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A 10-gene expression signature of Notch pathway predicts recurrence in ovarian carcinoma

FANG CHEN[1] and NAIFU LIU[2]

[1]Department of Obstetrics and Gynecology, Weifang People's Hospital, Weifang, Shandong 261000;
[2]Department of Gynecological Oncology, Shandong Cancer Hospital and Institute, Jinan, Shandong 250117, P.R. China

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Abstract. Patients with ovarian carcinoma are at high risk of tumor recurrence. In the present study, 81 Notch pathway genes were selected to find recurrence-related genes in The Cancer Genome Atlas dataset. A 10-gene signature (FZD4, HES1, PSEN2, JAG2, PPARG, FOS, HEY1, CDC16, MFNG, and EP300) was identified and validated that is associated with recurrence-free survival time, but not with overall survival time, in the TCGA dataset and in other two independent datasets, GSE9891 and GSE30161. This gene signature gave a significant performance in discriminating patients at high risk of recurrence from those at low risk, as measured by the area under the receiver operating characteristic curve. Cox proportional hazards regression analyses demonstrated that the prognostic value of this 10-gene set is independent of other clinical variables in all three datasets. The potential as a biomarker for predicting high- and low-risk subgroups for recurrence in ovarian cancer patients deserves further investigation in prospective patient cohorts in the future.

Introduction

Ovarian cancer is one of the most lethal malignant gynecological cancers worldwide (1). Compared with other cancers in women, ovarian carcinoma confers a relatively high risk of recurrence. Although there is a high initial response rate to standard surgery and chemotherapy, 30-40% of patients relapse within one year (2,3). Therefore, the prediction of patients at a high risk of recurrence may provide novel therapeutic avenues to improve their outcomes. Although common clinicopathological parameters, such as stage and histological grade, and several biomarkers were proposed for recurrence prediction, these factors demonstrated insufficient sensitivity and specificity (4). Thus, there is an urgent requirement to identify novel markers or models to increase the power of recurrence prediction for patients with ovarian carcinoma.

The Notch pathway and its abundant associated genes comprise a complicated network, which plays a significant role in the progressive growth of tumor cells in multiple cancer types (5). The Notch pathway alterations are prevalent and significantly associated with poor outcomes, including early recurrence in ovarian carcinoma (6,7). We speculate that Notch pathway associated molecular signatures may be useful for characterizing ovarian carcinomas at high risk of recurrence. In the present study, a 10-gene Notch pathway signature is defined that may assist in improved predictions of recurrence in ovarian carcinoma patients.

Materials and methods

Datasets. Three ovarian carcinoma gene expression datasets [The Cancer Genome Atlas (TCGA), GSE9891 and GSE30161] with documented recurrence information were selected for analysis in the present study (8-10). The expression data together with the curated and documented clinical metadata were extracted by the R curated Ovarian Data Bioconductor package, as previously described (11). Microarray platforms used in these datasets were Affy HT U133a (TCGA) and Affy U133 Plus 2.0 (GSE9891 and GSE30161). TCGA dataset comprises the whole-genome mRNA expression data of 522 ovarian carcinoma samples. The GSE9891 dataset comprises the gene expression microarray data of 275 ovarian carcinoma samples, including 40 early-stage and 257 late-stage tumors. The GSE30161 dataset was generated from 58 late-stage ovarian cancer samples, and 5 arrays were excluded due to the lack of complete recurrence information.

Finding of genes correlated with recurrence in TCGA ovarian carcinoma dataset. The expression data of a subset of 81 Notch pathway-associated genes (including core Notch pathway members, Notch pathway target genes, genes that crosstalk with Notch pathway and other genes involved in Notch pathway) were selected from TCGA ovarian carcinoma dataset. Cox proportional hazards model was used to test whether the gene expression of a particular gene significantly influenced

Correspondence to: Dr Naifu Liu, Department of Gynecological Oncology, Shandong Cancer Hospital and Institute, 440 Jiyan Road, Jinan, Shandong 250117, P.R. China
E-mail: naifuliu@163.com

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Table I. Notch pathway genes correlated with recurrence in The Cancer Genome Atlas ovarian carcinoma dataset.

Notch pathway genes	Gene names	Accession no.	Hazard ratio	FDR	Permutation P-value
FZD4	Frizzled family receptor 4	NM_012193	0.786	0.0466	0.0010
HES1	Hairy and enhancer of split 1, (*Drosophila*)	NM_005524	0.874	0.259	0.0091
PSEN2	Presenilin 2 (Alzheimer disease 4)	NM_000447	0.471	0.259	0.0094
JAG2	Jagged 2	NM_002226	0.819	0.286	0.0241
PPARG	Peroxisome proliferator-activated receptor gamma	NM_005037	0.741	0.286	0.0284
FOS	FBJ murine osteosarcoma viral oncogene homolog	NM_005252	1.094	0.286	0.0266
HEY1	Hairy/enhancer-of-split related with YRPW motif 1	NM_001040708	0.879	0.286	0.0298
CDC16	Cell division cycle 16	NM_001078645	1.202	0.286	0.0296
MFNG	MFNG O-fucosylpeptide 3-β-N-acetylglucosaminyltransferase	NM_001166343	1.323	0.286	0.0357
EP300	E1A-binding protein p300	NM_001429	0.818	0.321	0.0429

FDR, false discovery rate.

recurrence using the BRB-Arraytools software (12). The tests were performed at a significance threshold of univariate tests of 0.05 using permutation tests. The number of permutation tests was set as 10,000.

Recurrence-free survival (RFS) and overall survival (OS) time prediction based on the supervised principal components method using the 10-Notch pathway gene signature. After subseting the gene expression data using the 10-Notch pathway gene signature, recurrence and survival risk prediction in TCGA, GSE9891 and GSE30161 datasets were performed based on principal components using the BRB-ArrayTools software. 10-fold cross validation was selected, and the number of principal components was set as 2. The prognostic index percentile was used to separate arrays into high- and low-risk groups.

Statistical analysis. Distributions of RFS and OS were assessed using the Kaplan-Meier curve method and evaluated by the log-rank test. Multivariate analyses of prognostic factors were based on the Cox proportional hazards model. The receiver operating characteristic (ROC) curve was constructed using R package survival ROC and determined by permutation testing. The difference in clinicopathological characteristics between the high- and low-risk subgroups was determined by χ^2 test. All the statistical analyses were performed with Medcalc 11.4 Software unless otherwise specified. $P<0.05$ was considered to indicate a statistically significant difference.

Results

We hypothesized that Notch pathway genes correlated with recurrence may be clinically useful to differentiate between high- and low-risk ovarian carcinoma tumors. To investigate this, the BRB-Arraytool package was used to find Notch pathway genes with expression that was correlated with RFS time by univariate tests. As shown in Table I, using a significance threshold at 0.05, a list of 10 Notch pathway genes was determined to be associated with RFS time in TCGA ovarian carcinoma dataset.

Using a principal components model based on these 10 Notch pathway genes as a signature, a predictor was generated and TCGA ovarian cancer samples were classified into high-risk (n=263) and low-risk (n=259) subgroups. As shown in Fig. 1A, in TCGA dataset, the high-risk ovarian carcinomas demonstrated a significantly shorter RFS time than the low-risk cases (hazard ratio, 1.3656; 95% confidence interval, 1.0739-1.7364; P=0.0104). In order to evaluate the performance of this novel gene signature, its performance in predicting recurrence in two other independent ovarian carcinoma datasets, GSE9891 and GSE30161, was further validated. As shown in Fig. 1B and C, the signature was independently predictive of recurrence in the two validation datasets. In addition, this gene signature gave a significant value for the area under the curve when discriminating between high- and low-risk ovarian carcinomas in all three datasets (TCGA, P=0.0167; GSE9891, P=0.04; GSE30161, P=0.01) (Fig. 1D-F).

Next, the association between the predicted recurrence risk subgroups and the known prognostic factors was analyzed. In GSE9891, but not the other two datasets, the high recurrence risk subgroup exhibited a significant association with stage and debulking level (Table II). The multivariate Cox proportional hazards regression analyses found that the prognostic value on recurrence of the 10-Notch gene signature was independent of other known predictors in all three datasets (Table III).

Finally, it was evaluated whether this 10-gene signature could predict OS time for ovarian carcinomas. In contrast to RFS, no significant difference was found in OS time between the high- and low- risk subgroups in all three datasets (Fig. 2).

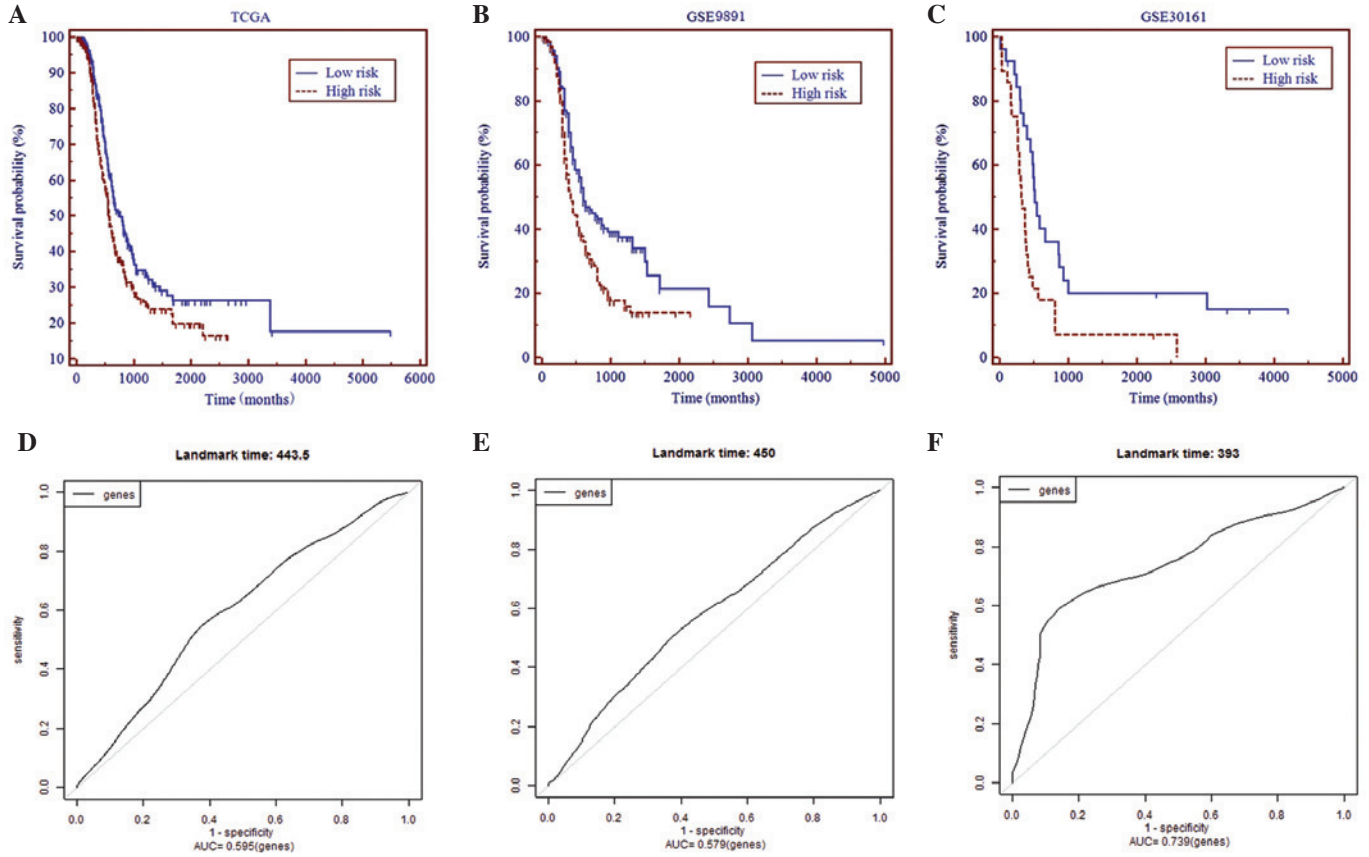

Figure 1. Recurrence prediction and receiver operating characteristic curves in 3 ovarian carcinoma gene expression datasets based on a 10-Notch pathway gene classifier. High-risk subgroups showed a significantly shorter recurrence-free survival time than low-risk groups in (A) The Cancer Genome Atlas (TCGA), (B) GSE9891 and (C) GSE30161 datasets. This gene signature gave an area under the curve (AUC) of (D) 0.595 in TCGA dataset; (E) 0.579 in the GSE9891 dataset and (F) 0.739 in the GSE30161 dataset.

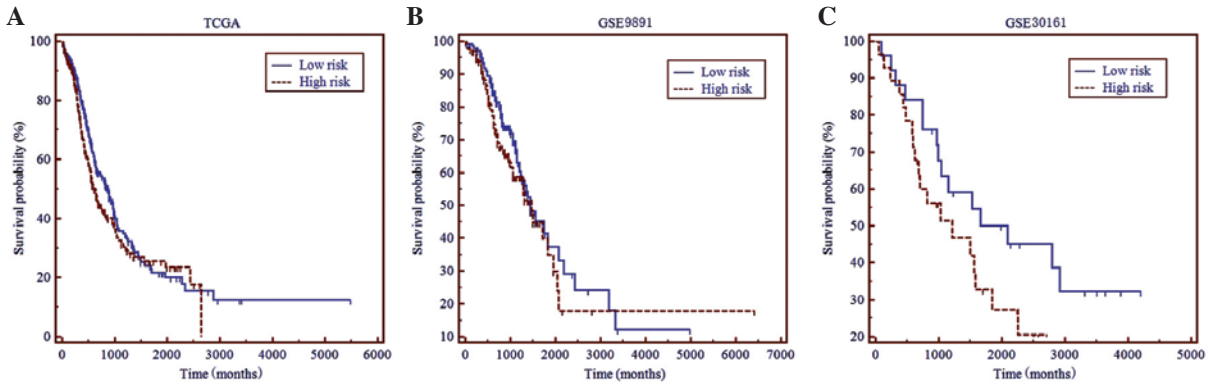

Figure 2. Overall survival (OS) time prediction in 3 ovarian carcinoma gene expression datasets based on the 10-Notch pathway gene classification. No difference was found in OS time between high- and low-risk subgroups in (A) The Cancer Genome Atlas (TCGA) dataset; (B) the GSE9891 dataset and (C) the GSE30161 dataset.

Discussion

In the current study, 10 Notch pathway genes were identified to significantly correlate with recurrence in ovarian carcinoma. This 10-gene signature could classify ovarian carcinoma into high- and low-risk recurrence subgroups, and showed a significant performance to predict recurrence in independent cohorts. Moreover, the prognostic value of this gene signature is independent of the common clinicopathological predictors of ovarian cancers.

Prognostic gene signatures based on microarray data have been recently developed to identify subgroups with a more aggressive phenotype or poor outcomes in ovarian carcinoma. For example, Gillet *et al* (13) found 3 multidrug resistance gene signatures with a statistically significant correlation with OS and progression-free survival time. Cheon *et al* (14) identified and validated a 10-gene signature regulated by transforming growth factor-β signaling that is associated with poor OS time in patients with high-grade serous ovarian cancer. Compared with the previous gene signatures, the present 10-gene classifier

Table II. Difference in clinicopathological characteristics between high- and low-risk recurrence subgroups defined by a 10-Notch pathway gene signature in 3 ovarian carcinoma gene expression datasets.[a]

Characteristics	Low risk	High risk	P-value
TCGA dataset			0.9589
Number	259	263	
Grade			
1-2	33	34	
3	224	220	
Stage			0.6486
Early	18	22	
Late	240	239	
Debulking			0.1067
Optimal	184	167	
Suboptimal	52	68	
GSE9891 dataset			
Number	142	133	0.0114
Grade			
1-2	68	44	
3	71	89	
Stage			0.0008
Early	31	9	
Late	111	123	
Debulking			0.8884
Optimal	41	41	
Suboptimal	83	77	
GSE30161 dataset			
Number	26	28	1.0000
Grade			
1-2	11	10	
3	14	15	
Debulking			0.9485
Optimal	14	15	
Suboptimal	10	13	

[a]Cases with unavailable clinicopathological data were not included.

Table III. Multivariate Cox proportional hazards regression analyses on recurrence-free survival time in 3 ovarian carcinoma gene expression datasets.

Covariate	P-value	Exp(b)	95% CI of Exp(b)
TCGA dataset			
Grade	0.5624	1.1112	0.7792-1.5848
Stage	0.0155	2.0291	1.1473-3.5887
Debulking	0.6801	0.9404	0.7032-1.2575
Predicted risk	0.0083	1.4119	1.0944-1.8215
GSE9891 dataset			
Grade	0.6509	0.9377	0.7106-1.2373
Stage	0.0001	5.0027	2.3040-10.8624
Debulking	0.0010	0.5829	0.4228-0.8035
Predicted risk	0.0382	1.3984	1.0200-1.9171
GSE30161 dataset			
Grade	0.7870	1.0997	0.5537-2.1841
Debulking	0.0160	0.4235	0.2113-0.8488
Predicted risk	0.0088	2.3312	1.2413-4.3781

TCGA, The Cancer Genome Atlas; CI, confidence interval.

exhibited a better performance on predicting recurrence, but not OS time for ovarian carcinoma. Moreover, for the first time, a Notch pathway gene signature is reported to have clinical significance in the prognosis prediction for patients with ovarian carcinoma. The use of this 10-Notch pathway gene signature should be investigated in prospective patient cohorts in the future.

Of the 10 Notch pathway genes identified in the present study, the functional and clinical significance has only been investigated in FZD4, HES1, PPARG and FOS. Only FZD4 has been found to be associated with recurrence in a previous study. Dai *et al* (15) found that DNA methylation and reduced expression of FZD4 are indicators of early disease relapse in ovarian tumors, which is consistent with the present finding that FZD4 gene expression is negatively associated with recurrence in ovarian carcinoma. Increased expression of HES1 and PPARG were validated to be predictors for poor OS in ovarian cancers (16,17). High expression of FOS was significantly associated with advanced clinical stage and chemoresistance (18). Considering that this 10-gene signature has clinical significance in classifying different subgroups with high and low recurrence risk, it is also possible that these Notch pathway genes may act as important mediators during ovarian carcinogenesis, and may represent novel therapeutic targets. Therefore, the biological significance of these 10 Notch signaling genes also deserves further investigation.

In conclusion, in the present study, a novel Notch pathway gene signature that is useful for predicting recurrence in ovarian cancers was developed. If prospectively validated, it would provide a reference for informing treatment decisions for patients with ovarian carcinoma. The biological significance of this signature and its potential as a biomarker also deserve further investigation in future studies.

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