A Gene Signature Predicting for Survival in Suboptimally Debulked Patients with Ovarian Cancer

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Abstract

Despite the existence of morphologically indistinguishable disease, patients with advanced ovarian tumors display a broad range of survival end points. We hypothesize that gene expression profiling can identify a prognostic signature accounting for these distinct clinical outcomes. To resolve survival-associated loci, gene expression profiling was completed for an extensive set of 185 (90 optimal/95 suboptimal) primary ovarian tumors using the Affymetrix human U133A microarray. Cox regression analysis identified probe sets associated with survival in optimally and suboptimally debulked tumor sets at a *P* value of <0.01. Leave-one-out cross-validation was applied to each tumor cohort and confirmed by a permutation test. External validation was conducted by applying the gene signature to a publicly available array database of expression profiles of advanced stage suboptimally debulked tumors. The prognostic signature successfully classified the tumors according to survival for suboptimally (*P* = 0.0179) but not optimally debulked (*P* = 0.144) patients. The suboptimal gene signature was validated using the independent set of tumors (odds ratio, 8.75; *P* = 0.0146). To elucidate signaling events amenable to therapeutic intervention in suboptimally debulked patients, pathway analysis was completed for the top 57 survival-associated probe sets. For suboptimally debulked patients, confirmation of the predictive gene signature supports the existence of a clinically relevant predictor, as well as the possibility of novel therapeutic opportunities. Ultimately, the prognostic classifier defined for suboptimally debulked tumors may aid in the classification and enhancement of patient outcome for this high-risk population. [Cancer Res 2008;68(13):5478–86]

Introduction

Ovarian cancer is the fifth most common form of cancer in women in the United States, accounting for 26% of those cases occurring in the female genital tract. It is estimated that 15,280 deaths will result from ovarian cancer in the year 2007, making it the most lethal of all gynecologic cancers (1). Of these deaths, the vast majority (60%) will be attributed to serous carcinoma of the surface epithelium (2). Most patients will present at an advanced stage with metastases present beyond the ovaries precluding curative treatment (3). Standard disease management includes complete cytoreduction followed by administration of platinum- and taxane-based chemotherapy (4). Despite the fact that 80% of advanced stage papillary serous ovarian cancers (stages III/IV) initially respond to primary treatment with surgery and chemotherapy, most recur with a drug resistant phenotype. In contrast, subsets of patients with clinically and morphologically indistinguishable disease, develop a more chronic form of ovarian cancer, and may survive 5 or more years with treatment. This observation suggests that patients with aggressive disease have a biologically different disease from patients that display more indolent forms of ovarian cancer. Consequently, there is a critical need for diagnostic classifiers that can reliably stratify patients for therapy, as well as novel targets for therapeutic intervention.

It has been shown that high-density transcription profiling can identify differentially expressed genes and establish molecular signatures in numerous biological systems including ovarian cancer (5–9). Recent efforts to derive clinical predictors for survival in ovarian cancer from gene expression data have focused on discrete patient groups clustered at either end of the survival spectrum (10–12). We hypothesize that correlating survival, as a continuous variable, with gene expression will provide a predictive signature for advanced stage serous ovarian cancer patients who are likely to develop aggressive disease.

In this study, we describe an analysis of a series of advanced stage, high-grade ovarian cancer specimens using the Affymetrix human U133A GeneChip oligonucleotide expression array to identify a prognostic gene signature correlating with survival. To account for the effect of debulking status on survival, we assessed optimally and suboptimally debulked tumors independently. Validation of the gene signature included reverse transcription-PCR (RT-PCR) of specific genes and successful application of the signature to an external publicly available set of gene array data on similar patients. Finally, pathway analysis of the expression data identified survival-associated signaling cascades underlying the prognostic signature. Together, these data have defined a prognostic signature relevant for suboptimally debulked ovarian cancer patients, and identified candidate genes whose expression may mediate survival in this high-risk subpopulation.

Materials and Methods

Tissue specimens. Tumor specimens were obtained from 185 previously untreated late-stage (III–IV) high-grade (2, 3) ovarian cancer patients hospitalized at the Memorial Sloan-Kettering Cancer Center between 1990 and 2003. Optimally debulked was defined as the removal of all tumor nodules >1 cm in maximal dimension. All specimens consisted of ≥80% tumor and were reviewed for histology and tumor grade. A set of 10 normal

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

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ovarian surface epithelium (OSE) cytobrushing specimens was also obtained as previously described (6). All specimens, and their corresponding clinical information, were collected under protocols approved by the institutional review boards of the institution.

Total RNA isolation and amplification for Affymetrix GeneChip hybridization and image acquisition. Total RNA was subsequently isolated using the RNeasy Micro kit (Qiagen), quantified, and checked for quality with a Bioanalyzer 2100 system (Agilent) before further manipulation. Sufficient labeled amplified RNA (aRNA) for microarray analysis was generated from tumor or OSE-derived total RNA using a single round of amplification as previously described (13). Array images were acquired using a GeneChip Scanner 3000 (Affymetrix). All microarray experiment was done in a single laboratory.

Data normalization and survival association. Robust Multi-Array pre-processing was completed for all 185 tumor and 10 OSE arrays under consideration using the Bioconductor suite of array analysis tools running in R version 2.0.1 (R Development Core Team, 2004). To establish whether gene expression was associated with survival, a univariate Cox proportional hazards model (Wald statistic) was fit for each gene to test for an association between gene expression and survival ($P < 0.01$). The resulting list underwent leave-one-out cross-validation (LOOCV) and permutation analysis with a log-rank test to confirm the utility of the list as described in the Supplementary Methods.

Hierarchical clustering. Hierarchical clustering of the suboptimally debulked patients used gene expression data from the corresponding survival signature. Both specimens and genes were clustered with a 1-correlation metric using average linkage in R.

Quantitative RT-PCR validation. Quantitative RT-PCR (qRT-PCR) was performed on 50 ng of total RNA from 30 suboptimally debulked specimens using primer sets specific for 19 select genes and the house keeping genes *GAPDH, GUSB*, and *cyclophillin* as previously described (13). Primer sequences can be seen in Supplementary Table S4.

External validation. To show the predictive ability of the gene signature, a predictor was built by our entire expression profile data of 95 suboptimally debulked tumors and validated by the gene expression profiles of 29 ovarian cancer patients with suboptimally debulked tumors (10). Using the signature of 572 genes associated with survival ($P < 0.01$) in the training set, patients in the validation set with compound covariate score above the median value obtained in the training set were classified into a poor-prognosis group, whereas those below the median were classified into the good-prognosis group. Because the training and validation sets were generated in different institutions, to make the data comparable with each other, the expression measurements for each probe set in each data set were median centered. We evaluated the predictive power of the prognosis profile using univariate and multivariate statistical analyses. For the univariate analysis, the odds ratio for long survival in the group with a good-prognosis signature, relative to the group with a poor-prognosis signature was estimated. For the multivariate analysis, logistic regression analysis was used to adjust the association between survival (short versus long) and prognosis signature for other clinical variables.

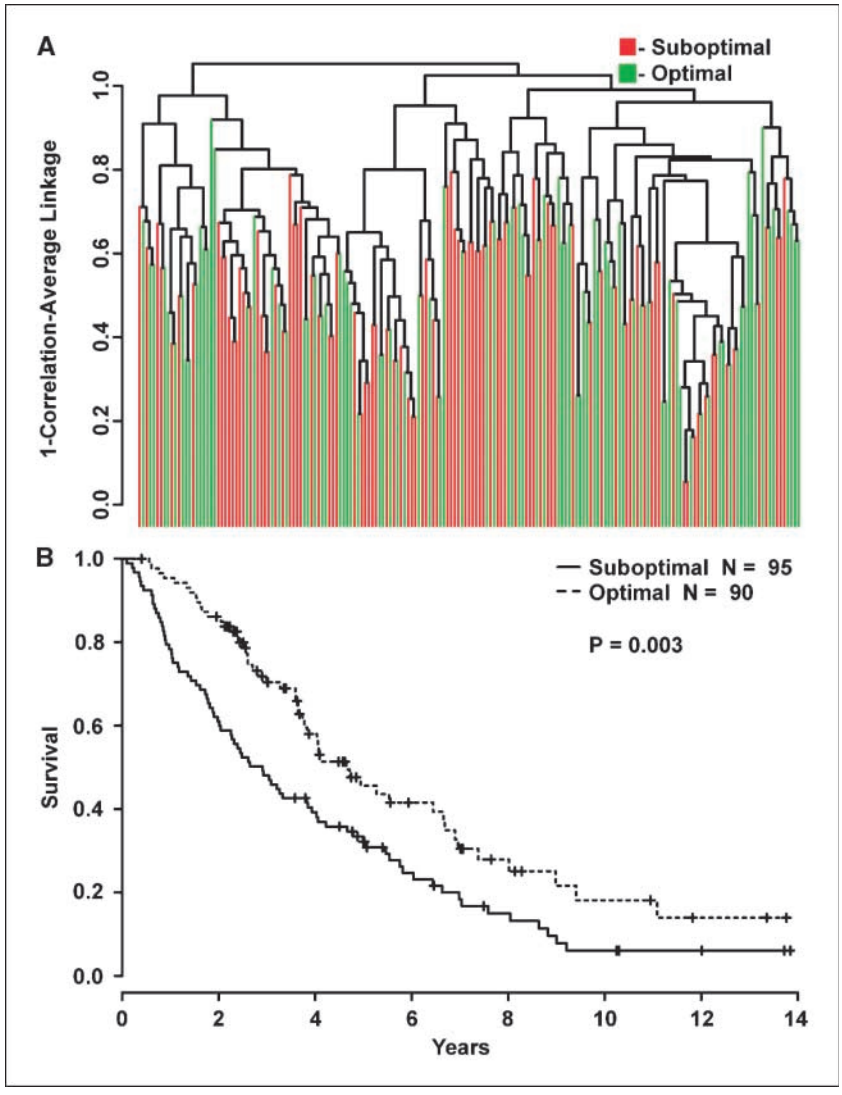

Figure 1. Association between debulking status and patient survival. *A,* hierarchical clustering of all 185 specimens using 1-correlation with average linkage for probe sets possessing a variance in the top two thirds. *B,* overall survival was analyzed relative to debulking status for all 185 tumors using a Kaplan-Meier survival curve.

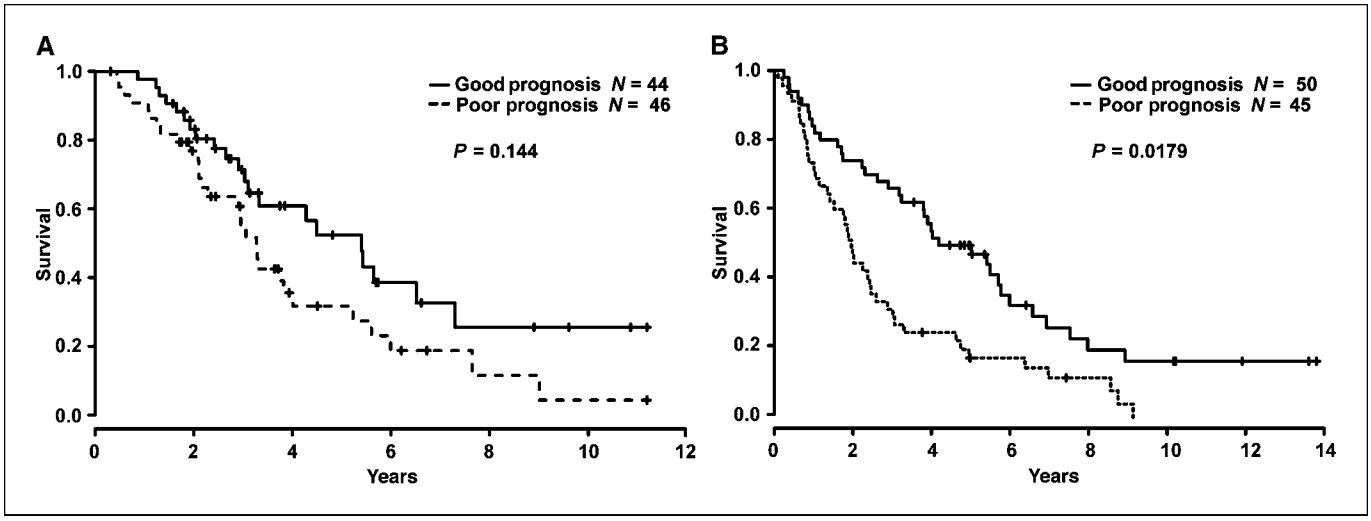

Figure 2. Identification and validation of gene signatures correlating with survival in papillary serous ovarian cancer patients. *A,* for optimally debulked tumors, the predictive classifier unsuccessfully classified tumors according to survival ($P_{permutation}$ = 0.144). *B,* in contrast, significant association was found for suboptimally, debulked patients ($P_{permutation}$ = 0.0179).

Class comparison and pathway analyses. Differentially expressed genes were identified for the 95 suboptimally debulked tumor and OSE specimens using a multivariate permutation test in BRB-ArrayTools version 3.2.2 software developed by Dr. Richard Simon and Amy Peng Lam of the Biometrics Research Branch of the National Cancer Institute. A random variance *t* test using 2,000 permutations was completed to identify the list of probe sets containing fewer than 10 false positives at a confidence of 95%. Differential expression was considered significant at a *P* value of <0.001.

To identify coregulated pathways affecting the survival of suboptimally debulked patients, lists containing differentially regulated probe sets in tumor versus OSE and identifiers highlighting survival associated genes were analyzed using PathwayStudio version 4.0 software (Ariadne Genomics).

Results

Clinical characterization of the tumor isolates. A total of 185 stage III, high-grade primary tumor specimens were obtained from patients with papillary serous tumors of the ovary whose survival spanned a spectrum of 13.6 years. With a median age of 64 years (range, 26–85 years), the median survival time was 2.7 years postsurgery. The overall response rate was 83% with all but one patient receiving a platinum-based primary chemotherapy (see Supplementary Table S1). Partitioning the sample according to debulking status yielded 90 optimally and 95 suboptimally debulked tumors with a median survival time of 3.5 and 2.2 years, respectively. Of the patients with optimally and suboptimally debulked tumors, 39 and 17 were still alive at the time of analysis, respectively.

Total RNA was isolated from these specimens, amplified, and hybridized to Affymetrix human U133A GeneChip oligonucleotide microarrays. The resulting microarray signal intensities for all 22,823 probe sets were normalized. To assess whether a prominent survival signature was evidenced in the array data, hierarchical clustering was conducted for probe sets possessing a variance in the top third across the specimens (Fig. 1*A*). From this analysis, distinct clustering based on survival (see Supplementary Fig. S1) or debulking status was not observed for the 185 tumors. Further analysis using additional filtering criteria showed a similar result (see Supplementary figure). Because debulking status is a robust predictor for prognosis in ovarian cancer, its contribution to survival was also evaluated. Generating a Kaplan-Meier plot distinguishing optimally from suboptimally debulked tumors yielded a significant difference ($P < 0.003$) in survival for the two groups by log-rank test (Fig. 1*B*). Given the significant contribution of debulking to outcome, the tumors were stratified according to debulking status for downstream analyses.

Derivation and validation of a gene signature predictive for survival in patients with papillary serous ovarian cancer. Among the optimally debulked tumors, 263 probe sets (Supplementary Table S2) were significantly associated with survival ($P < 0.01$; false discovery rate, 0.84) based on Cox regression analysis. Evaluation of the predictor using a compound covariate algorithm failed to reliably distinguish good from poor-prognosis patients during permutation of the LOOCV analysis ($P_{permutation}$ = 0.144; Fig. 2*A*). For suboptimally debulked patients, 572 survival-associated probe sets (Supplementary Table S3) were identified by Cox regression analysis ($P < 0.01$; false discovery rate, 0.38). LOOCV of the survival analysis successfully discriminated between the two prognosis groups and withstood permutation analysis (permutation P = 0.0179; Fig. 2*B*). Repeating the analysis with a multivariate Cox regression analysis after adjusting for age also yielded a significant association (P = 0.03). Together, these data supports the existence of a prognostic gene expression signature for suboptimally debulked disease. Of note comparison of the gene list for the optimally debulked patients to the suboptimally debulked patients identified only a five-gene overlap, suggesting the effect of gene expression on survival is different in the two groups.

Expression profiling cannot distinguish between optimally and suboptimally debulked tumors. As presented above, hierarchical clustering did not reveal distinct clusters of optimal and suboptimal specimens. Supervised analysis of optimal and suboptimally debulked tumors revealed that of 22,283 probes, only 21 were differentially expressed between these two groups at the 0.001 significance level, which is less than what would be expected by chance alone. Furthermore, comparison of the survival gene lists

for the optimally debulked tumors to the suboptimally debulked tumors identified only a five-gene overlap, suggesting the effect of gene expression on survival is different in the two groups.

Validation of the suboptimal survival signature. To validate the suboptimal survival signature, qRT-PCR was performed for 30 total RNA samples, which were included in the microarray analysis, using gene-specific primers for 19 randomly selected genes. Assayable expression levels for each gene were obtained and correlated to the corresponding microarray signal intensities. There was favorable agreement between the microarray and qRT-PCR data by Pearson correlation in 17 of 19 genes (Supplementary Figs. S2 and S3; Fig. 3).

To provide an additional level of validation, we tested our gene signature against an independent set of expression arrays from suboptimally debulked patients. The database from this study was publicly available (10). Although this study dichotomized survival and did not provide the specific survival time, we hypothesize that our gene list should still be able to distinguish "long" survivors from "short" survivors. Our 572-gene signature for the suboptimally debulked tumors is associated with survival in the validation set ($P = 0.0149$, Pearson's χ^2test). The crosstabulation of prognosis and survival showed that 7 of 10 patients with long survival were classified as good prognosis, whereas 15 of 19 of patients with short survival were classified as poor prognosis by our gene signature. The estimated odds ratio for long survival in the group with a good prognosis relative to the group with poor prognosis was 8.75. The odds ratio remains significant ($P = 0.0146$) after adjusting for age and tumor grade.

Identification of signaling events affecting suboptimally debulked patient survival. To ascertain whether subsets of the survival-associated genes might be participating in coordinated signaling pathway(s) contributing to patient outcome in suboptimally debulked patients, highly correlated probe sets ($P < 0.001$) identified during Cox regression analysis were visualized by hierarchical clustering (Table 1). The 57 probe sets clustered the 95 suboptimally debulked specimens according to survival (Fig. 4*A*; $P = 0.001$). As detailed in Fig. 4*B*, the prognostic expression signature partitioned the tumors with good-prognosis patients clustering in group A and those with a poor prognosis in group B. In addition, the majority of living patients resided in group A (12 versus 5; $P < .05$).

Utilizing this subset of genes, a biological association network (BAN) describing the minimum number of connections to join all of the survival-associated probe sets was determined. Of the 57 probe sets under consideration, only 38 possessed at least one documented interaction in the database. To place the pathway in a

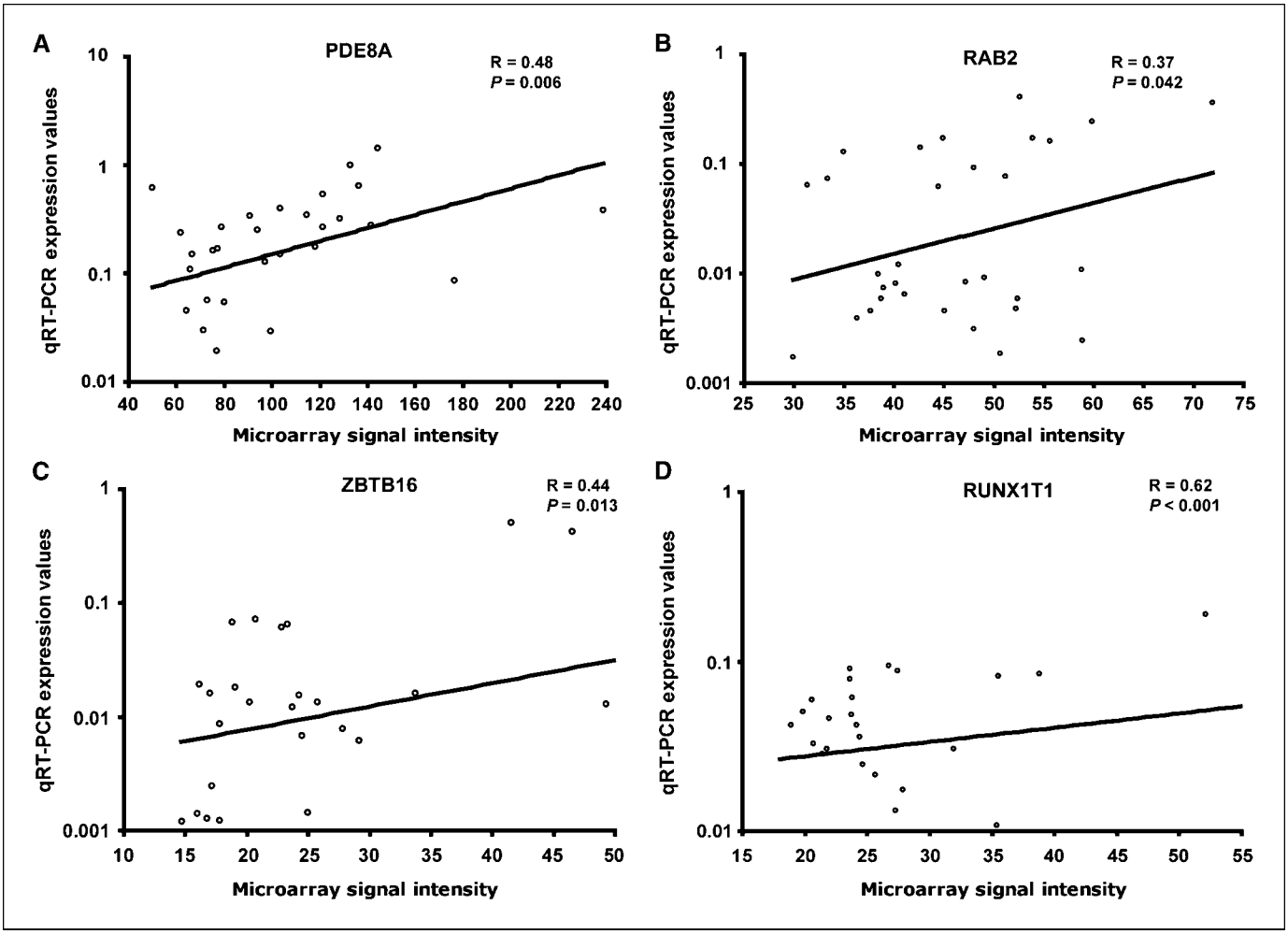

Figure 3. Validation of select survival-associated genes identified in suboptimally debulked patients. Microarray signal intensity values were confirmed using qRT-PCR for a subset of 30 patients by Pearson correlation for (*A*) *PDE8A,* (*B*) *RAB2,* (*C*) *ZBTB16,* and (*D*) *RUNX1T1*.

Table 1. Top survival-associated probe sets ($P < 0.001$) identified for suboptimally debulked patients by Cox regression analysis

Cox score coefficient	Probe set	Map	Gene	UniGene ID	Entrez ID
2	210078_s_at	3q26.1	*KCNAB1*	Hs.157818	7881
1.7	205978_at	13q12	*KL*	Hs.524953	9365
1.5	214152_at	15q21–q22	*PIGB*	Hs.126115	9488
1.5	208733_at	8q12.1	*RAB2*	Hs.369017	5862
1.3	213786_at	7p15	*TAX1BP1*	Hs.34576	8887
1.2	212522_at	15q25.3	*PDE8A*	Hs.9333	5151
1.1	203355_s_at	8pter–p23.3	*PSD3*	Hs.434255	23362
1.1	213894_at	7p21.3	*KIAA0960*	Hs.120855	23249
1.1	219427_at	4q28.1	*FAT4*	Hs.269121	79633
1.1	218306_s_at	15q22	*HERC1*	Hs.210385	8925
1	218878_s_at	10q21.3	*SIRT1*	Hs.369779	23411
1	211698_at	15q21.1–q21.2	*CRI1*	Hs.255973	23741
1	200685_at	1p31	*SFRS11*	Hs.479693	9295
0.9	214151_s_at	15q21–q22	*PIGB*	Hs.126115	9488
0.9	201535_at	13q12–q13	*UBL3*	Hs.145575	5412
0.8	202034_x_at	8p22–q21.13	*RB1CC1*	Hs.196102	9821
0.8	203970_s_at	6q23–q24	*PEX3*	Hs.7277	8504
0.8	218421_at	22q13.31	*CERK*	Hs.200668	64781
0.7	203049_s_at	5q15	*KIAA0372*	Hs.482868	9652
0.7	217731_s_at	13q14.3	*ITM2B*	Hs.446450	9445
0.7	205529_s_at	8q22	*RUNX1T1*	Hs.368431	862
0.6	208732_at	8q12.1	*RAB2*	Hs.369017	5862
0.6	208070_s_at	6q21	*REV3L*	Hs.232021	5980
0.6	212327_at	4p13	*KIAA1102*	Hs.335163	22998
0.6	205407_at	9p13–p12	*RECK*	Hs.388918	8434
0.6	206167_s_at	Xp22.3	*ARHGAP6*	Hs.435291	395
0.5	201916_s_at	6q21	*SEC63*	Hs.529957	11231
0.5	205883_at	11q23.1	*ZBTB16*	Hs.171299	7704
0.4	202242_at	Xp11.4	*TSPAN7*	Hs.441664	7102
0.4	201843_s_at	2p16	*EFEMP1*	Hs.76224	2202
0.4	204237_at	2q32.3–q33	*GULP1*	Hs.470887	51454
0.4	209894_at	1p31	*LEPR*	Hs.23581	54741
0.3	211959_at	2q33–q36	*IGFBP5*	Hs.369982	3488
−0.9	218908_at	17q25	*ASPSCR1*	Hs.298351	79058
−0.9	218473_s_at	19p13.11	*GLT25D1*	Hs.418795	79709
−1	211084_x_at	2p21	*PRKD3*	Hs.173536	23683
−1	221649_s_at	19p13	*PPAN*	Hs.14468	56342
−1	213669_at	19p13.11	*FCHO1*	Hs.96485	23149
−1	209831_x_at	19p13.2	*DNASE2*	Hs.118243	1777
−1.1	206723_s_at	19p12	*EDG4*	Hs.122575	9170
−1.1	221256_s_at	9q32	*HDHD3*	Hs.7739	81932
−1.1	216821_at	12q13	*KRT8*	Hs.307126	3856
−1.2	218161_s_at	15q23	*CLN6*	Hs.554828	54982
−1.2	218590_at	10q23.3–24.3	*PEO1*	Hs.22678	56652
−1.2	206722_s_at	19p12	*EDG4*	Hs.122575	9170
−1.2	216684_s_at	18q11.2	*SS18*	Hs.404263	6760
−1.2	216397_s_at	8q24.3	*BOP1*	Hs.535901	23246
−1.2	201679_at	7q21	*ARS2*	Hs.111801	51593
−1.3	219021_at	11q13.4	*RNF121*	Hs.368554	55298
−1.3	221941_at	10q26.3	*PAOX*	Hs.532469	196743
−1.4	215728_s_at	1p36.31–p36.11	*BACH*	Hs.126137	11332
−1.4	207484_s_at	6p21.31	*EHMT2*	Hs.520038	10919
−1.5	202848_s_at	5q35	*GRK6*	Hs.235116	2870
−1.6	215869_at	9q31.1	*ABCA1*	Hs.429294	19
−1.8	216855_s_at	1q44	*HNRPU*	Hs.166463	3192
−2	211363_s_at	9p21	*MTAP*	Hs.193268	4507
−3.3	208547_at	6p21.3	*HIST1H2BB*	Hs.553494	3018

disease-specific context, the 95 suboptimally debulked tumor specimens were compared with 10 normal OSE brushings analyzed with the identical Affymetrix array platform. This identified a total of 3341 differentially regulated probe sets. Overlaying differential gene expression data onto the BAN identified subsets of coregulated pathways implicated in cell proliferation, motility, apoptosis, chemoresistance, secretion, and chromatin maintenance with distinct sets of the genes associating with discrete patient groups when clustering probe set expression (Fig. 5*A*). As highlighted in Fig. 5*B*, survival signature genes *KL, SIRT1, RAB2, IGFBP5, REV3L, LEPR, MTAP, DNASE2,* and *BAT8* featured prominently in these processes.

Discussion

Transcription profiling has been used to derive molecular signatures associated with ovarian cancer (6, 9, 13, 14). However, only a few methods have been proposed to analyze survival outcome using microarray data. The first approach uses hierarchical clustering complemented by an assessment of survival distributions among clusters using traditional approaches, e.g., log-rank test (5, 15). However, our samples were homegenous in relation to histology, grade, and stage. Furthermore, subgroups in this histotype have not been reported precluding use of a cluster-based strategy. The second genre of analysis attempts to reduce data dimensionality by clustering gene expression including partial least squares or support vector machines (16, 17). These methods use a combination of all the genes to predict survival adding noise to the model because the vast majority of the genes should be unrelated to survival impeding the identification of genes that are the optimal predictors. Indeed, studies have shown that using only a subset of genes generally performs better than using all genes. Recent work by Berchuck and colleagues, as well as other groups (10–12), has used this type of approach to identify genes associated with long-term survival in serous ovarian cancer by comparing patient groups at opposing ends of the survival spectrum. However, it remains unclear whether these predictors are applicable to the majority of patients who will eventually die at an intermediate time point. In addition, their algorithms did not account for censoring and/or debulking status.

To overcome these limitations, our approach relied upon a Cox proportional hazards model evaluating survival as a continuous variable relative to gene expression (18). In this way, top survival-associated genes were easily selected for analysis with a compound covariate predictor and evaluated as a complex multigene signature applicable to a broad range of survival end points. LOOCV combined with a permutation test was applied across each set of tumors to estimate the prediction error. This approach

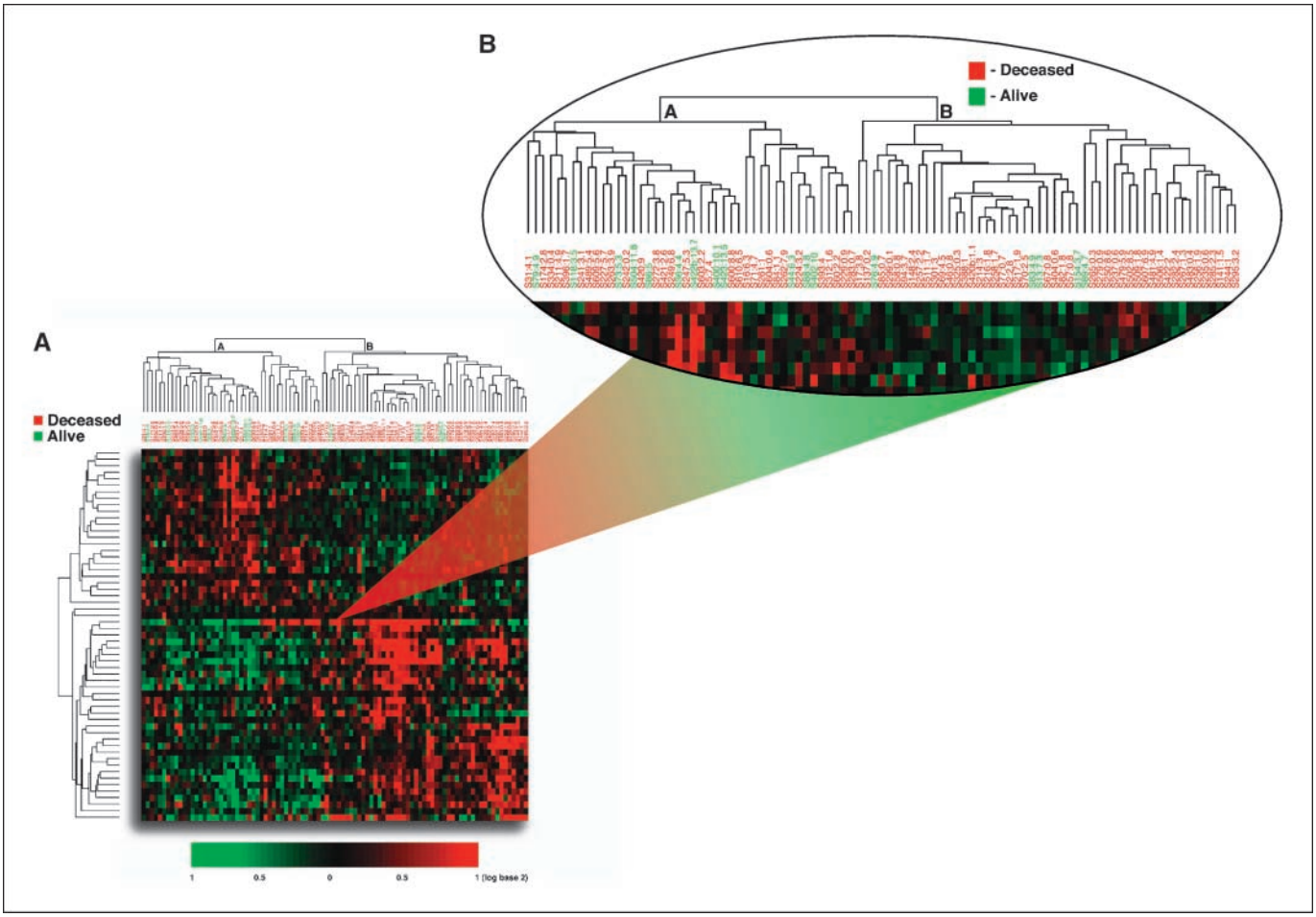

Figure 4. Clustering of highly correlated survival-associated probe sets in suboptimally debulked patients. *A,* median-centered expression data for the top 57 probe sets (*y* axis) associated with survival by Cox regression analysis ($P < 0.001$; *red,* overexpression; *green,* underexpression). *B,* the majority of living patients (12 versus 5; $P < 0.05$, χ^2) clustered with deceased patients possessing extended survival times (y) in group A (*x* axis; $P = 0.005$, Student's *t* test).

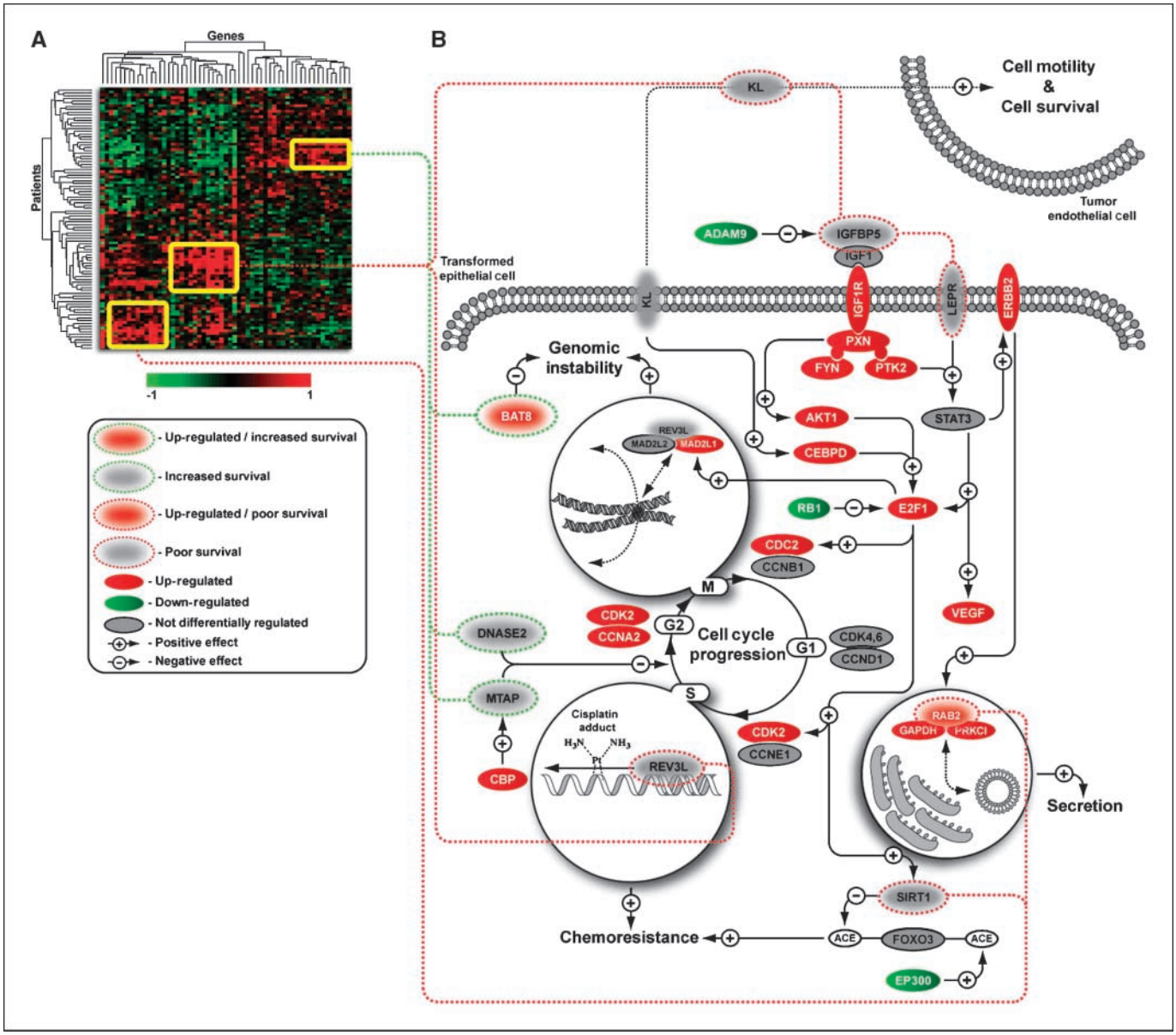

Figure 5. Assessment of putative signaling events contributing to patient survival in suboptimally debulked patients. *A,* 1-correlation clustering with average linkage of the 57 survival-associated genes identified several patient subgroups with discrete sets of coregulated genes. *B,* pathway analysis of the prognostic signature identified signaling events implicated in cell proliferation, motility, apoptosis, chemoresistance, secretion, and chromatin maintenance.

ensured the classifier was properly validated, and eliminated any bias associated with partitioning specimens into arbitrary training and validation cohorts (19). A recent retrospective analysis of publicly available microarray data sets has shown that in all but the largest studies random allocation of patients into test and validation groups may result in incorrect prediction error estimates (20).

To fully validate our signature, we tested it against an independent set of expression arrays from suboptimally debulked patients (10). The gene signature was statistically significantly associated with survival in this external validation set despite the fact that these cases had been dichotomized into short and long survivors. We think this reflects the robustness of the signature and its broad applicability.

Although this algorithm successfully identified a validated signature for suboptimal tumors, it was unable to do so for optimally debulked patients. This discrepancy is not a limitation of the statistical approach chosen here but rather a direct effect of the censoring status for a large proportion of the optimally debulked patients (43.3%). Because optimal debulking is generally associated with increased survival, a considerable number of patients were alive at the time of the analysis diminishing its power. Our decision to stratify based on debulking status resulted from the significant effect of this feature on patient outcome. Interestingly, direct comparison of these two groups of patients did not yield a statistically significant gene list. This is consistent with the inability of clustering to distinguish these groups of tumors. This striking finding does not diminish the important clinical effect of debulking but suggests possible mechanisms by which debulking improves survival. The clinical effect of debulking could relate to the surgical procedure itself either directly to tumor tissue removed or

indirectly via an inflammatory/immune response rather than any biological difference between these sets of tumors. Conversely, it is possible that there are not sufficient numbers of specimens in this study to identify subtle gene expression differences among these two sets of tumors.

When comparing the suboptimal survival signature to previous analyses relating gene expression to survival in the context of ovarian cancer, overlap in the prognostic lists was not observed (10–12). Indeed, even among these earlier studies, which all derived their predictors by evaluating short- and long-term survivors, coexpressed genes were not evidenced. These discrepancies are likely related to the algorithm(s) selected for derivation of the signature. For this evaluation, the application of Cox regression analysis to identify genes correlating with survival across a range of distinct survival end points precludes direct comparison to these prior studies.

By using pathway analysis software to identify signaling events in ovarian tumor specimens, we applied our classifier to an extensive database of tumor versus normal to identify major tumor-specific signaling pathways (13). Using this approach, we identified alterations in pathways modulating cell proliferation, motility, chemoresistance, secretion, apoptosis, and chromatin maintenance as prominent survival associated events. For instance, *IGFBP5* is significantly up-regulated in ovarian cancer. Insulin-like growth factor (IGF)-1 is a potent mitogenic factor in both tumor tissue (21, 22) and is modulated by IGF-binding protein (IGFBP) family members. Interestingly, *IGFBP5* has been identified in a poor-prognostic gene expression signature in breast cancer, and its overexpression has been implicated in tumor metastasis in patients with the disease (23, 24). Another example is a pathway involving *E2F1* expression. *E2F1* expression is stimulated by Klotho (KL) through CEBPD-mediated transcription (25). Together with activated AKT signaling, increased expression of *E2F1* by KL may promote chemoresistance reducing patient survival. E2F1 induces expression the of *SIRT1,* which permits tumor cells to overcome the affects of toxic stress caused by chemotherapeutic agents through deacetylation of FOXO survival proteins (26, 27). E2F1 also induces aberrant expression of *MAD2,* a component of the centromere during cell division, leading to deleterious chromosomal division (28). Furthermore, MAD2 complexes with REV3L a DNA repair polymerase that retains a high degree of processivity in the presence of cisplatin-induced adducts linking the spindle assembly checkpoint with DNA repair (29, 30). These alterations in *KL, SIRT1,* and *REV3L* expression may allow ovarian cancer cell to overcome cellular stress and the cytotoxic effects associated with chemotherapeutic treatment, which in turn may be critical determinants in the poor outcome of these patients.

Among suboptimally debulked patients with a good prognosis, antiproliferative, antiapoptotic, and chromatin maintenance signaling events were evidenced. MTAP is a key enzyme in the methionine salvage pathway, which resides in a genomic region that is frequently deleted in primary cancers and cell lines (31, 32). Also exerting an antiproliferative effect are G9A together with DNMT1, which ensure chromatin methylation patterns are perpetuated during cell divisions stabilizing the genome (33, 34). Despite a cellular environment that is conducive to cellular proliferation and genomic instability, differential regulation of key genes that mitigate these events may enhance patient survival.

This work identified a validated prognostic gene expression signature in suboptimally debulked patients with serous ovarian cancer. The expression of these genes significantly affects the survival of distinct patient groups by modulating tumor biology. The signaling events affected by many of these genes occur in pathways that are essential for tumor survival and expansion. These genes may serve as suitable targets alone or in combination for individualized therapeutic intervention. Ultimately, defining clinically relevant diagnostic signatures that are relevant across a broad spectrum of survival end points will not only facilitate management of the disease but will identify the optimal set of therapeutic targets.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin 2007;57:43–66.
2. Boring CC, Squires TS, Tong T, Montgomery S. Cancer statistics, 1994. CA Cancer J Clin 1994;44:7–26.
3. Boente MP, Hamilton TC, Godwin AK, et al. Early ovarian cancer: a review of its genetic and biologic factors, detection, and treatment. Curr Probl Cancer 1996;20:83–137.
4. Markman M, Bundy BN, Alberts DS, et al. Phase III trial of standard-dose intravenous cisplatin plus paclitaxel versus moderately high-dose carboplatin followed by intravenous paclitaxel and intraperitoneal cisplatin in small-volume stage III ovarian carcinoma: an intergroup study of the Gynecologic Oncology Group, Southwestern Oncology Group, and Eastern Cooperative Oncology Group. J Clin Oncol 2001;19:1001–7.
5. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000;403:503–11.
6. Bonome T, Lee JY, Park DC, et al. Expression profiling of serous low malignant potential, low-grade, and high-grade tumors of the ovary. Cancer Res 2005;65:10602–12.
7. DeRisi JL, Iyer VR, Brown PO. Exploring the metabolic and genetic control of gene expression on a genomic scale. Science 1997;278:680–6.
8. Golub TR, Slonim DK, Tamayo P, et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. Science 1999;286:531–7.
9. Zorn KK, Bonome T, Gangi L, et al. Gene expression profiles of serous, endometrioid, and clear cell subtypes of ovarian and endometrial cancer. Clin Cancer Res 2005;11:6422–30.
10. Berchuck A, Iversen ES, Lancaster JM, et al. Patterns of gene expression that characterize long-term survival in advanced stage serous ovarian cancers. Clin Cancer Res 2005;11:3686–96.
11. Lancaster JM, Dressman HK, Whitaker RS, et al. Gene expression patterns that characterize advanced stage serous ovarian cancers. J Soc Gynecol Investig 2004;11:51–9.
12. Spentzos D, Levine DA, Ramoni MF, et al. Gene expression signature with independent prognostic significance in epithelial ovarian cancer. J Clin Oncol 2004;22:4700–10.
13. Donninger H, Bonome T, Radonovich M, et al. Whole genome expression profiling of advance stage papillary serous ovarian cancer reveals activated pathways. Oncogene 2004;23:8065–77.
14. Wamunyokoli FW, Bonome T, Lee JY, et al. Expression profiling of mucinous tumors of the ovary identifies genes of clinicopathologic importance. Clin Cancer Res 2006;12:690–700.
15. Sorlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001;98:10869–74.
16. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2006. CA Cancer J Clin 2006;56:106–30.
17. Nguyen DV, Rocke DM. Partial least squares

proportional hazard regression for application to DNA microarray survival data. Bioinformatics 2002; 18:1625–32.
18. Cox DR. Regression models and life tables (with discussion). J R Stat Soc Series B 1972;34:248–75.
19. Dupuy A, Simon RM. Critical review of published microarray studies for cancer outcome and guidelines on statistical analysis and reporting. J Natl Cancer Inst 2007;99:147–57.
20. Molinaro AM, Simon R, Pfeiffer RM. Prediction error estimation: a comparison of resampling methods. Bioinformatics 2005;21:3301–7.
21. Adams TE, Epa VC, Garrett TP, Ward CW. Structure and function of the type 1 insulin-like growth factor receptor. Cell Mol Life Sci 2000;57:1050–93.
22. Grimberg A, Cohen P. Role of insulin-like growth factors and their binding proteins in growth control and carcinogenesis. J Cell Physiol 2000;183:1–9.
23. Hao X, Sun B, Hu L, et al. Differential gene and protein expression in primary breast malignancies and their lymph node metastases as revealed by combined cDNA microarray and tissue microarray analysis. Cancer 2004;100:1110–22.
24. van 't Veer LJ, Dai H, van de Vijver MJ, et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature 2002;415:530–6.
25. Chihara Y, Rakugi H, Ishikawa K, et al. Klotho protein promotes adipocyte differentiation. Endocrinology 2006; 147:3835–42.
26. Chu F, Chou PM, Zheng X, Mirkin BL, Rebbaa A. Control of multidrug resistance gene mdr1 and cancer resistance to chemotherapy by the longevity gene sirt1. Cancer Res 2005;65:10183–7.
27. Ford J, Jiang M, Milner J. Cancer-specific functions of SIRT1 enable human epithelial cancer cell growth and survival. Cancer Res 2005;65:10457–63.
28. Hernando E, Nahle Z, Juan G, et al. Rb inactivation promotes genomic instability by uncoupling cell cycle progression from mitotic control. Nature 2004;430: 797–802.
29. Murakumo Y, Roth T, Ishii H, et al. A human REV7 homolog that interacts with the polymerase ζ catalytic subunit hREV3 and the spindle assembly checkpoint protein hMAD2. J Biol Chem 2000;275:4391–7.
30. Wu F, Lin X, Okuda T, Howell SB. DNA polymerase ζ regulates cisplatin cytotoxicity, mutagenicity, and the rate of development of cisplatin resistance. Cancer Res 2004;64:8029–35.
31. Della Ragione F, Russo G, Oliva A, et al. 5′-Deoxy-5′-methylthioadenosine phosphorylase and p16INK4 deficiency in multiple tumor cell lines. Oncogene 1995;10: 827–33.
32. Stadler WM, Sherman J, Bohlander SK, et al. Homozygous deletions within chromosomal bands 9p21–22 in bladder cancer. Cancer Res 1994;54:2060–3.
33. Esteve PO, Chin HG, Smallwood A, et al. Direct interaction between DNMT1 and G9a coordinates DNA and histone methylation during replication. Genes Dev 2006;20:3089–103.
34. Rice JC, Briggs SD, Ueberheide B, et al. Histone methyltransferases direct different degrees of methylation to define distinct chromatin domains. Mol Cell 2003;12:1591–8.