Implementing an online tool for genome-wide validation of survival-associated biomarkers in ovarian-cancer using microarray data from 1287 patients

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Abstract

The validation of prognostic biomarkers in large independent patient cohorts is a major bottleneck in ovarian cancer research. We implemented an online tool to assess the prognostic value of the expression levels of all microarray-quantified genes in ovarian cancer patients. First, a database was set up using gene expression data and survival information of 1287 ovarian cancer patients downloaded from Gene Expression Omnibus and The Cancer Genome Atlas (Affymetrix HG-U133A, HG-U133A 2.0, and HG-U133 Plus 2.0 microarrays). After quality control and normalization, only probes present on all three Affymetrix platforms were retained ($n=22\,277$). To analyze the prognostic value of the selected gene, we divided the patients into two groups according to various quantile expressions of the gene. These groups were then compared using progression-free survival ($n=1090$) or overall survival ($n=1287$). A Kaplan–Meier survival plot was generated and significance was computed. The tool can be accessed online at www.kmplot.com/ovar. We used this integrative data analysis tool to validate the prognostic power of 37 biomarkers identified in the literature. Of these, *CA125* (*MUC16*; $P=3.7\times10^{-5}$, hazard ratio (HR)=1.4), *CDKN1B* ($P=5.4\times10^{-5}$, HR=1.4), *KLK6* ($P=0.002$, HR=0.79), *IFNG* ($P=0.004$, HR=0.81), *P16* ($P=0.02$, HR=0.66), and *BIRC5* ($P=0.00017$, HR=0.75) were associated with survival. The combination of several probe sets can further increase prediction efficiency. In summary, we developed a global online biomarker validation platform that mines all available microarray data to assess the prognostic power of 22 277 genes in 1287 ovarian cancer patients. We specifically used this tool to evaluate the effect of 37 previously published biomarkers on ovarian cancer prognosis.

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Introduction

With a mortality of 8.4 per 100 000 women, ovarian cancer is the most common cause of death among gynecological malignancies (http://seer.cancer.gov) with a 5-year survival rate of 10–30%. Relative to breast cancer, the molecular characteristics of epithelial ovarian cancer (EOC) are more heterogeneous. Despite extensive research, clinical–pathological factors including tumor stage, residual disease after surgery, histological type, and tumor grade are still the most important features related to patient outcome. To date, only two biomarkers have been approved by the Food and Drug Administration (FDA) for monitoring patients with EOC: *CA125* (*MUC16*; Gadducci *et al*. 1995, 2004, Cooper *et al*. 2002, Riedinger *et al*. 2006) and *HE4* (*WFDC2*; Huhtinen *et al*. 2009, Moore *et al*. 2009, 2010).

Several additional genes have been suggested as potential biomarkers for the progression of EOC. Low expression of p21 (Ferrandina *et al*. 2000, Plisiecka-Halasa *et al*. 2003, Bali *et al*. 2004), bax

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(Tai *et al*. 1998, Skirnisdottir *et al*. 2001), and hTERT (Brustmann 2005) and high expression of survivin (Sui *et al*. 2002), VEGFR (Hefler *et al*. 2006), p53 (Buttitta *et al*. 1997, Reles *et al*. 2001), human kallikrein 6 (Diamandis *et al*. 2003), human kallikrein 10 (Luo *et al*. 2001), Interleukin 6 (Scambia *et al*. 1995), p27 (Newcomb *et al*. 1999, Masciullo *et al*. 2000, Korkolopoulou *et al*. 2002, Schmider-Ross *et al*. 2006), cyclin D1 (Bali *et al*. 2004, Barbieri *et al*. 2004), cyclin D3 (Levidou *et al*. 2007), cyclin E (Sui *et al*. 2001, Farley *et al*. 2003, Rosen *et al*. 2006, Bedrosian *et al*. 2007), Bcl-xL (Materna *et al*. 2007), cIAP (Psyrri *et al*. 2006), and ERBB1 (Skirnisdottir *et al*. 2004, Psyrri *et al*. 2005) could represent prognostic variables for poor clinical outcome. In addition, the genome-wide investigation of adequate clinical cohorts delivers unprecedented amount of potential new biomarkers (Denkert *et al*. 2009).

However, most of these potential biomarkers have neither been validated in multivariate analyses nor was their discriminative power validated in large clinical cohorts. Even more alarmingly, many reports have questioned or rejected a correlation between a proposed biomarker and clinical outcome. Doubts were raised regarding the markers *CA125* (Cruickshank *et al*. 1987, van der Burg *et al*. 1988, Rustin *et al*. 1989, Sevelda *et al*. 1989), cyclin D1 (Masciullo *et al*. 1997, Dhar *et al*. 1999), p16 (Milde-Langosch *et al*. 2003, Khouja *et al*. 2007), p21 (Baekelandt *et al*. 1999, Levesque *et al*. 2000, Schuyer *et al*. 2001), p27 (Schmider *et al*. 2000), p53 (Smith-Sorensen *et al*. 1998, Wang *et al*. 2004, Green *et al*. 2006), Bcl-xl (Baekelandt *et al*. 2000), cIAP (Kleinberg *et al*. 2007), survivin (Cohen *et al*. 2003, Ferrandina *et al*. 2005), hTERT (Wisman *et al*. 2003, Widschwendter *et al*. 2004), ERBB1 (Berchuck *et al*. 1991, Meden *et al*. 1995, Nielsen *et al*. 2004), and ERBB2 (Rubin *et al*. 1993, Meden *et al*. 1995, Ross *et al*. 1999, Nielsen *et al*. 2004, Riener *et al*. 2004).

Given the large number of potential biomarkers for EOC, the immediate challenge is to validate the most robust candidates eligible for further investigation. Recent advances in genomic technologies together with powerful bioinformatic tools can enable us to deliver this prerequisite. We recently developed an online biomarker validation tool using microarray data of 2000 breast cancer patients (Gyorffy *et al*. 2010). In this, the expression of a selected gene can be used to split patients into groups, and the proportional survival of these groups is compared to each other.

In this study, our aim was to implement an online survival analysis tool for the rapid assessment of prognosis-related genes in ovarian cancer and to test the validity of previously proposed biomarkers. Furthermore, we also developed additional analysis options including the computation of multigenic prognosis predictors and the option of grouping patients based on applied treatment protocols.

Materials and methods

Collection of ovarian cancer microarray data sets

We searched Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) and The Cancer Genome Atlas (TCGA; http://cancergenome.nih.gov) to identify data sets suitable for the analysis. In this, the keywords 'ovarian', 'cancer', 'survival', 'gpl96', 'gpl570', and 'gpl571' were used. Only publications with available raw microarray gene expression data, clinical survival information, and at least 20 patients were included. Only three microarray platforms, GPL96 (Affymetrix HG-U133A), GPL570 (Affymetrix HG-U133 Plus 2.0), and GPL571/GPL3921 (Affymetrix HG-U133A 2.0), were considered because they are frequently used and because these particular arrays have 22 277 probe sets (representing 13 435 unique genes) in common. The use of almost identical platforms and identical probe sets is vital because different platforms for gene expression profiling measure expression of the same gene with varying accuracy, on different relative scales, and with diverse dynamic ranges (Tan *et al*. 2003). Finally, we controlled all samples using the ranked expression of all genes to identify repeatedly published microarrays.

Setup of server for online survival calculation

The raw.CEL files were MAS5 normalized in the R statistical environment (www.r-project.org) using the affy Bioconductor library (Gautier *et al*. 2004). MAS5 can be applied to individual chips, making future extensions of the database uncomplicated. Furthermore, MAS5 ranked among the best normalization methods when compared with the results of RT-PCR measurements in our recent study (Gyorffy *et al*. 2009). For the analysis, only probes measured on GPL96, GPL570, and GPL571/GPL3921 were retained ($n = 22\,277$). At this stage, we performed a second scaling normalization to set the average expression on each chip to 1000. Although this technique cannot remove all, but it can significantly reduce batch effects (Sims *et al*. 2008). We integrated the gene expression and clinical data using PostgreSQL, an open-source object-relational database system (www.postgresql.org). Data security is ensured through PostgreSQL

permissions that are imposed on individual tables in the project databases.

The KMplot web application can be reached in a platform-independent user interface. The interactivity of the service is increased by the usage of JavaScript and Ajax technologies. The server is hosted on Debian Linux (www.debian.org) and is powered by Apache (www.apache.org). he server-side scripts were developed in hypertext preprocessor (PHP), which controls the analysis requests and delivers the results. Open Database Connectivity is used as a middleware layer between the R and the PostgreSQL database via the RODBC package (cran.r-project.org/package=RODBC). The package 'survival' is used to calculate and plot Kaplan–Meier survival curves, and the number-at-risk is indicated below the main plot. Hazard ratio (HR; and 95% confidence intervals) and logrank *P* are calculated and displayed. The central server for the Kaplan–Meier plotter for ovarian cancer can be reached at www.kmplot.com/ovar.

Probe set options

We also implemented a set of probe-set-related options, including the option to use all probe sets available for a given gene on the microarray simultaneously and to use a combined expression of several probe sets. Using this option, it is possible to assess the effect of the mean expression of gene combinations on survival.

In addition, bee swarm plot can be drawn using the beeswarm package (www.cbs.dtu.dk/~eklund/beeswarm/). The bee swarm plot is capable of visualizing gene expression as nonoverlapping points in a one-dimensional scatter plot. A bee swarm plot can be used to quickly identify outlier samples and genes with bimodal distribution.

Validation of previously published EOC biomarkers

A PubMed search was performed using the keywords 'ovarian cancer', 'survival', 'biomarker', and 'gene expression' to identify genes described in the literature as potential EOC biomarkers. Then, using PubMed gene, we added a unique gene symbol for each of the genes and identified the corresponding Affymetrix probe set IDs. The capability of these genes to predict survival was measured by using the probe set IDs in the online analysis tool.

In the combination of several markers, their mean expression is first computed for each sample. Then, the median of these is used for splitting the patients into cohorts during the analysis.

Table 1 Clinical properties of the ovarian cancer patients used in the analysis

GEO ID	Reference	GEO platform	No. of samples in data set	Death event	Median overall survival	Serous/ endometrioid	Grade (1/2/3)	Stage (1/2/3/4)	Debulk optimal (/out of)	Treatment contains platin (/out of)	Treatment contains Taxol (/out of)
GSE14764	Denkert *et al.* 2009	GPL96	80	21	35.2	68/7	NA	NA	27/29	78/79	79/79
GSE15622	Ahmed *et al.* 2007	GPL571	35	28	27.0	31/0	0/7/28	0/0/26/9	NA	20/35	15/35
GSE19829	Konstantinopoulos *et al.* 2010	GPL570	28	17	35.0	NA	NA	NA	NA	NA	NA
GSE3149	Bild *et al.* 2006	GPL96	116	69	34.0	NA	4/55/54	0/1/96/14	64/117	115/115	94/115
GSE9891	Tothill *et al.* 2008	GPL570	285	110	28.0	264/20	24/18/217	19/97/164/0	160/229	242/282	195/282
GSE18520	Mok *et al.* 2009	GPL570	53	41	22.0	53/0	0/0/53	NA	NA	NA	NA
GSE26712	NA	GPL96	185	129	38.7	NA	NA	NA	90/185	NA	NA
TCGA	TCGA 2011	GPL3921	505	277	30.6	505/0	5/62/427	15/24/386/81	333/452	458/473	233/468
		Total	1287	692	31.0	925/27	33/142/779	34/122/672/109	674/1121	914/985	616/980

NA, data not available; /out of, total number of patients with available clinical data.

Table 2 The association between prognostic markers and progression-free survival. The patients were divided into two groups as having higher or lower expression as compared to the median. The markers were analyzed in subsets of patients with equivalent clinical characteristics to the cohorts in which the association has previously been described

Symbol	Gene	Reference	Survival	Analyzed in the cohort of	Affymetrix ID	Q	HR	*P*
CA(MUC 16)125	CA 125	Gadducci *et al.* 1995, Cooper *et al.* 2002, Gadducci *et al.* 2004, Riedinger *et al.* 2006	PFS	All patients	220196_at	2	NS	NS
					201384_s_at	1	**1.3**	**0.0003***
					201383_s_at	1	**1.4**	**3.7×10^{-5}***,a
KRT19	Cytokeratin 19	Tempfer *et al.* 1998, Gadducci *et al.* 2001	PFS	Debulk=subopt.	201650_at	1	NS	NS
KLK6	Kallikrein 6	Diamandis *et al.* 2003	PFS	All patients	216699_s_at	2	**0.79**	**0.002***
					204733_at	1	NS	NS
KLK10	Kallikrein 10	Luo *et al.* 2001	PFS	Stage=3+4	209792_s_at	1	NS	NS
					215808_at	3	NS	NS
IL6	Interleukin 6	Scambia *et al.* 1995	OS	All patients	205207_at	2	NS	NS
IL7	Interleukin 7	Lambeck *et al.* 2007	OS	All patients	206693_at	3	NS	NS
IFNG	γ-Interferon	Marth *et al.* 2004	PFS	All patients	210354_at	3	**0.81**	**0.004***
FAS	sFas	Hefler *et al.* 2000, Konno *et al.* 2000	PFS	All patients	204780_s_at	1	1.2	0.017
					204781_s_at	1	NS	NS
					212218_s_at	1	0.84	0.024
					215719_x_at	2	NS	NS
					216252_x_at	2	NS	NS
					217006_x_at	3	NS	NS
VEGFR	VEGFR	Hefler *et al.* 2006	OS	All patients	203934_at	2	1.2	0.064
CCND1	Cyclin D1	Bali *et al.* 2004, Barbieri *et al.* 2004	OS	Stage=3+4	208711_s_at	1	NS	NS
					208712_at	1	NS	NS
CCND3	Cyclin D3	Levidou *et al.* 2007	OS	All patients	201700_at	1	NS	NS
CCNE	Cyclin E	Sui *et al.* 2001, Farley *et al.* 2003, Rosen *et al.* 2006, Bedrosian *et al.* 2007	OS	Debulk=subopt.	213523_at	2	NS	NS
					205034_at	2	NS	NS
					211814_s_at	3	NS	NS
P(CDK N2B)15	p15	Kudoh *et al.* 2002	PFS	All patients	204599_s_at	1	NS	NS
					212857_x_at	1	1.3	**0.0005***
					214512_s_at	1	1.2	0.01
					221727_at	3	NS	NS
					218708_at	1	NS	NS
P(CDK N2A)16	p16	Katsaros *et al.* 2004, Kommoss *et al.* 2007	PFS	Debulk=subopt.	207039_at	2	**0.66**	**0.002***
					209644_x_at	1	NS	NS
					211156_at	3	0.69	0.009
CDKN1A	p21	Ferrandina *et al.* 2000, Plisiecka-Halasa *et al.* 2003, Bali *et al.* 2004	PFS	Histology=serous	202284_s_at	1	NS	NS
CDKN1B	p27	Newcomb *et al.* 1999, Masciullo *et al.* 2000, Korkolopoulou *et al.* 2002, Schmider-Ross *et al.* 2006	PFS	All patients	209112_at	1	**1.4**	**5.4×10^{-5}***,a
RB1	pRB	Dong *et al.* 1997, Konstantinidou *et al.* 2003	OS	Stage=1	203132_at	1	NS	NS
					211540_s_at	3	NS	NS
E2F1	E2F1	Suh *et al.* 2008	PFS	All patients	2028_s_at	1	0.83	0.017
						3	NS	NS
E2F2	E2F2	Reimer *et al.* 2007	PFS	All patients	207042_at	3	0.86	0.037
E2F4	E2F4	Reimer *et al.* 2007	PFS	All patients	202248_at	3	0.85	0.034
					38707_r_at	1	NS	NS
TP53	p53	Buttitta *et al.* 1997, Reles *et al.* 2001	PFS	Stage=3+4	211300_s_at	2	NS	NS
					201746_at	1	0.84	0.075
TP73	p73	Becker *et al.* 2006	OS	All patients	220804_s_at	3	NS	NS
BAX	bax	Tai *et al.* 1998, Skirnisdottir *et al.* 2001	PFS	Therapy=contains Taxol	208478_s_at	2	NS	NS
					211833_s_at	2	NS	NS

Table 2 *continued*

Symbol	Gene	Reference	Survival	Analyzed in the cohort of	Affymetrix ID	Q	HR	*P*
BCL2L1	Bcl-xl	Materna *et al.* 2007	PFS	All patients	212312_at	1	0.86	0.04
					215037_s_at	2	NS	NS
					206665_s_at	3	NS	NS
BIRC2	cIAP	Psyrri *et al.* 2006	OS	Stage=3+4	202076_at	1	NS	NS
BIRC5	Survivin	Sui *et al.* 2002	PFS	All patients	210334_x_at	2	**0.75**	**0.00017***
					202094_at	2	0.84	0.018
					202095_s_at	1	0.84	0.018
TERT	hTERT	Brustmann 2005	OS	Histology=serous	207199_at	3	NS	NS
EGFR	ERBB1	Skirnisdottir *et al.* 2004, Psyrri *et al.* 2005	PFS	Stage=1+2	201983_s_at	1	NS	NS
					201984_s_at	1	NS	NS
					211551_at	2	NS	NS
					210984_x_at	3	NS	NS
					211550_at	3	NS	NS
					211607_x_at	3	NS	NS
ERBB2	ERBB2	Lassus *et al.* 2004	PFS	Histology=serous	210930_s_at	3	NS	NS
					216836_s_at	1	NS	NS
MET	c-Met	Sawada *et al.* 2007	OS	Stage=3+4	217828_at	1	NS	NS
					203510_at	1	NS	NS
					211599_x_at	1	NS	NS
					213807_x_at	2	NS	NS
					213816_s_at	3	NS	NS
MMP2	MMP-2	Torng *et al.* 2004	PFS	Histology=endom.	201069_at	1	0.33	0.05
MMP9	MMP-9	Sillanpaa *et al.* 2007	OS	Stage=1	203936_s_at	1	NS	NS
MMP14	MT1-MMP	Kamat *et al.* 2006	OS	Stage=2+3+4	160020_at	1	NS	NS
					202828_s_at	1	NS	NS
					202827_s_at	2	NS	NS
					217279_x_at	3	NS	NS
WFDC2 (HE4)	Epididymis protein 4	Huhtinen *et al.* 2009, Moore *et al.* 2009, 2010	PFS	All patients	203892_at	1	NS	NS
SERPINB5	Maspin	Secord *et al.* 2006	PFS	Debulk=subopt.	204855_at	1	NS	NS
BRCA1	BRCA1	Thrall *et al.* 2006	OS	All patients	211851_x_at	3	0.82	0.01
					204531_s_at	2	NS	NS
ERCC1	ERCC1	Darcy & Tian 2007	PFS	Stage=3	203719_at	1	NS	NS
				Therapy=Tax+Plat	203720_s_at	1	NS	NS

PFS, progression-free survival; OS, overall survival, HR, hazard ratio; Q, quality score for the probe set as measured after normalization across the entire data set (1, average expression over 500 or maximal expression over 1000; 2, intermediate probes; 3, average expression below 100); NS, not significant (significance over 0.05). *bold indicates $P<0.005$.
[a]See Kaplan–Meier plots in Fig. 1.

Results

Construction of combined ovarian cancer microarray database

We identified 1287 unique patients in eight data sets meeting our criteria in GEO and TCGA. In the GSE3149 data set, we found two samples repeatedly published (GSM70546=GSM70547 and GSM70511=GSM 70512). As for these samples the unique recognition of the appropriate clinical information was not possible, we removed them from the final database. Of the above, 72% have serous and 2% have endometrioid tumors. Patients are distributed across stage 1 ($n=34$, 3.6%), stage 2 ($n=122$, 13%), stage 3 ($n=672$, 71.7%), and stage 4 ($n=109$, 11.6%). Debulking was optimal (residual tumor <1 cm) in 674 out of 1119 patients. The median overall survival is 31.0 months, 1090 patients have progression-free survival data, and 1287 have overall survival data. (note: some publications report 'disease-free survival' (Konstantinopoulos *et al.* 2010) or 'relapse-free survival' (Tothill *et al.* 2008) instead of 'progression-free survival'. These were merged as 'progression-free survival' to enable a meta-analysis of the complete database.). A summary of the clinical characteristics of the patients in each data set used in the analysis is shown in Table 1.

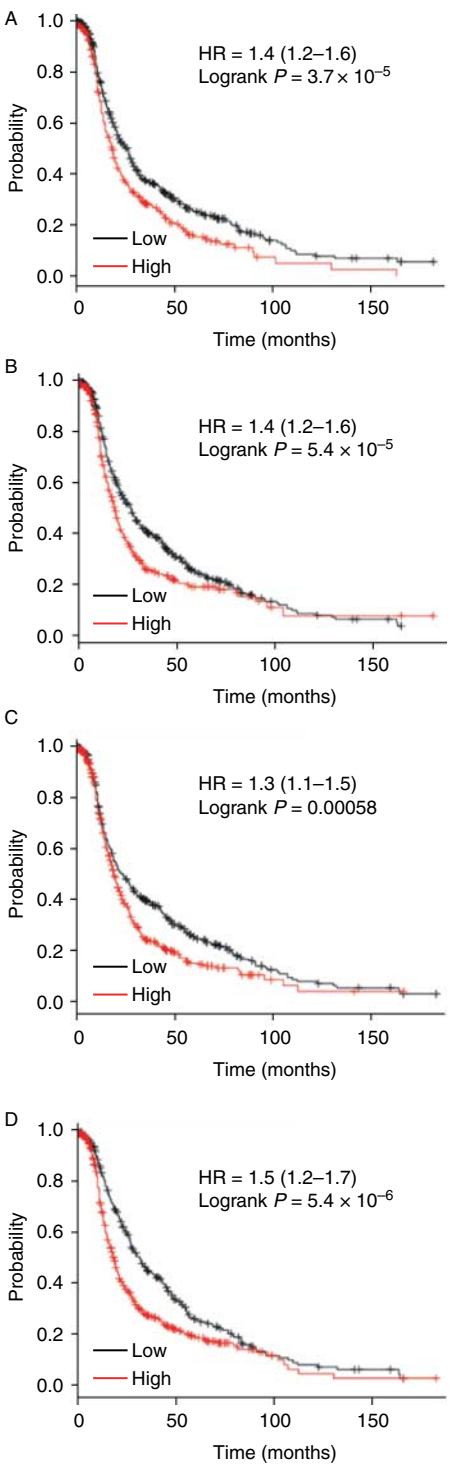

Figure 1 Survival plots depicting the good prognostic effect on progression-free survival of the lower expression of *CA125* (A, 201383_s_at), *CDKN1B* (B, 209112_at), and *P15* (C, 212857_x_at). Classification using the mean expression of two genes (*CA125*+*CDKN1B*) with a cutoff at the lower quartile results in increased discriminative power (D).

Setup of online survival analysis platform

The Kaplan–Meier plot shows the association between the investigated marker and survival in which the samples are grouped according to the median (or upper or lower quartile) expression of the selected gene. Before running the analysis, the patients can be filtered using stage, histology, grade, and treatment parameters including debulking status and applied chemotherapy. In addition, as an alternative to progression-free survival, overall survival can also be investigated.

Since there is an already established biomarker (*CA125*), a clinician might be interested in a specific clinical cohort of patients having low *CA125* levels. Therefore, we added an additional filtering option in which only patients having an average *CA125* expression (average of the two reliable probe sets) below the lower quartile of all patients are included. We must note that while this study measured tissue levels of *CA125*, the FDA-approved test for ovarian cancer is serum based.

Validation of previously published EOC biomarkers

Markers of ovarian cancer prognosis have been identified using literature search. We computed Kaplan–Meier plots for 37 proposed biomarkers to assess their effect on prognosis (for the complete results see Table 2 and Fig. 1). All biomarkers were investigated in the same cohort in which they were discovered. High significance was achieved for *CA125, KLK6, IFNG, P15, P16, CDKN1B*, and *BIRC5*. In addition, we have also run the analysis for predicting progression-free survival in all patients.

In an effort to improve accuracy, a pair-wise combination of the three best performing probe sets was assessed independently. The combination of *CA125* and *CDKN1B* with a cutoff at the lower quartile resulted in classification significance superior to the power of the markers independently (HR = 1.5 and $P = 5.4 \times 10^{-6}$ vs HR = 1.4, $P = 3.7 \times 10^{-5}$ and HR = 1.4, $P = 5.4 \times 10^{-5}$ for *CA125* and *CDKN1B*, respectively, see Table 2 and Fig. 1).

Discussion

The validation of prognostic biomarkers is a major bottleneck in ovarian cancer research. Here, we combined multiple large microarray data sets to increase the statistical power for a meta-analysis of 22 277 genes. We developed a freely accessible online tool to estimate the prognostic value of any selected gene in a large cohort of clinical patients. After dividing

the patients into two groups based on the expression of the selected gene, a Kaplan–Meier plot is generated. The implemented computations are performed in real time on our server. This enables seamless future extension using new data sets or new filtering options.

We have integrated data sets from GEO and TCGA – ~40% of samples used by www.kmplot.com/ovar are from the TCGA repository. For the TCGA samples alone, there is an option to perform analyses in the caIntegrator website (https://caintegrator.nci.nih.gov). The samples in TCGA are open access or restricted (access is granted to NIH staff and to eRA Commons principal investigators) – however, the Affymetrix HG-U133 microarray profiles for the ovarian cancer patients are publicly available. We plan to continuously incorporate new GEO data sets as well as new TCGA samples in www.kmplot.com.

In contrast to breast cancer, where several already approved markers are in clinical use, in ovarian cancer only minimal progress has been made in recent years. When investigating the previously proposed biomarkers, we found that only few genes are actually capable of predicting outcome in our combined data set: *CA125, P15, KLK6, IFNG, P16, CDKN1B*, and *BIRC5*. Of these, *CA125* and *CDKN1B* resulted in very robust significance. These results may reflect the high genetic heterogeneity of ovarian cancer (Gyorffy *et al*. 2008) and emphasize the importance of potential improvements in prognosis.

The most extensively studied marker for EOC is *CA125* (Gadducci *et al*. 1995, 2004, Cooper *et al*. 2002, Riedinger *et al*. 2006), and determining its concentration in serum is essential for monitoring ovarian cancer progression. Fifty percent increase in serum *CA125* level has been correlated to progression, and present progression definition of the Gynecological Cancer Intergroup defines progression based on two elevated serum *CA125* levels. According to our results, tumor level of *CA125* gene was able to predict later clinical outcome. Notably, we observed two different probe sets representing *CA125* as significant. A third probe set did not show significant prognostic power, but it has also displayed low quality in terms of average expression as compared to the probe sets with significant prognostic power.

The role of the cell cycle control gene p27 (*CDKN1B*) as a prognostic marker in ovarian cancer was suggested in several studies (Newcomb *et al*. 1999, Masciullo *et al*. 2000, Korkolopoulou *et al*. 2002, Schmider-Ross *et al*. 2006). In addition, numerous recent analyses also confirmed its role in 205 (Lee *et al*. 2011), 131 (Skirnisdottir *et al*. 2011), and 339 (Duncan *et al*. 2010) patients. p27 is measured by only one probe set on the microarrays, and this probe set delivered high prognostic power in our analysis.

P15 is a tumor suppressor gene previously associated with ovarian cancer progression in 45 patients (Kudoh *et al*. 2002). The methylation status of *P15* has also been investigated but was not an independent prognostic factor in 145 patients (Tam *et al*. 2007). One of the probe sets measuring *P15* (212857_x_at) delivered a high prognostic potential.

To this point, we have investigated the prognostic power of individual probe sets. However, recent reports based on genomic technologies use not only single selected genes, but also a combination of these. In addition, some of the markers are not related to ovarian cancer prognosis in general, but have discriminative potential in one of the subgroups, or are related to different treatment regimens. While the evaluation of all potential markers and all eligible combinations is beyond the scope of this study, our online tool was set up exactly to enable researchers to perform these tests on our database.

In summary, we reviewed previously reported biomarkers of ovarian cancer prognosis and assessed their performance in a meta-analysis of 1297 ovarian cancer patients. We also developed an online biomarker validation platform to mine all available microarray data to assess the prognostic power of 22 277 genes.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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