The Inhibitor of DNA Binding Family Regulates the Prognosis of Ovarian Cancer

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Abstract

Background: The inhibitor of DNA binding or differentiation (*ID*) protein family contributes to the carcinogenesis and progression of various cancers. However, its mechanistic role in tumor initiation and progression of ovarian cancer (OC) has remained unclear.

Methods: We used the Oncomine, GEPIA, Kaplan-Meier plotter, cBioPortal, SurvExpress, PROGgene V2 server, TIMERdatabase, and FunRich to evaluate the expression and predictive prognostic value of individual IDs members' mRNA in patients with OC.

Results: Our results revealed that the mRNA transcripts of all *ID* family members were markedly downregulated in OC compared to normal tissue. Aberrant expression of *ID 1/3/4*correlated with cancer aggressiveness and clinical in OC patients. The prognostic value of *ID* members was also explored within the subtypes, pathological stages, clinical stages, and TP53 mutational status. The group with a low risk *IDs* showed a relatively good overall survival (OS) in comparison to the high-risk group. In contrast, the expression level of *IDs* was significantly associated with the levels of infiltrating B cells and macrophages. Finally, enrichment analysis showed that *ID* co-expressed genes were involved in *ID-*, *c-MYC*, *TNF-*, and Wnt signaling pathways.

Conclusion: These results indicate that *ID1/3/4* may be exploited as promising prognostic biomarkers and therapeutic targets in OC patients.

Introduction

Key among the genes that encode helix-loop-helix (HLH) family of transcription factors is the inhibitor of DNA binding or differentiation *(ID*), abundant in stem and progenitor cells (1). To date, ID proteins are encoded by four *ID* genes in the *ID* family in vertebrates: *ID1-4*, all of which encode the corresponding (1, 2). As negative regulators of basic HLH proteins, *ID* proteins are potent suppressors of typical HLH proteins, and this is achieved via the formation of non-functional heterodimers (3). Recently, numerous studies have reported aberrant expression of *ID* proteins in different human malignancies. It has also been associated with advanced tumor metastasis and development of multiple carcinomas (1, 2, 4). In addition, *ID* proteins are involved in virtually all tumor-associated processes, including cell differentiation, cycle regulation, angiogenesis, stemness, epithelial-mesenchymal transition, chemoresistance, and immunomodulation (1–3, 5). More importantly, findings from prior studies reveal the regulation of *ID* proteins expression and function via *ID*-specific antisense oligonucleotides, small interfering RNAs, or nanocomplexes, which in turn affect micro-angiogenesis and apoptosis in various types of tumor cells (4, 6, 7). Taken together, ID proteins may be promising and effective predictive biomarkers and anti-angiogenic or anti-apoptotic agents for cancer management.

The incidence of OC has been on the rise; in 2018, it was estimated that 184,799 million deaths and 295,414 new cases of OC were recorded worldwide (8). This situation is expected to worsen globally. Although standard tumor reduction surgery combined with chemotherapy, and recent application of targeted therapies have significantly improved the survival chances of OC patients, this condition still shows a 5-year survival rate below 40% (9). The poor prognosis and high mortality rate are largely a result of less sensitive and suboptimal tools for early diagnosis, high recurrence following surgery resection, distant metastasis, and resistance to systemic chemotherapy and molecular drugs (10, 11). Therefore, effective prognostic markers and promising molecular therapeutic strategies for OC patients are highly desirable.

Recently, it was reported that several *ID* proteins are aberrantly expressed in OC samples in comparison with normal tissues, and the level of *ID* is closely related to poor differentiation, advanced stage, enhanced malignant potential, and worse clinical pathological features of OC (12–14) (15). Elevated expression levels of *ID1* and *ID3* were found to be a strong predicator of shorter survival in OC (14, 15). These reports show that *ID* could be a promoter of OC progression and tumorigenesis. More importantly, animal experiments showed that partial loss function or knockdown of *ID1* and *ID3* decreased proliferation, anchorage-independent growth, increased apoptosis, and reduced survival in various human cancer cells (16, 17). There is reason to believe that *IDs* may be novel therapeutic genes and potentially versatile therapeutic targets for OC. Regrettably, the distinct roles of the individual *ID* proteins in OC are not fully known. In our study, we comprehensively analyzed the relationships between the four *ID* subtypes and OC based on several large databases such as cBioPortal, Kaplan-Meier plotter (KM plotter), Gene Expression Profiling Interactive Analysis (GEPIA), SurvExpress, TIMER, and FunRich, to determine the expression patterns, genetic alterations, immune infiltrations, molecular function, and prognostic signature of ID proteins in OC.

Materials And Methods

Ethics statement

All protocols and experiments in this study conformed to the Declaration of Helsinki and were approved by the Academic Committee of the First People's Hospital of Yichang. The data used in this study were obtained from published reports.

Oncomine analysis

The Oncomine (www.oncomine.org) contains massive cancer-related microarray datasets of DNA or RNA sequences. It is frequently used in genome-wide expression studies (18). Herein, it was employed to reveal the transcriptional profile of *ID* family members in patient specimens from different cancer types and healthy controls. Moreover, the Student's t-test was used to compare the expression levels between the two groups. Significant expressions were those with fold-change = 1.5; *P*-value = 0.001.

GEPIA dataset analysis

GEPIA (http://gepia.cancer-pku.cn) provides a platform for analyzing RNA sequencing dataset covering on 9,736 tumors and 8,587 normal specimens in the Genotype-tissue Expression dataset (GTEx) and the Cancer Genome Atlas (TCGA) projects. GEPIA is highly interactive and enables users to adjust various functions, such as dimensionality reduction analysis, correlation analysis, survival analysis, tumor/normal differential expression analysis, similar gene detection, and profiling plotting based on the pathological stage or type of cancer (19).

TCGA and cBioPortal analysis

TCGA (http://cancergenome.nih.gov/) comprises pathological and sequencing datasets for 30 types of cancers (20). On the other hand, cBioPortal is a freely-accessible cancer genomic web platform (http://www.cbioportal.org/), which may be used for integrative analysis and multi-functional visualization for clinical profiles and data of cancer genomics (21). In this study, the dataset "Ovarian Serous Cystadenocarcinoma (TCGA, Provisional)" was used. The frequency of ID family gene alterations, copy number variance, and mRNA expression z-scores (RNA Seq V2 RSEM) were assessed using cBioPortal in line with the guidelines provided on the cBioPortal webpage.

Functional enrichment analysis

FunRich is an open access gene interaction network analysis tool and enables comprehensive functional annotation of various biological processes (22). In the present study, processes and pathways enrichment analyses of ID family proteins were performed using FunRich to identify genes associated with ID expression. In addition, the Gene Ontology (GO) terms for cellular component (CC), molecular function (MF), and biological process (BP) categories, as well as the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enrichment analysis of the 50 closely related genes significantly associated with ID expression were performed through FunRich.

Kaplan–Meier plotter analysis

KM plotter (www.kmplot.com) platform to estimate the prognostic performance of ID mRNA expression. This database constitutes the survival information and gene expression datasets of 1,816 patients with OC (23). We then subcategorized patients into low and high expression groups on the basis of the median expression value, and assessed the progression-free survival (PFS), and overall survival (OS) of OC patients employed the Kaplan-Meier survival plot, with log-rank p-value, a hazard ratio (HR) with 95% confidence intervals (CI). Further sub-classification was performed; TP53 mutation status, histological subtypes and pathological subtypes, foe subgroup analysis.

SurvExpress analysis

The SurvExpress (http://bioinformatica.mty.itesm.mx/SurvExpress), a web-based resource, is commonly used for risk assessment and survival multivariate analysis using gene expression data (24). This database was employed herein for risk assessment and survival analysis to identify key *ID* gene signatures in OC. A prognostic index established was utilized to group patient samples into high or low-risk groups in reference to the median value of the index by employing the maximized risk algorithm. The log-rank *p*-value, log-rank test with HR with 95% CI was utilized for statistical analysis of the equality of survival curves.

PROGgeneV2 analysis

The PROGgeneV2 is a web based tool available at www. compbio.iupui.edu/proggene. It contains data from 134 cohorts from 21 cancer types based on the Gene Expression Omnibus (GEO), the European Bioinformatics Institute (EBI) and TCGA. In the present study, PROGgeneV2 was used to validate the relationship between the expression of IDs and prognostic outcomes in OC. The HRs and the corresponding 95% CIs were used to assess the prognostic efficiency of IDs on OC. HRs and 95% CIs for OS were directly obtained from PROGgeneV2. Different data sets were meta-synthesized using STATE 14.0 software (State Corporation, College Station, TX, USA). The heterogeneity among studies was estimated with the v2-based Q-test and Higgins' I^2 statistic. A p-value < 0.05 for the Q-test or I2 > 30% indicated significant heterogeneity, and the random-effects model was used; otherwise, the fixed-effects model was used.

TIMER analysis

The extent of immune infiltration among various types is often estimated using the TIMER (https://cistrome.shinyapps.io/timer platform) (25). This tool was therefore utilized to assess the correlation of *IDs* expression with six immune infiltrates (DCs, macrophages, neutrophils, CD4 + T cells, B cells, and CD8 + T cells) in OC using Spearman's correlation analysis. On the basis of this correlation module, we established scatter plots between a pair of user-defined genes for each type of cancer, and the expression of each gene was presented with log2 RSEM.

Results

Mapping the mRNA expression profile to IDs in OC samples

The human genome contains genes encoding four *ID* family members. The Oncomine database was employed to compare the expression pattern of *ID* genes in cancer samples and normal tissue samples, and the results are presented in Fig. 1 and Table 1. Notably, cancer samples displayed the lowest expression of ID1 mRNA among the three datasets (26, 27). It was reported that *ID1* is decreased in ovarian serous adenocarcinoma when compared to normal samples in the Yoshihara datasets (27) and Hendrix (26). In the TCGA dataset also, *ID1* also downregulated in ovarian serous cystadenocarcinoma in comparison to normal samples, with a fold change of -2.901. Similarly, the transcriptional level of *ID2* was significantly downregulated in patients with OC in the three datasets (26–28). In the Hendrix (26) and Yoshihara dataset (27), *ID2* was significantly downregulated with fold changes of -1.523 and –6.008, respectively, in ovarian serous adenocarcinoma while it was downregulated in ovarian serous surface papillary carcinoma with a fold change of -11.999 in the Welsh dataset (28). A similar trend was also found for *ID3*. The *ID3* mRNA expression was markedly lower in multiple types of ovarian cancer compared to that in normal tissues in the Welsh (28), Yoshihara (27), and Hendrix (26) datasets. The mRNA level of *ID4* was significantly lower in ovarian carcinoma and ovarian serous cystadenocarcinoma than that in the normal samples in the Bonome (29) and TCGA datasets.

Table 1
The mRNA levels of IDs in between ovarian normal tissues and different types of OC (ONCOMINE).

ID family	Types of Ovarian cancer vs. Norma	t-test	Fold change	*P* value	Ref	PMID
ID1	Ovarian Serous Adenocarcinoma vs. Normal	-9.123	-1.531	**7.32E-10**	Hendrix Ovarian	16452189
	Ovarian Serous Cystadenocarcinoma vs. Normal	-6.753	-2.901	**6.20E-05**	TCGA Ovarian	-
	Ovarian Serous Adenocarcinoma vs. Normal	-6.084	-4.067	**9.16E-8**	Yoshihara Ovarian	19486012
ID2	Ovarian Serous Adenocarcinoma vs. Normal	-13.880	-1.523	**1.11E-16**	Hendrix Ovarian	16452189
	Ovarian Serous Surface Papillary Carcinoma vs. Normal	-8.342	-11.999	**7.84E-09**	Welsh Ovarian	11158614
	Ovarian Serous Adenocarcinoma vs. Normal	-10.929	-6.008	**3.25E-15**	Yoshihara Ovarian	19486012
ID3	Ovarian Serous Surface Papillary Carcinoma vs. Normal	-7.992	-6.756	**1.46E-07**	Welsh Ovarian	11158614
	Ovarian Serous Adenocarcinoma vs. Normal	-11.731	-9.796	**6.38E-15**	Yoshihara Ovarian	19486012
	Ovarian Clear Cell Adenocarcinoma vs. Normal	-8.933	-1.946	**4.14E-06**	Hendrix Ovarian	16452189
	Ovarian Serous Adenocarcinoma vs. Normal	-9.049	-1.785	**8.25E-06**	Hendrix Ovarian	16452189
ID4	Ovarian Carcinoma vs. Normal	-15.628	-7.350	**2.44E-11**	Bonome Ovarian	18593951
	Ovarian Serous Cystadenocarcinoma vs. Normal	-6.289	-3.362	**1.22E-04**	TCGA Ovarian	-

We also investigated the mRNA expression levels of *IDs* in OC compared to that in normal tissue using the GEPIA dataset. As shown in Fig. 2A **-** **D**, *ID1-ID3* mRNA transcripts were relatively low in OC tissues compared to normal ovarian tissues, however, only the levels of *ID2* and *ID3* showed marked differences between OC and normal tissues. In addition, analysis of the GEPIA dataset indicated that the mRNA level of *ID* was not related to the different stages of OC (Fig. 2E - H).

Genetic alteration rate of IDs and co-expressed genes in OC patient samples

Gene variations of *IDs* in OC were examined on the cBioPortal. As shown in Figure. 3, a total of 594 patients and 606 samples from the TCGA provisional dataset of ovarian serous carcinoma were analyzed. The genetic alteration rates of *ID1*, *ID2*, *ID3*, and *ID4* were 10, 6, 3, and 15%, respectively (Fig. 3A). We further explored the impact of *IDs* genetic alterations on the prognosis of OC. Notably, no significant association between the prognosis of OC with *ID* gene alteration or without alteration based on the TCGA provisional dataset (*p* values, 0.404 and 0.759, Fig. 3B, C).

Functional enrichment analysis of IDs and co-expressed genes in patients with OC

We subsequently compiled a list of the expressed *IDs* and the 50 closest co-expressed genes predicted by analyzing GO and KEGG in Funrich. As shown in Fig. 4, the BP of IDs and their co-expressed genes were dramatically concentrated in processes related to regulation of nucleic acid metabolism, nucleobase, nucleoside nucleotide and regulation of gene expression, peptidolysis, proteolysis, organogenesis, and regulation of immune response (Fig. 4A). The MF of these genes were mainly transcription regulator activity, protease inhibitor activity, protein binding, antigen binding, and protein serine/threonine phosphatase activity (Fig. 4B). For the CC, the genes were correlated with nuclear membrane, protein kinase, CK2 complex, nucleus, junctional sarcoplasmic reticulum membrane, and connexon complex (Fig. 4C). Additionally, the KEGG analysis revealed significant enrichment of genes in *ID-, c-MYC-, TNF-*, and Wnt signaling pathways (Fig. 4D).

Prognostic value of IDs in OC samples

We subsequently assessed the correlation of individual *IDs* with different clinical pathology parameters such as pathological grade, clinical stage, and TP53 mutation status of OC. The results presented in Fig. 5 and Table 2 indicates that high mRNA expression of *ID1* and *ID3* predicted worse PFS and OS in serous OC patients. In contrast, the mRNA level of *ID4* predicted favorable OS. In endometrioid OC, the expression of *ID1* and *ID3* showed a strong correlation with good PFS. As shown in Table 3, in OC patients with pathological grade III, elevated *ID1* and *ID3* correlated with poor PFS and OS. In patients with pathological grade II, *ID3* correlated with poor PFS and OS. In addition, upregulated *ID1* was linked to poor OS and upregulated *ID2* correlated with poor OS in pathological grade I patients. As shown in Table 4, in clinical stage III patients, increased expression of *ID1* and *ID3* was associated with worse OS, and elevated *ID2* was associated with poor PFS. In clinical stage IV OC patients, elevated ID1 was associated with worse OS and high *ID3* expression was related to poor OS and PFS in this subgroup. As shown in Table 5, Moreover, high expression of *ID1* and *ID3* was related was associated with worse PFS and OS in OC patients carrying mutated TP53, and high *ID1*, *ID2*, and *ID3* expression was associated with worse OS in OC patients with wild-type TP53.

Table 2
Correlation of the mRNA expression level of IDs with overall or different pathological histology OC prognosis (Kaplan-Meier plotter).

ID family	Affymetrix ID	Pathological histology	OS				PFS			
			Cases	HR	95% CI	p-value	Cases	HR	95% CI	p-value
ID1	208937_s_at	Overall	**1656**	**1.23**	**1.08–1.41**	**0.0023**	1453	1.09	0.96–1.24	0.1700
		Serous	**1207**	**1.23**	**1.04–1.46**	**0.0170**	**1104**	**1.29**	**1.12–1.49**	**0.0005**
		Endometrioid	37	0	0-inf	0.0180	**51**	**0.18**	**0.07–0.48**	**0.0001**
ID2	213931_at	Overall	1656	0.87	0.75–1.01	0.0590	**1435**	**1.18**	**1.04–1.34**	**0.0110**
		Serous	1207	1.13	0.97–1.33	0.1100	**1104**	**1.18**	**1.02–1.36**	**0.0300**
		Endometrioid	37	0	0-inf	0.1300	51	0.46	0.18–1.18	0.0990
ID3	207826_s_at	Overall	**1656**	**1.35**	**1.16–1.56**	**0.0001**	**1435**	**1.19**	**1.05–1.35**	**0.0076**
		Serous	**1207**	**1.42**	**1.19–1.69**	**0.0001**	**1104**	**1.34**	**1.14–1.57**	**0.0004**
		Endometrioid	37	0	0-inf	0.0710	**51**	**0.21**	**0.07–0.59**	**0.0011**
ID4	209291_at	Overall	**1656**	**0.82**	**0.71–0.95**	**0.0071**	1435	1.09	0.96–1.23	0.1900
		Serous	**1207**	**0.84**	**0.72–0.98**	**0.0240**	1104	0.89	0.76–1.04	0.1300
		Endometrioid	37	0	0-inf	0.0920	51	4.28	0.97–18.93	0.0380

Table 3
Correlation of the mRNA expression level of IDs with different pathological grade OC prognosis (Kaplan-Meier plotter).

ID family	Affymetrix ID	Pathological grades	OS				PFS			
			Cases	HR	95% CI	p-value	Cases	HR	95% CI	p-value
ID1	208937_s_at		56	1.78	0.65–4.83	0.2500	37	1.74	0.48–6.32	0.4000
			324	**1.5**	**1.06–2.12**	**0.0200**	256	1.2	0.89–1.61	0.2200
			1015	**1.23**	**1.04–1.45**	**0.0140**	**837**	**1.25**	**1.06–1.48**	**0.0078**
			20	2.05	0.76–5.53	0.1500	19	-	-	-
ID2	213931_at		**56**	**3.38**	**1.18–9.72**	**0.0170**	37	2.48	0.76–8.08	0.1200
			324	1.12	0.81–1.55	0.4800	**256**	**1.36**	**1.02–1.82**	**0.0380**
			1015	0.93	0.78–1.1	0.3700	837	1.09	0.93–1.29	0.2900
			20	0.35	0.1–1.23	0.0880	19	-	-	-
ID3	207826_s_at		56	1.6	0.53–4.79	0.4000	37	3.7	0.48–28.49	0.1800
			324	**1.5**	**1.11–2.04**	**0.0087**	**256**	**1.42**	**1.02-2.00**	**0.0390**
			1015	**1.3**	**1.08–1.57**	**0.0063**	**837**	**1.33**	**1.11–1.61**	**0.0023**
			20	1.69	0.64–4.44	0.2800	19	-	-	**-**
ID4	209291_at		56	0.62	0.24–1.59	0.3200	37	0.35	0.11–1.08	0.0570
			324	1.16	0.85–1.58	0.3400	256	1.26	0.93–1.71	0.3100
			1015	**0.85**	**0.75-1**	**0.0480**	837	0.91	0.75–1.09	0.3000
			20	3.06	0.91–10.34	0.0610	19	-	-	-

Table 4
Correlation of the mRNA expression level of IDs with different clinical stage OC prognosis (Kaplan-Meier plotter).

ID family	Affymetrix ID	clinical stage	OS				PFS			
			Cases	HR	95% CI	p-value	Cases	HR	95% CI	p-value
ID1	208937_s_at		74	2.9	0.93–9.09	0.0550	96	2.21	0.62–7.92	0.2100
			61	1.89	0.58–6.2	0.2800	67	0.45	0.18–1.09	0.0700
			1044	**1.45**	**1.23–1.7**	**0.0000**	**919**	**1.24**	**1.07–1.45**	**0.0056**
			176	0.78	0.52–1.16	0.2100	**162**	**1.91**	**1.30–2.80**	**0.0007**
ID2	213931_at		74	0.46	0.14–1.52	0.1900	96	0.44	0.15–1.32	0.1300
			61	2.76	0.92–8.28	0.0590	67	2.29	1.1–4.76	0.0220
			1044	0.87	0.73–1.02	0.0840	**919**	**1.19**	**1.01–1.41**	**0.0380**
			176	0.71	0.48–1.05	0.0880	162	0.71	0.48–1.05	0.0810
ID3	207826_s_at		74	3.78	0.49–29.37	0.1700	96	0.51	0.17–1.53	0.2200
			61	0.54	0.17–1.73	0.2900	67	0.45	0.18–1.08	0.0670
			1044	**1.48**	**1.23–1.78**	**0.0000**	**919**	**1.26**	**1.08–1.47**	**0.0031**
			176	**1.67**	**1.12–2.49**	**0.0110**	**162**	**1.62**	**1.10–2.38**	**0.0140**
ID4	209291_at		74	2.3	0.5–10.5	0.2700	96	0.52	0.18–1.48	0.2100
			61	2.32	0.52–10.42	0.2600	67	2.23	0.99–4.99	0.0470
			1044	0.83	0.69–1.01	0.0560	919	0.91	0.77–1.08	0.2800
			176	1.47	0.94–2.31	0.0920	162	1.53	0.96–2.43	0.0690

Table 5
Correlation of the mRNA expression level of IDs with different TP53 mutation status OC prognosis (Kaplan-Meier plotter).

ID family	Affymetrix ID	TP53 mutation	OS				PFS			
			Cases	HR	95% CI	p-value	Cases	HR	95% CI	p-value
ID1	208937_s_at	mutated	**506**	**1.38**	**1.1–1.74**	**0.0052**	**483**	**1.47**	**1.17–1.84**	**0.0007**
		wild type	**94**	**1.74**	**1.01-3**	**0.0450**	84	0.68	0.39–1.2	0.1900
ID2	213931_at	mutated	506	1.21	0.96–1.52	0.0990	483	0.85	0.68–1.06	0.1500
		wild type	**94**	**2.12**	**1.13–3.97**	**0.0170**	84	1.34	0.76–2.37	0.3200
ID3	207826_s_at	mutated	**506**	**1.51**	**1.17–1.94**	**0.0012**	**483**	**1.37**	**1.07–1.76**	**0.0120**
		wild type	**94**	**1.99**	**1.13–3.51**	**0.0160**	84	1.72	0.98–3.02	0.0560
ID4	209291_at	mutated	506	0.83	0.66–1.04	0.1000	483	1.32	1.04–1.68	0.0240
		wild type	94	0.56	0.3–1.03	0.0570	84	1.47	0.84–2.6	0.1800

Prognostic value of ID signatures in patients with OC

The SurvExpress platform was used to establish a prognostic index based on *ID* expression. A total of 1,609 patients from three ovarian cancer datasets with large sample sizes were analyzed using the SurvExpress platform. High/low risk groups were categorized by prognostic risk algorithm in each dataset. The survival analysis and Kaplan–Meier plotter between high risk (red) and low risk (green) groups and the heat map of the expression of *IDs* in each dataset are shown in Fig. 6. The results showed that the expression of each *ID* member was distributed between high and low risk groups. More importantly, the low risk group displayed a significantly good OS in comparison with the high risk group in the ovarian Meta-base: 6 cohorts with 22 K genes (HR = 1.44, 95% CI = 1.19–1.75), ovarian serous cystadenocarcinoma TCGA (HR = 1.28, 95% CI = 1.02–1.60) and OV – TCGA - ovarian serous cystadenocarcinoma June 2016 (HR = 1.40, 95% CI = 1.01–1.95) datasets, respectively.

Validate the prognostic value of IDs in patients with OC in different data sets

We applied PROGgeneV2 to validate the he prognostic value of *IDs* in patients with OC in different data sets. The results showed that seventeen data sets with 2,585 subjects reported the data of relationship between *ID1* and OS in patients with OC. The pooled result showed that increased *ID1* expression was significantly correlated with worse OS (HR: 1.08, 95% CI: 1.01–1.14, $p = 0.017$), with significant heterogeneity (I^2: 36.5%, $Ph = 0.065$) (Fig. 7A). In addition, the same 17 data sets reported data on the association between *ID2* and OS in patients with OC. Meta-analysis of these 17 sets showed that there was no significant correlation between the expression of *ID2* and the OS of OC patients (HR: 1.02, 95% CI: 0.96–1.09, $p = 0.$ 0.617), and with no significant heterogeneity (I^2: 10.3%, $Ph = 0.334$)

(Fig. 7B). Simultaneously, there were 18 data sets containing 2,663 OC patients the prognostic value of *ID3* and *ID4* in OS. As shown in Fig. 7C, the elevated *ID3* expression was significantly associated with unfavorable OS (HR: 1.10, 95% CI: 1.04–1.16, $p < 0.001$) and no significant heterogeneity was observed (I^2: 11.5%, *Ph* = 0.317). At last, the results presented in Fig. 7D indicates that increased *ID4* expression was positively correlated with better OS (HR: 0.90, 95% CI: 0.84–0.97, $p < 0.001$), with extreme heterogeneity (I^2: 55.0%, *Ph* = 0.003).

Immune infiltration analysis of IDs in patients with OC

We explored the correlation between *ID* expression and immune infiltration levels in OC using correlation modules in TIMER. As shown in Fig. 8, *ID1* expression level showed a significant negative correlation with infiltrating levels of B cells (r = -0.252, p = 2.07e-08) and DCs (r = -0.113, p = 1.30e-02). In contrast, it showed a positive correlation with infiltrating levels of macrophages (r= 0.15, p = 9.95e-04). *ID2* level showed a negative correlation with infiltrating levels of B cells (r = -0.110, p = 1.55e-02), and positive correlation with infiltrating levels of macrophages (r= 0.150, p = 9.95e-04) and neutrophils (r= 0.097, p = 3.39e-02) in OC. For *ID3*, there was a significant correlation with infiltrating levels of B cells (r = -0.184, p = 5.18e-5) and macrophages (r= 0.217, p = 1.68e-06). In addition, there was a negative correlation with infiltrating levels of macrophages (r = -0.098, p = 3.10e-02) and neutrophils (r = -0.14, p = 2.15e-03) in OC.

Discussion

Key among the genes that encode the helix-loop-helix (HLH) family of transcription factors is the *ID*, abundant in stem and progenitor cells (1). To date, it is known that ID proteins are encoded by four *ID* genes in the *ID* family in vertebrates: *ID1-4*, all of which encode the corresponding four *ID* family members (1, 2). These genes are located in different chromosomes and show inconsistent expression profiles and functions (30). Emerging evidence suggests that *ID* proteins play vital roles in tumorigenesis in several types of malignancies mediated by their ability to regulate cell-cycle, cell differentiation, epithelial-mesenchymal transition, chemoresistance, and immunomodulation (1–3, 5). Yet, the specific roles of the four *ID* members in OC are obscure. This study evaluated the prognostic value and expression of ID family genes by investigating various large databases. Our study presents the first silico and bioinformatics analysis of the ID family.

ID1 is the most widely characterized component of the HLH transcription factor family (31). Studies show that the molecular functions of *ID-1* included induction of cell proliferation, increasing DNA synthesis, and interaction with various oncogenes (32). Aberrant expression of the *ID1* protein has not only been detected in multiple types of human cancers, but is also correlated with tumor stages and clinical outcome (33, 34). Furthermore, ectopic expression of *ID1* in human cancer cells increases serum-independent cell growth, enhances primary tumor G1/S phase formation and metastatic potential, and protects tumor cells against apoptosis. Conversely, inhibition or inhibition of *ID1* in human cancer cells has been shown to suppress cell proliferation, induce cellular senescence, induce G2/M cell-cycle arrest, reduce tumor colony formation or multiplicity, and increase lifespan (35, 36). In OC, Schindl et. al. found that *ID1* expression correlates with the malignant potential of OC and is correlated with aggressive behavior, differentiation of tumor cells, and clinical prognosis (12). Several studies have found that increased *ID1* may promote cancer cell proliferation and enhance endothelial progenitor cell angiogenesis through regulation or facilitation of EGFR and TGFβ1 expression, and activation NF-κB/MMP-2 and PI3K/Akt signaling pathways in OC cells (6, 37–39). In addition, the study by Li ZD et al demonstrated that apigenin can suppress the expression of *ID1*, resulting in inhibition of tumorigenesis in human OC A2780 cells (40). Thus, *ID1* represents a promising therapeutic target for OC. In our study, the Oncomine and GEPIA datasets indicated that the expression of *ID1* was suppressed in human OC.

The Kaplan–Meier plotter and PROGgeneV2 analysis revealed a high mRNA expression of *ID1*, and this was correlated with poor OS in all OC patients. These data reflect the heterogeneity of *ID1* expression in mRNA and protein levels, and point to the oncogenic function of *ID1*.

ID2 belongs to the HLH transcription factor family, which promotes proliferation and invasive growth in multiple solid cancers, e.g., hepatocellular cancer, breast cancer, thyroid cancer, pancreatic cancer, and OC (1, 41). Like *ID1*, several studies have shown that *ID2* promotes the proliferation of human cancer cells by inhibiting cell apoptosis, enhancing cancer stemness of pre-malignant cells, or mediating m6A modifications (42–44). Conversely, reduced *ID2* expression increases apoptosis, reduces cell proliferation, and decreases tumor initiation in human cancer cells (16, 45). However, currently there are very few reports on *ID2* and OC development in the literature. An earlier study showed the *ID2* gene as a candidate for inherited predisposition to breast and ovarian cancer in Jewish women (46). Moreover, the study by Meng et. al. reported that elevated *ID2* expression in ERα-positive epithelial tumor cells promoted the invasiveness of cells via a non-canonical pathway independent forming dimers with basic helix-loop-helix factors (47). In this study, *ID2* mRNA expression was found to be lower in OC samples than in normal ones, and elevated *ID2* expression was strongly related to poor PFS in all patients with OC. Prognostic analysis in patients with OC in different data sets, however, overall effect did not show any significant correlation between *ID2* expression and OS. The oncogenic effects, predictive value, and potential molecular targets of *ID2* in OC remain to be investigated further.

ID3, associated with HLH transcription factors, has been recognized as a key regulator of cell development, senescence, differentiation, proliferation, stemness, and migration (1, 48). It has been confirmed that *ID3* and *ID1* can compensate for each other and have similar biological functions (5). Previous studies have demonstrated that aberrant expression of *ID3* is associated with advanced tumor stage and poor prognosis in many types of human cancers. In animal experiments, although Id1+/-Id3-/- or Id1-/-Id3+/- mice grow to adulthood, they are unsuitable to implanted tumor xenografts because these mice lack the capacity to recruit hematopoietic precursors and endothelial (48, 49). Furthermore, double knockdown of *ID1* and *ID3* has been shown to decrease proliferation and anchorage-independent growth, increase apoptosis, and reduce survival in various human cancer cells (16, 17). More importantly, *ID3* knockdown improved the survival duration of animals in a seeding model of medulloblastoma. It also compromised the progression of leptomeningeal seeding and the growth of primary tumors (50). Elsewhere, it was recognized that BMP4 signaling is active in ovarian cancer cells where it maintains *ID3* expression. This was confirmed by the use of BMP4 blocker Noggin, which decreased endogenous *ID3* expression (51). In this study, we also demonstrated that the expression of *ID3* in OC tissues was lower than that in normal tissues, and *ID3* overexpression was associated with reduced OS and PFS in OC patients. Because *ID3* undergoes epigenetic inhibition in multiple cancers, it is therefore thought to be a tumor suppressor.

In comparison with the other *ID* proteins, *ID4* possesses a polyproline domain at its C terminus and a polyalanine domain at its N terminus. Although it harbors the HLH domain, *ID4* does not display similar expression and function with D protein (5). Numerous studies have shown that the phenotypic changes and molecular pathways regulated by *ID4* are, in general, not like those regulated by *ID1*, *ID2*, and *ID3*. Interestingly, *ID4* seems to function as a tumor suppressor in multiple cancers and as a tumor promoter in a small subset of cancers (30, 52, 53). The proposed tumor-suppressing effects of *ID4* draw on observations that ID4 undergoes epigenetic silencing in several solid cancers such as esophageal, gastric, pancreatic, colorectal, cholangiocarcinoma and lung cancer. However, ID4 has been reported to be elevated in some small cancers, such as OC, prompting researchers to re-classify it as a tumor promoter (4, 52, 54, 55). Mice deficient in *ID4* develop some types of cancers in their lifetime, and the lack of *ID4* results in follicular dysplasia and increased atretic follicles due to decreased estrogen biosynthesis (30). It is worth

noting that a recent study showed that administration to mice harboring an ovarian tumor with an *ID4*-specific tumor-penetrating nanocomplex was capable of suppressing the growth of established tumors and significantly improved survival (7). In the current study, unlike *ID1, ID2*, and *ID3*, the expression of *ID4* was higher in OC tissues than that in normal tissues, and high *ID4* expression was significantly correlated with better OS in OC patients, thereby indicating its tumor promoter role in OC.

We also attempted to examine the mechanisms and roles of members of the *ID* family, we also used the cBioPortal database to explore the mutations in the *ID* family. The results showed that the genetic alteration rate of the *ID* family members varied from 3% to 15% for individual genes based on the TCGA provisional dataset, however, there was no significant association between the prognosis of OC with *ID* gene alteration or without alteration. We then constructed a network of *ID* family members and 50 of the closest co-expressed genes. The results of the functional analysis indicated that these genes were mainly enriched in tumor-related pathways, including the *ID-*, *c-MYC-*, *TNF-*, and Wnt signaling pathways. In addition, two major highlights of this study were the *ID* signature and immune infiltration analysis. In the *ID* signature analysis, the prognostic values of *ID* signature in patients with OC were evaluated in three datasets based on the SurvExpress platform. The method overcomes the problem single gene with the expression optimal cutoff for prognostic analysis cannot fully reflect the optimal differentiation of survival benefits and performance of potential biomarkers. In the immune infiltration analysis, we explored the correlation between *IDs* expression with six immune infiltration levels in OC via correlation modules in TIMER. Our results showed that *ID* expression showed a strong correlation with infiltrating levels of B cells and macrophages, which further confirmed that the biological role of *ID* may be associated with immune regulation. However, the underlying molecular mechanisms and regulation steps remain largely unexplored.

Conclusions

This study reveals that *IDs* exhibited diverse expression profiles between OC and normal samples. Aberrant expression of *ID1/3/4* was correlated with cancer aggressiveness and prognosis in OC patients. The group with low risk *ID* signature presented a markedly good OS relative to the high-risk group. In contrast, the expression levels of *IDs* were significantly correlated with the levels of infiltrating B cells and macrophages. Finally, enrichment analysis showed that ID co-expressed genes were involved in *ID-*, *c-MYC-; TNF-*, and Wnt signaling pathways. These results indicate that *ID1/3/4* may be exploited as promising prognostic biomarkers and therapeutic targets in OC patients.

Abbreviations:

ID, inhibitor of differentiation/DNA-binding; OC,ovarian cancer; HLH ,helix-loop-helix; GTEx, the Genotype-tissue Expression dataset; TCGA, the Cancer Genome Atlas; EBI, European Bioinformatics Institute; GO, Gene Ontology; CC, cellular component; MF, molecular function; BP, biological process; KEGG, Kyoto Encyclopedia of Genes and Genomes ; PFS, progression-free survival ; OS, overall survival; HR,hazard ratio ; CI, confidence intervals; GEPIA, Gene Expression Profiling Interactive Analysis.

Declarations

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publishedarticle. Ethics approval and consent to participate Not applicable. Consent for publication Not applicable. Competing interests The authors declare that they have no competing interests.

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Figures

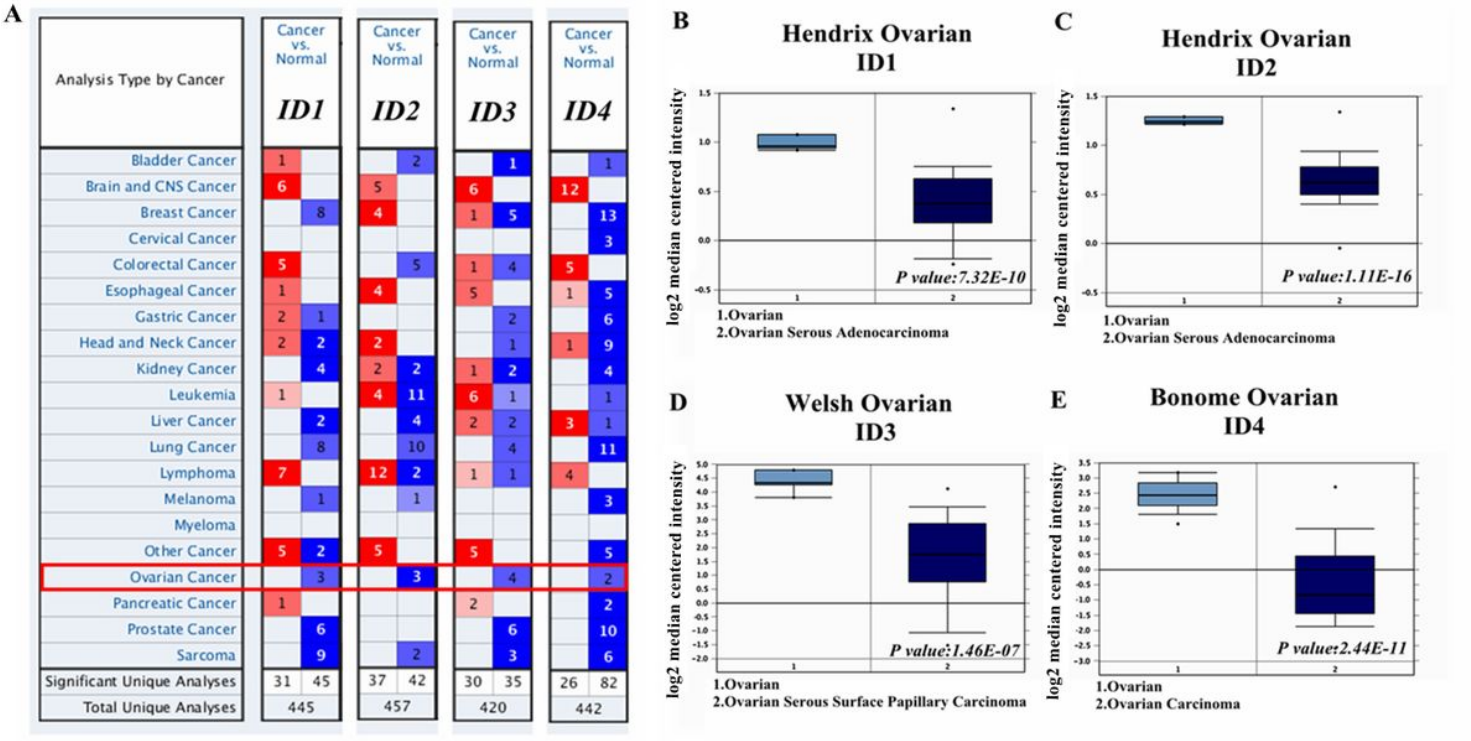

Figure 1

The mRNA levels of IDs in different types of cancers and OC (ONCOMINE). Note:The thresholds were restricted as follows: P value = 0.001; fold-change = 1.5; and data type, mRNA, respectively. (A) The mRNA levels of ID family members in different types of cancers. The graphic demonstrated the numbers of datasets with statistically significant mRNA over-expression (red) or down-expression (blue) of the target gene. (B)- (E) The mRNA levels of ID1-4 in human OC and normal tissue in four datasets, such as Hendrix Ovarian, Hendrix Ovarian, Welsh Ovarian, Bonome Ovarian, respectively.

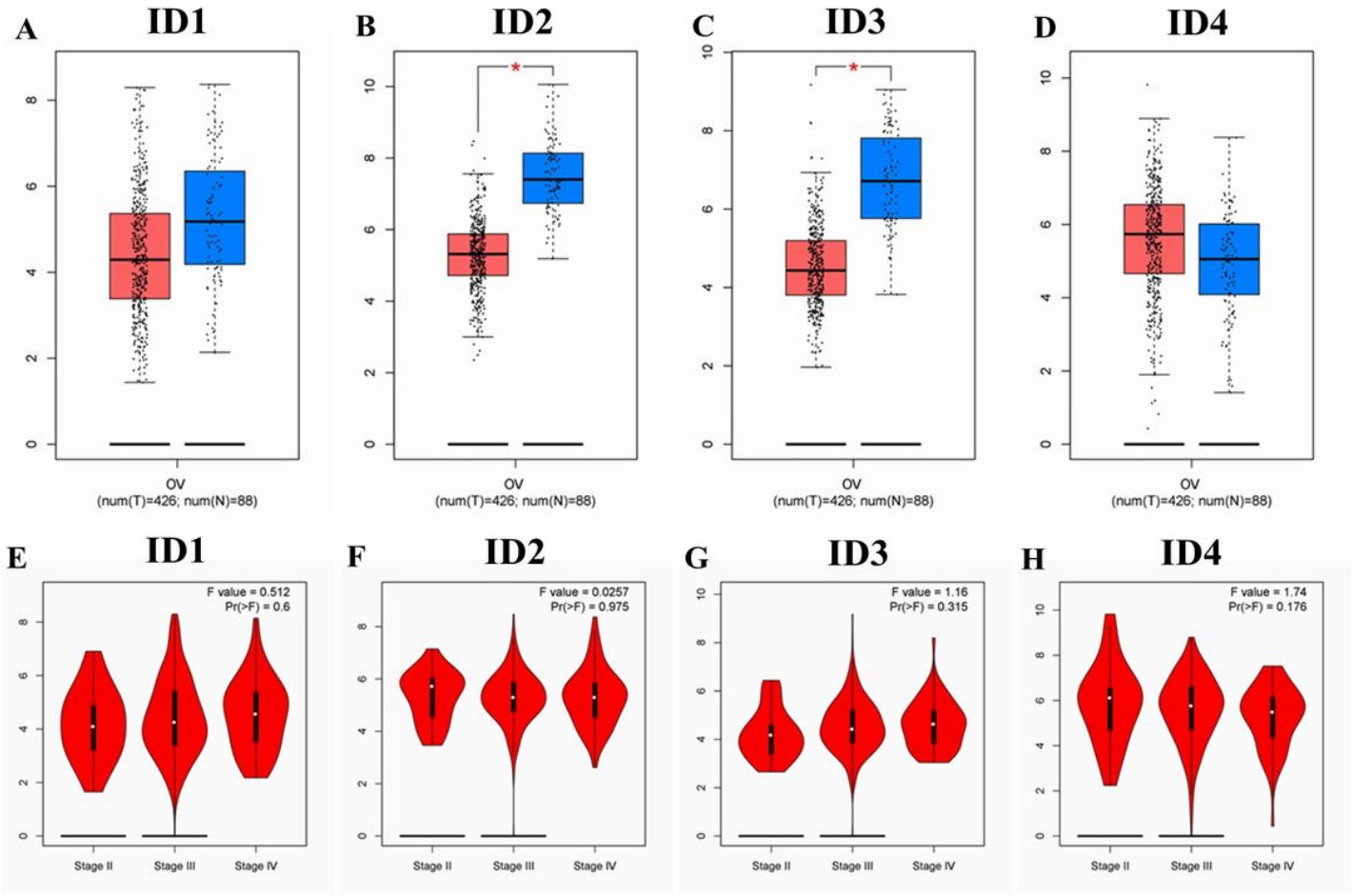

Figure 2

The mRNA expression levels of IDs in overall or subgroups of different stage OC patients (GEPIA database). Note: Box plots derived from gene expression data in GEPIA comparing expression of a specific ID family member in OC tissue and normal tissues, the p value was set up at 0.05. (A)- (D) The distribution of ID1-ID4 gene mRNA expression between OC tissue and normal tissues, respectively. (E)-(H) Boxplot showing relative expression of ID1-ID4 in OC patients in stages, 2, 3 or 4 using GEPIA, respectively. (*, P< 0.05; **, P< 0.01; ***, P < 0.001).

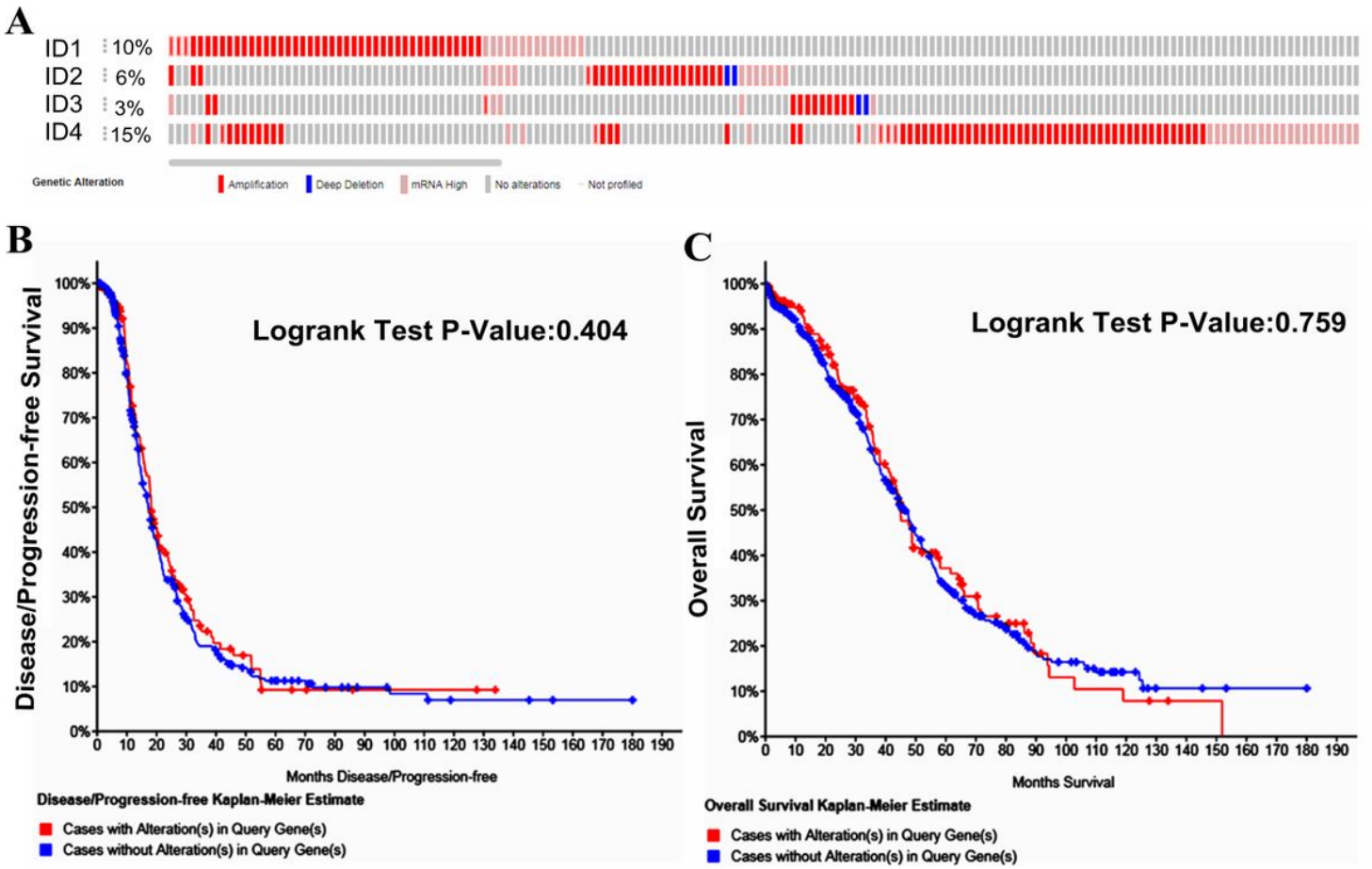

Figure 3

Alteration frequency and prognostic value of IDs in OC (TCGA and cBioPortal). (A) OncoPrint visual summary of alteration on a query of ID family members. (B) Kaplan-Meier plots comparing OS in cases with/without ID family members gene alterations. (C) Kaplan-Meier plots comparing disease free survival (DFS) in cases with/without E2F family members alterations.

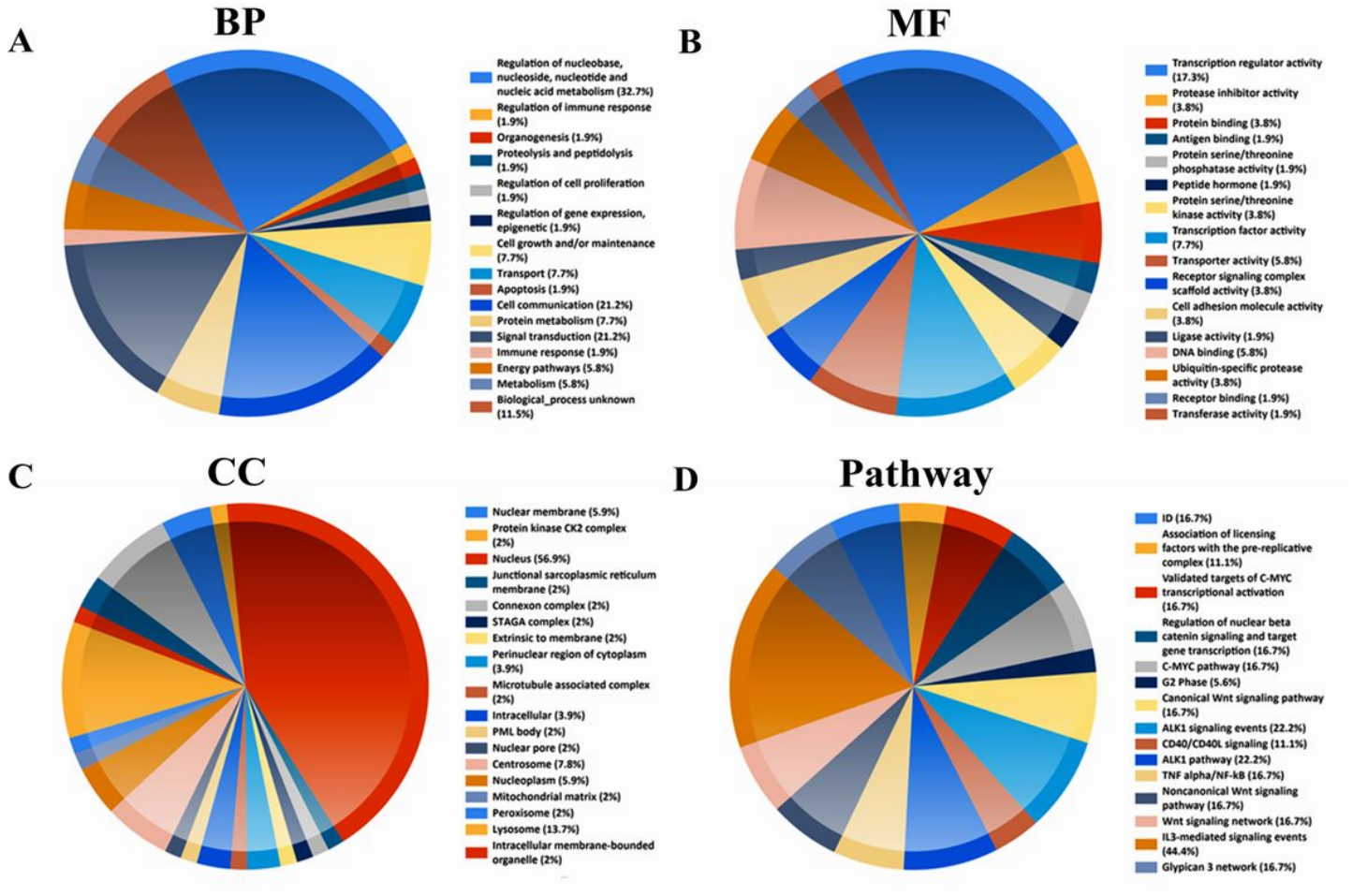

Figure 4

TheGene Ontology and KEGG enrichment analysis of IDs in OC (Funrich database). (A)-(D) The biological pathways and Gene Ontology (GO) terms for biological process (BP), molecular function (MF), cellular component (CC) categories and KEGG pathway enrichment analyses were performed through FunRich, respectively.

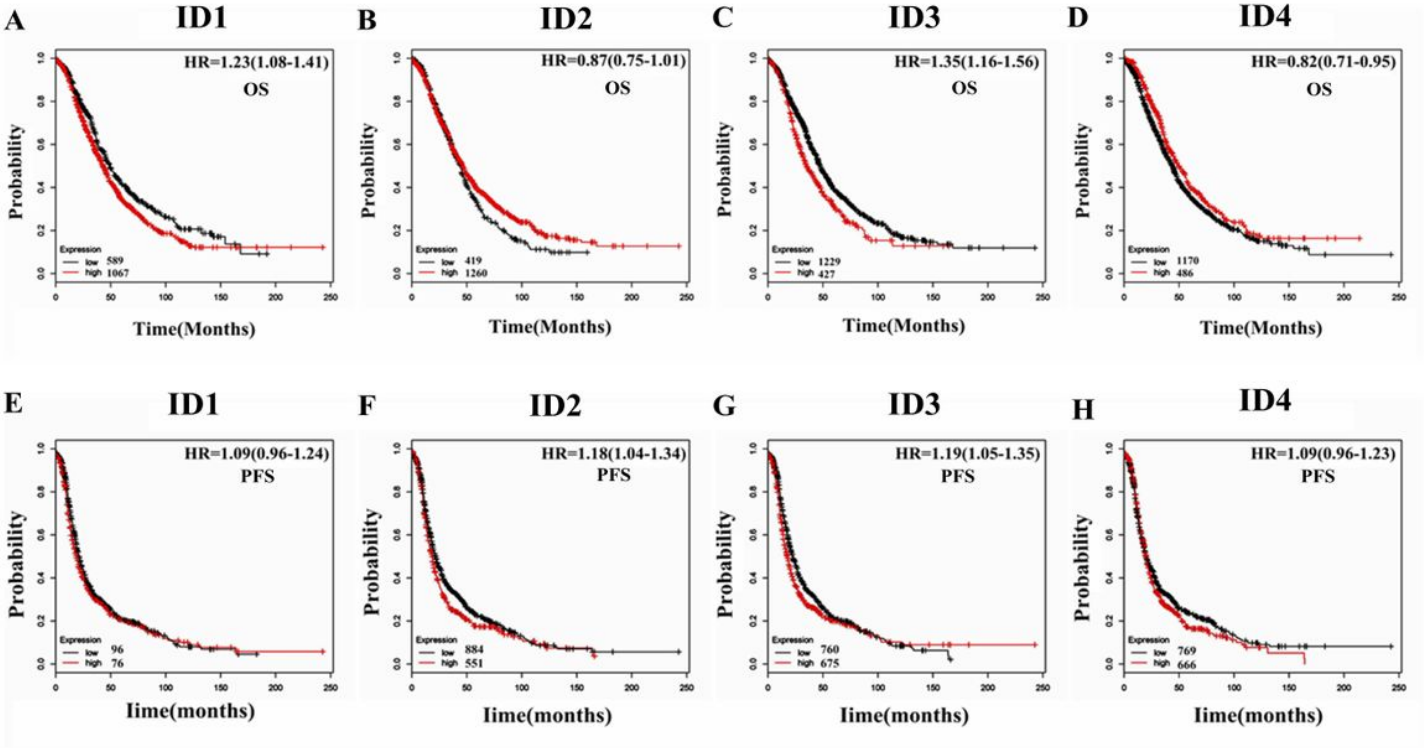

Figure 5

The prognostic value of the individual IDs (KM Plotter database). (A)- (D) The OS Kaplan-Meier survival curves of ID1-4 are plotted for OC patients by KM Plotter database; (E)- (H) The PFS Kaplan-Meier survival curves of ID1-4 are plotted for OC patients by KM Plotter database, respectively.

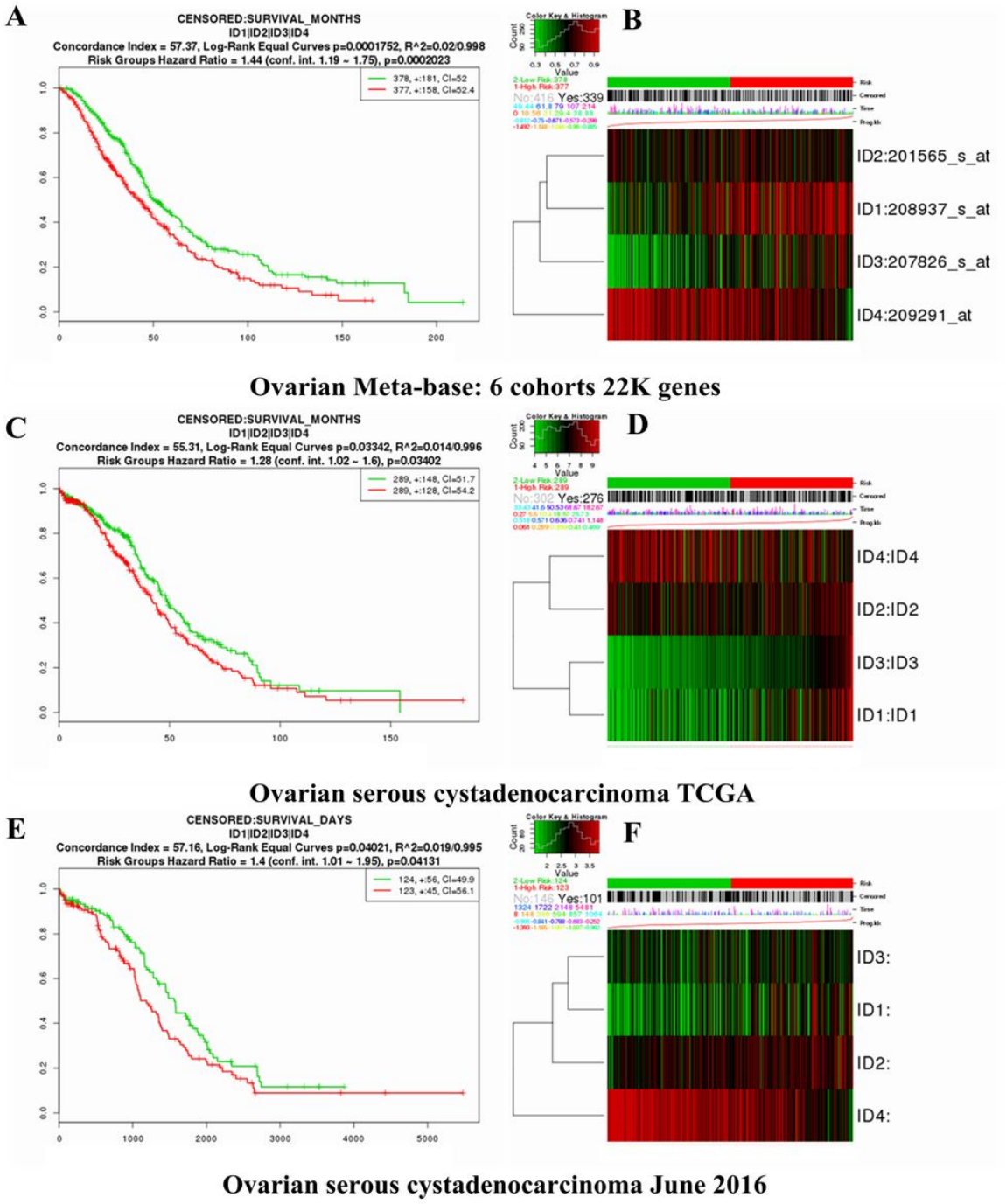

Figure 6

The genes signature of IDs in OC (SurvExpress database). (A)-(B) The Kaplan-Meier survival curves and heat maps of ID1-4 were explored in high risk and low risk group for ovarian Meta-base: 6 cohorts 22K genes; (C)-(D)The Kaplan-Meier survival curves and heat maps of ID1-4 were explored in high risk and low risk group forovarian serous cystadenocarcinoma TCGA; and (E)-(F) The Kaplan-Meier survival curves and heat maps of ID1-4 were explored in high risk and low risk groupfor OV - TCGA-ovarian serous cystadenocarcinoma June 2016 datasets, respectively.

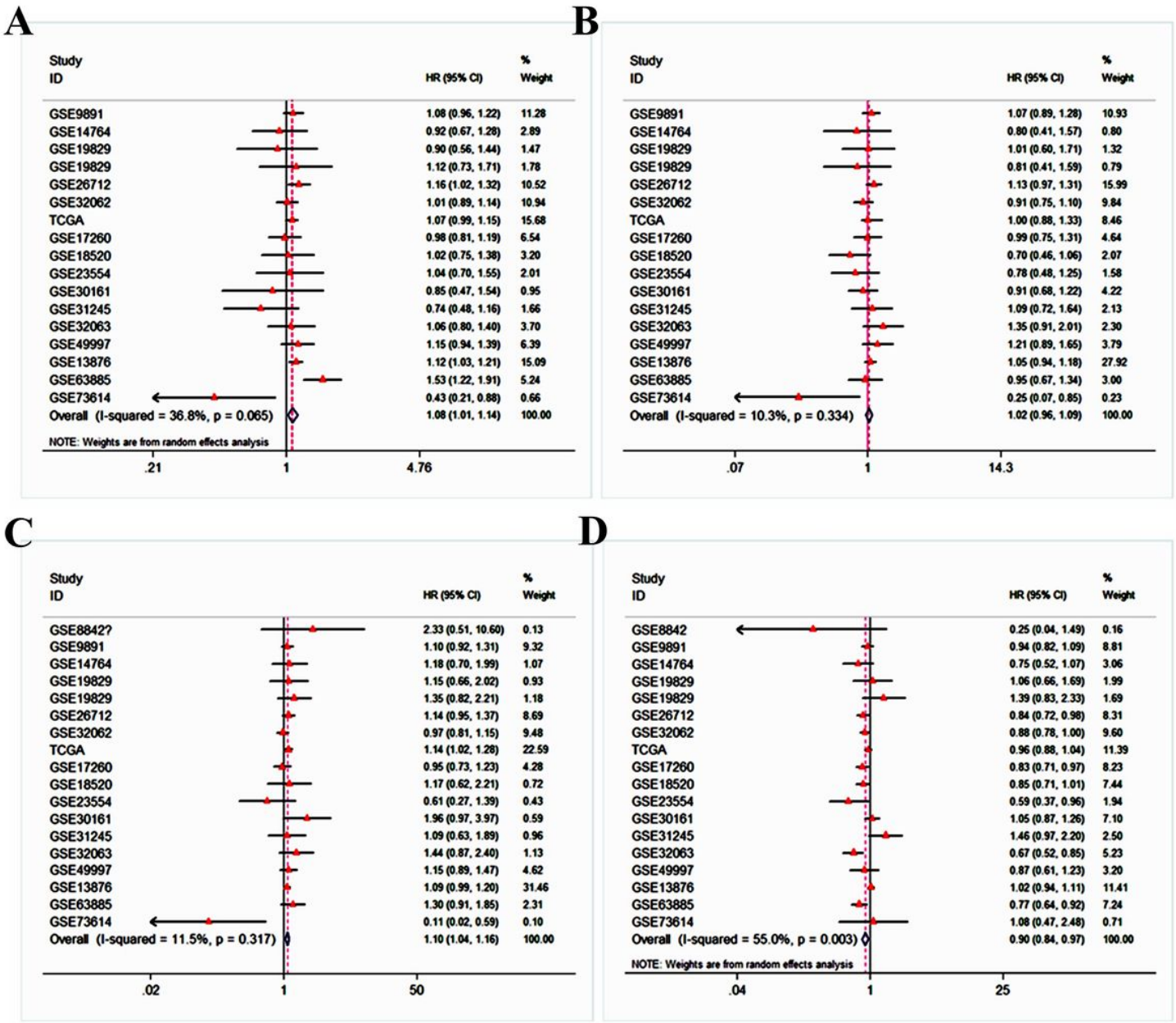

Figure 7

Validate the prognostic value of IDs in patients with OC in different data sets. (A)- (B) Validate the prognostic value of ID1-2 in patients with OC in 17 data setswith 2,585 patients, respectively. (C)- (D) Validate the prognostic value of ID1-2 in patients with OC in 18 data sets with 2,663 patients, respectively.

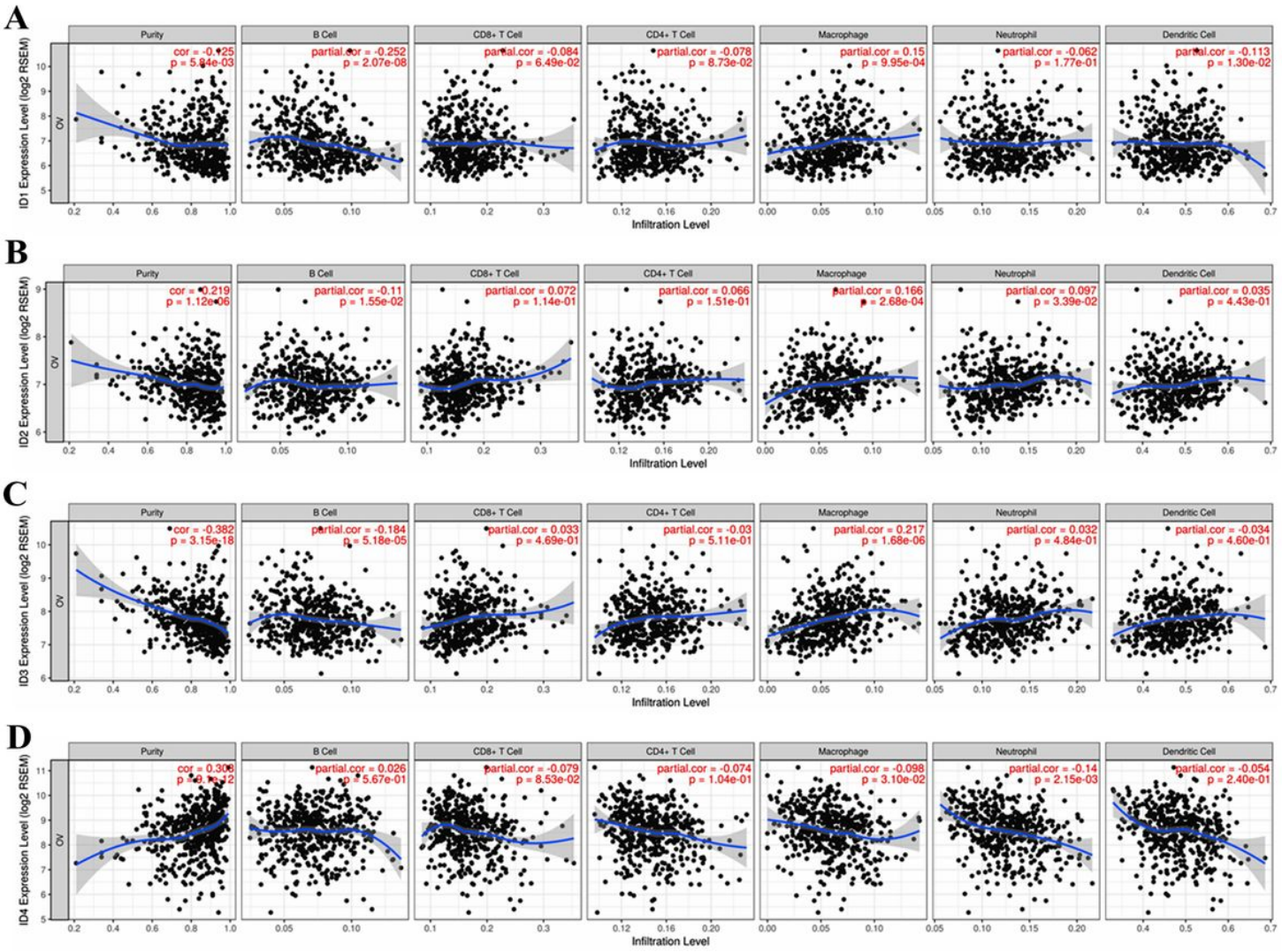

Figure 8

Correlation of the expression levels of ID family members with immune infiltration level in OC (TIMER database). (A)-(D) Correlation of ID1-4 expression with immune infiltration level in OC, respectively.

Supplementary Files

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- ID.xlsx
- TableS1.docx