes data are publicly available identified a subset of candidates that were significantly associated with clinically relevant measures of patient outcome including PFS and OS. Among these, elevated levels of AKAP12 transcript were found to consistently and significantly associate with worse PFS and OS in multiple independent, publicly available ovarian cancer tissue expression databases for which transcript abundances were determined by two hybridization-based methodologies and/or from RNA-seq. The association between elevated AKAP12 expression and poor patient outcome was observed in the Discovery Cohort based on Affy-mRNA and RNA-seq data, in Validation Cohort 1 based on Affy-mRNA data, and in Validation Cohort 2 based on Operon-mRNA data. Multivariate Cox modeling demonstrated that AKAP12 expression was an independent prognostic factor for PFS and OS. Receiver operator curves for predicting 5 year survival in HGSOC patients demonstrated that the predictive accuracy of AKAP12 transcript expression and FIGO stage were similar in the Discovery and Validation 1 cohorts. Potential concern regarding the reliability of hybridization-based transcript detection for AKAP12 was alleviated by performing a paired analysis of Affy-mRNA relative RNA-seq based transcript expression, which yielded a Spearman's correlation coefficient of 0.952 and a kappa statistic of 0.873 for categorized expression in paired aliquots evaluated by both platforms. Interestingly, elevated AKAP12 expression in women with SOC was associated with a loss of AKAP12 gene methylation in patient subsets. This observation prompted us to investigate whether differential AKAP12 gene methylation may in part explain the increase in transcript and protein abundance observed in taxol-resistant vs -sensitive HGSOC cells. Inhibition of DNA methyltransferase with 5-aza-2~ deoxycytidine in paclitaxel-sensitive but not -resistant HGSOC cells resulted in significant elevations in AKAP12 transcript levels, suggesting that loss of AKAP12 gene methylation may occur during the phenotypic acquisition of taxol resistance.

AKAP12 possesses multiple myristoylation motifs that target it to the plasma membrane.³³ Notably, AKAP12 lacks a canonical N-terminal signal peptide sequence to facilitate active secretion. However, AKAP12 has been shown by three previous investigations of the human plasma proteome to be present and detectable in peripheral circulation.³⁴⁻³⁷ AKAP12 has

diverse protein scaffolding functions, tethering the actin cytoskeleton to the signaling activity of multiple protein kinases, including v-Src, PKA, and PKC.³³ AKAP12 is classified as a tumor suppressor, as decreased expression in cancer cells is directly associated with an increased invasive and metastatic phenotype.³⁸ Furthermore, AKAP12 has been shown to attenuate pulmonary metastasis in a murine model of prostate cancer through inhibition of angiogenesis via modulation of vascular endothelial growth factor secretion. Clinical analyses of AKAP12 abundance has revealed low expression in various solid tumor types, including gastric, lung, ovarian and pancreatic cancers and considerable evidence implicates hypermethylation of the AKAP12 promoter sequence as the basis for loss of expression.^{33,40–45}

Previous work from our group demonstrated elevated AKAP12 protein in the mitochondrial fraction of platinum-resistant ovarian cancer cells.⁴⁶ Taking these data together with the present work, a suggestive trend of increased expression of AKAP12 in chemoresistant ovarian cancer cells is observed. We are actively pursuing the translational potential of AKAP12 as a biomarker of chemoresistance in ovarian cancer in an expanded cohort of chemoresistant/refractory versus sensitive ovarian cancer patient tissues using selected-reaction monitoring (SRM) mass spectrometry assays and enzyme-linked immunosorbent assay (ELISA) analyses to directly quantify AKAP12 abundance followed by correlation with clinically established measures of chemoresistance. The acquisition of a chemoresistant phenotype in ovarian cancer cells can occur through epithelial-to-mesenchymal transition (EMT).⁴⁷ Indeed, AKAP12 mRNA has been previously shown to be elevated in murine proximal tubular cells stimulated to undergo EMT.⁴⁸ Furthermore, the observed correlation of elevated AKAP12 expression with poor ovarian cancer patient OS and PFS is noteworthy, particularly in light of the canonical tumor suppressor functions previously reported for AKAP12. Progression-free survival can be impacted by a variety of clinical disease features including suboptimal debulking as well as characteristics of innate tumor cell biology.⁴⁹ Elevated AKAP12 transcript expression has been previously included in gene signatures predicting poor survival in ovarian cancer patients.^{50,51} Considering the significant association of elevated AKAP12 gene expression with ovarian cancer patient PFS and the observed increases in this protein in both cisplatin- and paclitaxel-resistant HGSOC cell line models, it is provocative to suggest that elevations in AKAP12 may support the acquisition of chemoresistance in ovarian cancer cells.

In conclusion, the data presented here, and recently by our group,⁴⁶ support the hypothesis that increases in AKAP12 expression accompanies acquisition of platinum- and taxol-resistance in HGSOC cell lines and, further, that elevated AKAP12 transcript expression in HGSOC patients is significantly associated with worse PFS and OS, has independent prognostic value for both of these outcome measures, and exhibits predictive value for 5 year survival equivalent to FIGO stage in this clinical setting. These observations, together with the increased shedding/secretion of AKAP12 by taxol-resistant ovarian cancer cells, warrant further investigations to characterize the clinical utility of AKAP12 as a circulating chemotherapeutic resistance biomarker and to delineate the mechanistic role(s) of this protein in conferring a chemoresistance phenotype in ovarian cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. A Snapshot of Ovarian Cancer. National Cancer Institute; Bethesda, MD: 2013.
2. Statistics. Ovarian Cancer National Alliance; Washington, DC: <http://www.ovariancancer.org/about/statistics/>
3. SEER Cancer Statistics Factsheets: Ovary Cancer. National Cancer Institute; Bethesda, MD: <http://seer.cancer.gov/statfacts/html/ovary.html>
4. McGuire WP, Hoskins WJ, Brady MF, Kucera PR, Partridge EE, Look KY, Clarke-Pearson DL, Davidson M. Cyclophosphamide cisplatin compared with paclitaxel cisplatin in patients with stage III and stage IV ovarian cancer. *N Engl J Med*. 1996; 334:1–6. [PubMed: 7494563]
5. Agarwal R, Kaye SB. Ovarian cancer: strategies for overcoming resistance to chemotherapy. *Nat Rev Cancer*. 2003; 3:502–16. [PubMed: 12835670]
6. Sung M, Giannakakou P. BRCA1 regulates micro tubule dynamics taxane-induced apoptotic cell signaling. *Oncogene*. 2014; 33:1418–28. [PubMed: 23524581]
7. Misiukiewicz K, Gupta V, Bakst R, Posner M. Taxanes in cancer of the head and neck. *Anti cancer Drugs*. 2014; 25:561–70. [PubMed: 24534821]
8. Schiff PB, Fant J, Horwitz SB. Promotion of microtubule assembly in vitro by taxol. *Nature*. 1979; 277:665–7. [PubMed: 423966]
9. Arnal I, Wade RH. How does taxol stabilize microtubules? *Curr Biol*. 1995; 5:900–8. [PubMed: 7583148]
10. Zasadil LM, Andersen KA, Yeum D, Rocque GB, Wilke LG, Tevaarwerk AJ, Raines RT, Burkard ME, Weaver BA. Cytotoxicity of paclitaxel in breast cancer is due to chromosome missegregation on multipolar spindles. *Sci Transl Med*. 2014; 6:229ra43.
11. Bani MR, Nicoletti MI, Alkharouf NW, Ghilardi C, Petersen D, Erba E, Sausville EA, Liu ET, Giavazzi R. Gene expression correlating with response to paclitaxel in ovarian carcinoma xenografts. *Mol Cancer Ther*. 2004; 3:111–21. [PubMed: 14985451]
12. Kumar S, Mahdi H, Bryant C, Shah JP, Garg G, Munkarah A. Clinical trials and progress with paclitaxel in ovarian cancer. *Int J Womens Health*. 2010; 2:411–27. [PubMed: 21270965]
13. Landen CN Jr, Goodman B, Katre AA, Steg AD, Nick AM, Stone RL, Miller LD, Mejia PV, Jennings NB, Gershenson DM, Bast RC Jr, Coleman RL, Lopez-Berestein G, Sood AK. Targeting aldehyde dehydrogenase cancer stem cells in ovarian cancer. *Mol Cancer Ther*. 2010; 9:3186–99. [PubMed: 20889728]
14. Goto T, Takano M, Sakamoto M, Kondo A, Hirata J, Kita T, Tsuda H, Tenjin Y, Kikuchi Y. Gene expression profiles with cDNA microarray reveal RhoGDI as a predictive marker for paclitaxel resistance in ovarian cancers. *Oncol Rep*. 2006; 15:1265–71. [PubMed: 16596196]
15. Lee DH, Chung K, Song JA, Kim TH, Kang H, Huh JH, Jung SG, Ko JJ, An HJ. Proteomic identification of paclitaxel resistance associated hnRNP A2 and GDI 2 proteins in human ovarian cancer cells. *J Proteome Res*. 2010; 9:5668–76. [PubMed: 20858016]
16. Cicchillitti L, Di Michele M, Urbani A, Ferlini C, Donat MB, Scambia G, Rotiho D. Comparative proteomic analysis of paclitaxel sensitive A2780 epithelial ovarian cancer cell line and its resistant counterpart A2780TC1 by 2D-DIGE: the role of ERp57. *J Proteome Res*. 2009; 8:1902–12. [PubMed: 19714814]

17. Zhang FF, Zhu YF, Zhao QN, Yang DT, Dong YP, Jiang L, Xing WX, Li XY, Xing H, Shi M, Chen Y, Bruce IC, Jin J, Ma X. Microvesicles mediate transfer of P-glycoprotein to paclitaxel-sensitive A2780 human ovarian cancer cells, conferring paclitaxel resistance. *Eur J Pharmacol.* 2014; 738:83–90. [PubMed: 24877693]
18. Domcke S, Sinha R, Levine DA, Sander C, Schultz N. Evaluating cell lines as tumour models by comparison of genomic profiles. *Nat Commun.* 2013; 4:2126. [PubMed: 23839242]
19. Teng PN, Wang G, Hood BL, Conrads KA, Hamilton CA, Maxwell GL, Darcy KM, Conrads TP. Identification of candidate circulating cisplatin-resistant biomarkers from epithelial ovarian carcinoma cell secretomes. *Br J Cancer.* 2014; 110:123–32. [PubMed: 24178762]
20. Bateman NW, Sun M, Hood BL, Flint MS, Conrads TP. Defining central themes in breast cancer biology by differential proteomics: conserved regulation of cell spreading and focal adhesion kinase. *J Proteome Res.* 2010; 9:5311–24. [PubMed: 20681588]
21. McDonald, JH. *Handbook of Biological Statistics.* Sparky House Publishing; Baltimore, MD: 2009. p. 160-4.
22. Storey JD, Tibshirani R. Statistical significance for genome wide studies. *Proc Natl Acad Sci USA.* 2003; 100:9440–5. [PubMed: 12883005]
23. Bonome T, Levine DA, Shih J, Randonovich M, Pise-Masison CA, Bogomolny F, Ozbun L, Brady J, Barrett JC, Boyd J, Birrer MJ. Agene signature predicting for survival in suboptimally debulked patients with ovarian cancer. *Cancer Res.* 2008; 68:5478–86. [PubMed: 18593951]
24. Denkert C, Budczies J, Darb-Esfahani S, Gyorffy B, Sehouli J, Konsgen D, Zeillinger R, Weichert W, Noske A, Buckendahl AC, Muller BM, Dietel M, Lage H. A prognostic gene expression index in ovarian cancer- validation across different independent data sets. *J Pathol.* 2009; 218:273–80. [PubMed: 19294737]
25. Dressman HK, Berchuck A, Chan G, Zhai J, Bild A, Sayer R, Cragun J, Clarke J, Whitaker RS, Li L, Gray J, Marks J, Ginsburg GS, Potti A, West M, Nevins JR, Lancaster JM. An integrated genomic based approach to individualized treatment of patients with advanced-stage ovariancancer. *J Clin Oncol.* 2007; 25:517–25. [PubMed: 17290060]
26. Mok SC, Bonome T, Vathipadiekal V, Bell A, Johnson ME, Wong Kk, Park DC, Hao K, Yip DKP, Donniger H, Ozbun L, Samimi G, Brady J, Randonovich M, Pise-Masison CA, Barrett JC, Wong WH, Welch WR, Berkowitz RS, Birrer MJ. A gene signature predictive for outcome in advanced ovarian cancer identifies a survival factor: microfibril associated glycoprotein 2. *Cancer Cell.* 2009; 16:521–32. [PubMed: 19962670]
27. Tothill RW, Tinker AV, George J, Brown R, Fox SB, Lade S, Johnson DS, Trivett MK, Etemadmoghadam D, Locandro B, Traficante N, Fereday S, Hung JA, Chiew YE, Haviv I, Gertig D, DeFazio A, Bowtell DDL. Novel molecular subtypes of serous and endometrioid ovarian cancer linked to clinical outcome. *Clin Cancer Res.* 2008; 14:5198–208. [PubMed: 18698038]
28. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. *Nature.* 2011; 474:609–15. [PubMed: 21720365]
29. Crijns APG, Fehrmann RSN, de Jong S, Gerbens F, Meersma GJ, Klip HG, Hollema H, Hofstra RMW, te Meerman GJ, de Vries EGE, van der Zee AGJ. Survival-related profile, pathways, and transcription factors in ovarian cancer. *PLoS Med.* 2009; 6
30. Yoshihara K, Tsunoda T, Shigemizu D, Fujiwara H, Hatae M, Fujiwara H, Masuzaki H, Katabuchi H, Kawakami Y, Okamoto A, Nogawa T, Matsumura N, Udagawa Y, Saito T, Itamochi H, Takano M, Miyagi E, Sudo T, Ushijima K, Iwase H, Seki H, Terao Y, Enomoto T, Mikami M, Akazawa K, Tsuda H, Moriya T, Tajima A, Inoue I, Tanaka K. Japanese Serous Ovarian Cancer Study Group. High risk ovarian cancer based on 126 gene expression signature is uniquely characterized by downregulation of antigen presentation pathway. *Clin Cancer Res.* 2012; 18:1374–85. [PubMed: 22241791]
31. Ganzfried BF, Riester M, Haibe-Kains B, Risch T, Tyekucheva S, Jazic I, Wang XV, Ahmadifar M, Birrer MJ, Parmigiani G, Huttenhower C, Waldron L. curatedOvarianData: clinically annotated data for the ovarian cancer transcriptome. *Database.* 2013; 2013:bat013. [PubMed: 23550061]
32. Lin X, Gelman IH. Reexpression of the major protein kinase C substrate, SSeCKS, suppresses v-src-induced morphological transformation and tumorigenesis. *Cancer Res.* 1997; 57:2304–12. [PubMed: 9187136]

33. Gelman IH. Emerging roles for SSeCKS/Gravin/AKAP12 in the control of cell proliferation, cancer malignancy, and barrierogenesis. *Genes Cancer*. 2010; 1:1147–56. [PubMed: 21779438]
34. Liu X, Valentine SJ, Plasencia MD, Trimpin S, Naylor S, Clemmer DE. Mapping the human plasma proteome by SCX-LC-IMS-MS. *J Am Soc Mass Spectrom*. 2007; 18:1249–64. [PubMed: 17553692]
35. Nanjappa V, Thomas JK, Marimuthu A, Muthusamy B, Radhakrishnan A, Sharma R, Ahmad Khan A, Balakrishnan L, Sahasrabudhe NA, Kumar S, Jhaveri BN, Sheth KV, Kumar Khatana R, Shaw PG, Srikanth SM, Mathur PP, Shankar S, Nagaraja D, Christopher R, Mathivanan S, Raju R, Sirdeshmukh R, Chatterjee A, Simpson RJ, Harsha HC, Pandey A, Prasad TS. Plasma Proteome Database as a resource for proteomics research: 2014 update. *Nucleic Acids Res*. 2014; 42:D959–65. [PubMed: 24304897]
36. Barnea E, Sorkin R, Ziv T, Beer I, Admon A. Evaluation of prefractionation methods as a preparatory step for multi dimensional based chromatography of serum proteins. *Proteomics*. 2005; 5:3367–75. [PubMed: 16047308]
37. Farrah T, Deutsch EW, Omenn GS, Campbell DS, Sun Z, Bletz JA, Maffick P, Katz JE, Malmstrom J, Ossola R, Watts JD, Lin B, Zhang H, Moritz RL, Aebersold R. A high-confidence human plasma proteome reference set with estimated concentrations in PeptideAtlas. *Mol Cell Proteomics*. 2011; 10:MI10.006353.
38. Gelman IH, Gao L. SSeCKS/Gravin/AKAP12 metastasis suppressor inhibits podosome formation via RhoA- and Cdc42-dependent pathways. *Mol Cancer Res*. 2006; 4:151–8. [PubMed: 16547152]
39. Su B, Zheng Q, Vaughan MM, Bu Y, Gelman IH. SSeCKS metastasis-suppressing activity in MatLyLu prostate cancer cells correlates with vascular endothelial growth factor inhibition. *Cancer Res*. 2006; 66:5599–607. [PubMed: 16740695]
40. Choi MC, Jong HS, Kim TY, Song SH, Lee DS, Lee JW, Kim TY, Kim NK, Bang YJ. AKAP12/Gravin is inactivated by epigenetic mechanism in human gastric carcinoma and shows growth suppressor activity. *Oncogene*. 2004; 23:7095–103. [PubMed: 15258566]
41. Jo U, Whang YM, Kim HK, Kim YH. AKAP12alpha is associated with promoter methylation in lung cancer. *Cancer Res Treat*. 2006; 38:144–51. [PubMed: 19771275]
42. Bonazzi VF, Irwin D, Hayward NK. Identification of candidate tumor suppressor genes inactivated by promoter methylation in melanoma. *Genes, Chromosomes Cancer*. 2009; 48:10–21. [PubMed: 18803327]
43. Mardin WA, Petrov KO, Enns A, Senninger N, Haier J, Mees ST. SERPINB5 and AKAP12-expression and promoter methylation of metastasis suppressor genes in pancreatic ductal adenocarcinoma. *BMC Cancer*. 2010; 10:549. [PubMed: 20939879]
44. Hayashi M, Nomoto S, Kanda M, Okamura Y, Nishikawa Y, Yamada S, Fujii T, Sugimoto H, Takeda S, Kodera Y. Identification of the A-kinase anchor protein 12 (AKAP12) gene as a candidate tumor suppressor of hepatocellular carcinoma. *J Surg Oncol*. 2012; 105:381–6. [PubMed: 22052684]
45. Goeppert B, Schmidt CR, Geiselhart L, Dutruel C, Capper D, Renner M, Vogel MN, Zachskorn C, Zinke J, Campos B, Schmezer P, Popanda O, Wick W, Weller M, Meyermann R, Schittenhelm J, Harter PN, Simon P, Weichert W, Schirmacher P, Plass C, Mittelbronn M. Differential expression of the tumor suppressor A-kinase anchor protein 12 in human diffuse and pilocytic astrocytomas is regulated by promoter methylation. *J Neuropathol Exp Neurol*. 2013; 72:933–41. [PubMed: 24042196]
46. Chappell NP, Teng PN, Hood BL, Wang G, Darcy KM, Hamilton CA, Maxwell GL, Conrads TP. Mitochondrial proteomic analysis of cisplatin resistance in ovarian cancer. *J Proteome Res*. 2012; 11:4605–14. [PubMed: 22900918]
47. Chen X, Zhang J, Zhang Z, Li H, Cheng W, Liu J. Cancer stem cells, epithelial-mesenchymal transition and drug resistance in high grade ovarian serous carcinoma. *Hum Pathol*. 2013; 44:2373–84. [PubMed: 23850493]
48. Higgins DF, Lappin DW, Kieran NE, Anders HJ, Watson RW, Strutz F, Schlondorff D, Haase VH, Fitzpatrick JM, Godson C, Brady HR. DNA oligonucleotide microarray technology identifies fisp-12 among other potential fibrogenic genes following murine unilateral ureteral obstruction (UUO): modulation during epithelial-mesenchymal transition. *Kidney Int*. 2003; 64:2079–91. [PubMed: 14633130]

49. Borley J, Wilhelm-Benartzi C, Brown R, Ghaem-Maghami S. Does tumour biology determine surgical success in the treatment of epithelial ovarian cancer? A systematic literature review. *Br J Cancer*. 2012; 107:1069–74. [PubMed: 22935582]
50. Rieger M, Wei W, Waldron L, Culhane AC, Trippa L, Oliva E, Kim SH, Michor F, Huttenhower C, Parmigiani G, Birrer MJ. Risk prediction for late-stage ovarian cancer by meta-analysis of 1525 patient samples. *J Natl Cancer Inst*. 2014; 106
51. Verhaak RG, Tamayo P, Yang JY, Hubbard D, Zhang H, Creighton CJ, Feraday S, Lawrence M, Carter SL, Mermel CH, Kostic AD, Etemadmoghadam D, Saksena G, Cibulskis K, Duraisamy S, Levanon K, Sougnez C, Tsherniak A, Gomez S, Onofrio R, Gabriel S, Chin L, Zhang N, Spellman PT, Zhang Y, Akbani R, Hoadley KA, Kahn A, Kobel M, Huntsman D, Soslow RA, Defazio A, Birrer MJ, Gray JW, Weinstein JN, Bowtell DD, Drapkin R, Mesirov JP, Getz G, Levine DA, Meyerson M. Cancer Genome Atlas Research Network Prognostically relevant gene signatures of high grade serous ovarian carcinoma. *J Clin Invest*. 2013; 123:517–25. [PubMed: 23257362]

Figure 1. Comparative analyses of taxol-resistant OV90-TR1 and E3 cells with taxol-sensitive OV90 cell secretomes. (A) Heatmap representing the 81 proteins identified to be cosignificantly altered in taxol-resistant (OV90-TR1/E3) vs -sensitive (OV90) cells (q -value < 0.05). (B) Top five Disease and Bio Functions enriched in proteins cosignificant between OV90-TR1 and E3 taxol resistant cells. N = number of proteins associated with functional category (Ingenuity Pathway Analysis).

Figure 2. Elevated AKAP12 gene expression is associated with poor overall and poor progression-free survival in ovarian cancer patients. (A) Overall survival (OS): Discovery Affymetrix (multivariate hazard ratio [HR] = 1.130, p -value = 0.015, n = 545), (B) OS: Validation 1 Affymetrix (multivariate HR = 1.183, p -value = 0.007, n = 638), (C) progression-free survival (PFS): Discovery Affymetrix (multivariate HR = 1.343, p -value = 0.019, n = 510), and (D) PFS: Validation 1 Affymetrix (multivariate HR = 1.465, p -value = 0.032, n = 213).

Figure 3. AKAP12 is elevated in the secretomes of taxol-resistant ovarian cancer cells. AKAP12 levels was assessed in equivalent amounts of total secretome protein harvested from two syngeneic cells line models (CaOV3-TRI and OV90-TRI) and one primary model of taxol-resistant ovarian cancer (E3). Total protein detailed for loading reference (Stain-Free gel, BioRad). AKAP12 peptide spectral matches (PSM) derived from global proteomic analyses.

Figure 4. AKAP12 mRNA and total protein is elevated in taxol-resistant ovarian cancer cells. A. AKAP12 mRNA was assessed by qPCR analysis of equivalent amounts of total RNA harvested from two syngeneic cell line models (CaOV3-TRI and OV90-TRI) and one primary model of taxol-resistant ovarian cancer (E3). Data reflects taxol-resistant versus sensitive fold-changes and triplicate technical replicate delta-Ct values of AKAP12 normalized to 18S rRNA * $P < 0.05$; Student's *T*-Test B. AKAP12 protein was assessed in equivalent amounts of total cell lysates by immunoblot analysis. GAPDH levels were utilized as the loading control.

Figure 5. Elevated AKAP12 expression correlates with methylation status. A. Categorized analysis of AKAP12 Copy Number Alterations (CNA, Log2) and AKAP12 methylation status versus AKAP12 Affymetrix mRNA level ($n = 533$ patients) ($P < 0.05$; ANOVA T -test). B. AKAP12 gene expression is elevated following 5-Aza-2'-deoxycytidine treatment for 48 hours in taxol-sensitive (OV90) versus resistant (OV90-TR1) ovarian cancer cells. Data reflects two biological replicates and triplicate technical replicates. $*P < 0.05$; Student's T -Test.

Table 1
Public Gene Expression Data Cohorts

cohort	citation	data set	data platform	cases
Discovery	TCGA Research Network 2011 ²⁸	TCGA	Affymetrix HT U133a	545 ^a
			Illumina RNASeqV2	251 ^a
			Infinium Methylation27 BeadChip	544
			ABI exome sequencing/mutation calling	536
			Affymetrix SNP6 Copy number	534
Validation 1	Bild 2007 ²⁵	GSE3149	Affymetrix U133a GPL96	116
	Bonome 2008 ²³	GSE26712	Affymetrix U133a GPL96	185
	Denkert 2009 ²⁴	GSE14764	Affymetrix U133a GPL96	68
	Tothill 2008 ²⁷	GSE9891	Affymetrix U133 GPL96	216
	Mok 2009 ²⁶	GSE18S20	Affymetrix U133 GPL96	53
Validation 2	Crijns 2009 ²⁹	GSE13876	Operon Human v3 GPL77S9	157

^aN = 155 with Affymetrix HT U133a, Illumina RNASeqV2, clinical, and outcome data.

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