Cancer Management and Research

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ORIGINAL RESEARCH

Identification of Prognostic Biomarkers Among FAM83 Family Genes in Human Ovarian Cancer Through Bioinformatic Analysis and Experimental Verification

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Purpose: Family with sequence similarity 83 (FAM83) is a newly discovered oncogene family, and the members of which can affect the prognosis of patients with malignant tumors via various mechanisms. However, the functions and molecular mechanisms of *FAM83* genes in ovarian cancer (OC) have not yet been investigated. This study aimed to explore the clinical significance and prognostic value of *FAM83* genes in OC.

Materials and Methods: We used a series of bioinformatics databases (Oncomine, GEPIA, cBioPortal, Kaplan–Meier plotter, DAVID and TIMER) to investigate the expression status, prognostic value, genetic alteration and biological function of all eight *FAM83* genes in OC. In addition, a tissue microarray cohort (TMA) comprising 99 ovarian tumor tissues and 19 normal ovarian tissues was used to validate the protein expression and clinicopathological significance of *FAM83H*.

Results: Several datasets demonstrated the mRNA levels of *FAM83A/D/E/F/H* were significantly higher in OC compared with that in normal tissue. Moreover, the upregulation of *FAM83D/H* has been mutually confirmed in the Oncomine and GEPIA datasets. Kaplan–Meier survival analysis indicated that the *FAM83D/H* upregulation could predict poor prognosis of OC patients who had shorter overall survival (OS) and progression-free survival (PFS). In addition, cBioportal analysis indicated that the genetic alterations of *FAM83* genes might affect the survival outcomes of patients with OC. Furthermore, KEGG analysis suggested that *FAM83D/H* are involved in the progression of OC through the cell cycle signaling pathway, and they had significant co-expression relationship with cell cycle-related genes. Finally, immunohistochemistry analysis confirmed the high expression of *FAM83H* protein in OC tissue, suggesting that its expression is positively correlated with the FIGO stage and pathological subtype of OC.

Conclusion: This study elucidated the expression status and prognostic value of *FAM83* genes in OC and identified that *FAM83D/H* might be potential targets for the prognostic monitoring and targeted therapy of OC.

Keywords: *FAM83s*, ovarian cancer, prognosis, immunohistochemistry, biomarker

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Introduction

Ovarian cancer (OC) is one of the most common gynecological malignancies and the leading cause of gynecological cancer-related worldwide, accounting for approximately 185,000 deaths annually.[1] Although surgical resection, chemoradiotherapy and immune targeted therapy have remarkably improved the survival

Received: 13 July 2021
Accepted: 23 October 2021
Published: 15 November 2021

of patients with OC in recent decades, the prognosis of these patients remains unsatisfactory, with 5-year overall survival (OS) rates being less than 30%.[2–4] The main reason for this situation is that the pathogenesis of OC is not yet completely clear.[5]

Cell signaling networks rigorously regulate numerous intracellular signaling pathways to precisely control the progression of cellular activities.[6] When these important signaling pathways are disrupted, abnormal cellular changes may occur, leading to tumorigenesis.[7] Therefore, the search for key factors involved in the intracellular regulatory networks of OC might proffer a theoretical basis for individualized diagnosis and treatment of OC. Recent evidence suggests that Family with sequence similarity 83 (FAM83) genes are aberrantly expressed in a variety of tumors and can facilitate aspects of their malignant progression, such as metastasis and drug resistance, leading to poor prognosis.[8,9] Additionally, these genes play crucial roles in several classical signaling pathways, such as the EGFR, MAPK and PI3K/AKT pathways, all of which are closely related to the occurrence and development of OC.[10,11] However, the relationship between *FAM83* genes and OC remains unclear. Thus, an in-depth exploration of the biological significance of *FAM83* genes in OC is warranted.

FAM83 is a newly discovered oncogene family consisting of eight genes (*FAM83A–H*), all of which have a highly conserved structural domain of unknown function (DUF1669) at their N-terminal ends.[12] The first family members to be identified and studied were *FAM83A* and *FAM83B*. Lee et al showed that *FAM83A* overexpression could make breast cancer cells resistant to tyrosine kinase inhibitors.[13] Meanwhile, Cipriano et al found that aberrant *FAM83B* expression induced carcinogenesis in breast epithelial cells by activating the RAS signaling pathway.[14] These studies demonstrated for the first time that members of this newly identified family play pivotal roles in cancer-related signaling pathways and can promote malignant transformation of cells when their protein function is dysregulated. Subsequently, the oncogenic effects of *FAM83* genes have been increasingly demonstrated in other cancer types, including OC. For instance, the upregulation of *FAM83B* in ovarian cancer cells increased their proliferation and growth rates and reduced the apoptosis rate.[15] Mechanistically, Zhu et al and Zhang et al verified that *FAM83D* overexpression could promote malignant progression of OC via the EGFR pathway or the PI3K/AKT/mTOR signaling pathway.[16,17] However, the clinical value and biological significance of other *FAM83* genes in OC have rarely been reported.

To our knowledge, this is the first study to comprehensively and systematically identify the expression status and prognostic significance of *FAM83* genes in OC by investigating several public databases. Besides that, *FAM83D* and *FAM83H* were identified, which could be potential biomarkers closely related to the malignant clinical features of OC, by immunohistochemistry (IHC) and clinicopathological analysis. In summary, the study provides a new viewpoint on the mechanisms of OC pathogenesis and novel targets for individualized diagnosis and treatment of OC.

Materials and Methods

Oncomine

Oncomine (https://www.oncomine.org/) is a mainstream public data-mining platform that contains gene expression data of 3606 OC samples and 12,764 normal samples for cancer genetic information analysis.[18] In this study, we compared the mRNA expression of the eight *FAM83* genes between OC tissue samples and normal tissue samples. The parameter-filtering criteria were as follows: p-value < 0.01, fold change > 1.5, gene rank in the top 10%, and data type: mRNA.

GEPIA

Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/) is a powerful bioinformatics analysis platform that contains extensive gene expression data of 9734 tumor samples and 8587 normal samples from TCGA and the GTEx projects.[19] It has notable advantages in revealing differences in expression of target genes between cancer and normal tissue. Hence, we compared the mRNA expression levels of *FAM83* genes between 426 OC and 88 normal ovarian tissue samples using GEPIA.

cBioPortal

The cBioportal (https://www.cbioportal.org/) database allows an in-depth investigation of tumor mechanisms from the DNA level, which greatly aids cancer researchers in revealing the relationship between molecular genomic profiles and clinical prognosis.[20] We thoroughly explored the genetic alterations, such as amplification, mutation and deep deletion, in the eight *FAM83* genes and investigated whether these gene alterations were associated with OS in patients with OC. In addition, the co-expression genes of

FAM83D/H were selected from cBioPortal database for functional enrichment analysis based on a Spearman correlation coefficient of > 0.35 or < −0.35.

Kaplan–Meier Plotter

Kaplan–Meier plotter (https://kmplot.com/analysis/) is a professional online tool that can clarify the effect of a certain gene on cancer prognosis.[21] We searched for *FAM83s* in the Kaplan–Meier plotter database to investigate their effect on OS and PFS in patients with OC. Considering their overexpression and significant relationship with poor prognosis, *FAM83D* and *FAM83H* were selected for further analysis in different subgroups with the aim to reveal the correlation of *FAM83D/H* with various clinicopathological factors.

GO Enrichment Analysis and KEGG Pathway Analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.abcc.ncifcrf.gov/) is a well-known online biological annotation tool that can identify the most significantly enriched processes from a large-scale gene or protein expression dataset.[22] We uploaded the *FAM83D/H* co-expression data into DAVID for Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. The results indirectly revealed the biological processes and signaling pathways in which that *FAM83D/H* are involved.

TIMER

The Tumor Immune Estimation Resource (TIMER, https://cistrome.shinyapps.io/timer/) database is a powerful tool to explore the relationship between gene expression and tumor immune infiltration.[23] We used the TIMER algorithm to assess the association of the expression of *FAM83D/H* with the abundance of infiltrated immune cells ($CD4^+$ cells, $CD8^+$ cells, macrophages, neutrophils, B cells and dendritic cells) in the OC tumor microenvironment. The TIMER 2.0 (http://timer.comp-genomics.org/) was further used to examine the effect of different copy numbers of *FAM83D/H* on immune infiltration in OC.

Patients and Clinical Tissue Samples

In this study, we collected 118 paraffin-embedded tissue samples from patients who underwent gynecological surgery at the Third Affiliated Hospital of Zhengzhou University from January 2016 to May 2021. Informed consent was obtained from patients and their families, and this study was approved by the Ethics Committee of the Third Affiliated Hospital of Zhengzhou University (NO.202105401) in accordance with the ethical guidelines of the Declaration of Helsinki. Tumor tissue derived from 99 patients who underwent surgical treatment for ovarian cancer, and normal ovary tissue derived from 19 patients who underwent hysterectomy for uterine fibroids. Table S1 summarizes the clinicopathological characteristics of study participants.

Immunohistochemistry (IHC)

Tissue microarray blocks were prepared by first fixing tissue samples in 10% formaldehyde for 24 hours and then embedding in paraffin. Paraffin-embedded 4-μm-thick tissue slices were obtained and treated with 0.3% H_2O_2 to quench the activity of endogenous peroxidases and for antigen retrieval. The sections were incubated overnight with an anti-FAM83H antibody (Bioss, bs-16-18R) at 4°C and then with a secondary antibody at room temperature. Two senior pathologists independently analyzed the IHC staining results under a double-blind situation. The samples were scored according to the proportion of positive cells as follows: 0, 0–10%; 1, 10–29%; 2, 30–49%; 3, 50–74%; and 4, 75–100%. The staining intensity was evaluated as follows: 0, no staining; 1, weak; 2, medium; and 3, strong. The final immunostaining score (ranging from 0 to 12) was calculated by multiplying the two scores, and samples were classified into four levels based on their final scores: 1+ group, 0–3; 2+ group, 4–6; 3+ group, 7–9; and 4+ group, 10–12.

Statistical Analysis

GraphPad Prism 7.00 software was used to conduct statistical analysis. Differences between the two groups were estimated using Student's *t*-test (unpaired, two-tailed). The Wilcoxon signed-rank test was used to analyze the relationships between *FAM83H* expression and cancer histology subtypes. OS and progression-free survival (PFS) were calculated using the Kaplan–Meier method. Spearman correlation analysis was used to measure the correlation between the expression of the two studied genes. In all analyses, p-value less than 0.05 was considered statistically significant.

Results

Expression Levels of FAM83 Members in OC

Abnormal activation and expression of oncogenes are crucial for malignant transformation of cells.

A comprehensive understanding of the expression of *FAM83* genes in multiple tumors is essential. As shown in Figure 1, abnormal expression of *FAM83* genes, especially *FAM83A, FAM83D* and *FAM83H*, is observed in various cancers. Regarding OC, Oncomine analysis revealed that the mRNA expression of *FAM83D/F/H* was significantly higher in OC tissue than in normal ovarian tissue ($p < 0.05$) (Table 1 and Figure S1). In the Yoshihara Ovarian dataset, *FAM83D* mRNA expression in serous ovarian adenocarcinoma was 6.972 times higher than that in peritoneum tissue ($p < 0.0001$). Similarly, the Lu Ovarian dataset demonstrated that *FAM83D* expression was significantly elevated in serous ovarian and endometrioid ovarian adenocarcinoma compared with that in normal ovarian surface epithelium ($p = 6.90E^{-3}$, fold change = 1.705; $p = 2.61E^{-2}$, fold change = 1.680). Moreover, the Yoshihara Ovarian dataset revealed that *FAM83F* mRNA expression was markedly increased by 4.497 times in serous ovarian adenocarcinoma in comparison with peritoneum tissue ($3.00E^{-4}$). Significant *FAM83H* overexpression in serous ovarian adenocarcinoma tissue in comparison with that in ovarian surface epithelium tissue was also found in the Lu Ovarian dataset ($p = 3.80E^{-3}$, fold change = 1.644).

Analysis type by cancer	Cancer vs. normal FAM83A		Cancer vs. normal FAM83B		Cancer vs. normal FAM83C		Cancer vs. normal FAM83D		Cancer vs. normal FAM83E		Cancer vs. normal FAM83F		Cancer vs. normal FAM83G		Cancer vs. normal FAM83H	
Bladder Cancer	1														1	
Brain and CNS Cancer							1	1								3
Breast Cancer	1						17	1			2	1			17	
Cervical Cancer																
Colorectal Cancer	2			3	3		13			5					1	
Esophageal Cancer		2		1		2		2	4							1
Gastric Cancer							7								6	
Head and Neck Cancer	2					1					1				1	
Kidney Cancer				2			1	1			1	1				1
Leukemia								6								1
Liver Cancer							2								2	
Lung Cancer	4				1		7		1		2				3	
Lymphoma						3		1				1				3
Melanoma	1					1						1				2
Myeloma																
Other Cancer	1						3			2		1			1	
Ovarian Cancer							3				1				1	
Pancreatic Cancer	2						1		1			1			1	
Prostate Cancer				2											4	
Sorcoma				1						4		1				1
Significant Unique Analyses	14	2		9	4	7	55	12	6	10	7	7			38	12
Total Unique Analyses	203		230		238		287		341		254		132		290	

10 5 1 % 1 5 10

Cell color is determined by the best gene rank percentile for the analyses within the cell.

Figure 1 Transcriptional level of *FAM83* family members in various types of cancer (ONCOMINE).

Notes: The graph shows the numbers of datasets with statistically significant mRNA overexpression (red) or down-regulated expression (blue) of the target gene. The threshold was designed with following parameters: p-value <0.001, fold change >1.5 and gene rank in the Top 10%. The number on each cell represents the number of datasets that meet the filtering threshold. Cell color is determined by the best gene rank percentile for the analyses within the cell. Student's *t*-test was used to identify the difference between two groups.

Table 1 *FAM83s* Expression in Different Type of Ovarian Cancer and Normal Ovarian Tissues (Oncomine)

Gene	Type of Ovary Cancer vs Ovary	Fold Change	P-value	Dataset
FAM83D	Ovarian Serous Adenocarcinoma vs Peritoneum	6.972	<1.00E-4	Yoshihara Ovarian
	Ovarian Serous Adenocarcinoma vs Ovarian Surface Epithelium	1.705	6.90E-3	Lu Ovarian
	Ovarian Endometrioid Adenocarcinoma vs Ovarian Surface Epithelium	1.680	2.61E-2	Lu Ovarian
FAM83F	Ovarian Serous Adenocarcinoma vs Peritoneum	4.497	3.00E-4	Yoshihara Ovarian
FAM83H	Ovarian Serous Adenocarcinoma vs Ovarian Surface Epithelium	1.644	3.80E-3	Lu Ovarian

Note: Statistic method: Student's *t*-test (unpaired, two-tailed).
Abbreviation: vs, versus.

Next, we used GEPIA to verify differences in the mRNA expression of *FAM83* genes between OC samples (n = 426) and normal ovarian samples (n = 88). As demonstrated in the box plots in Figure 2, the expression levels of *FAM83A/D/E/H* were significantly increased, whereas those of *FAM83B/C/F/G* showed no difference in the OC samples compared with the normal samples. Taken together, the high expression of *FAM83D/H* in OC was confirmed in the two independent databases.

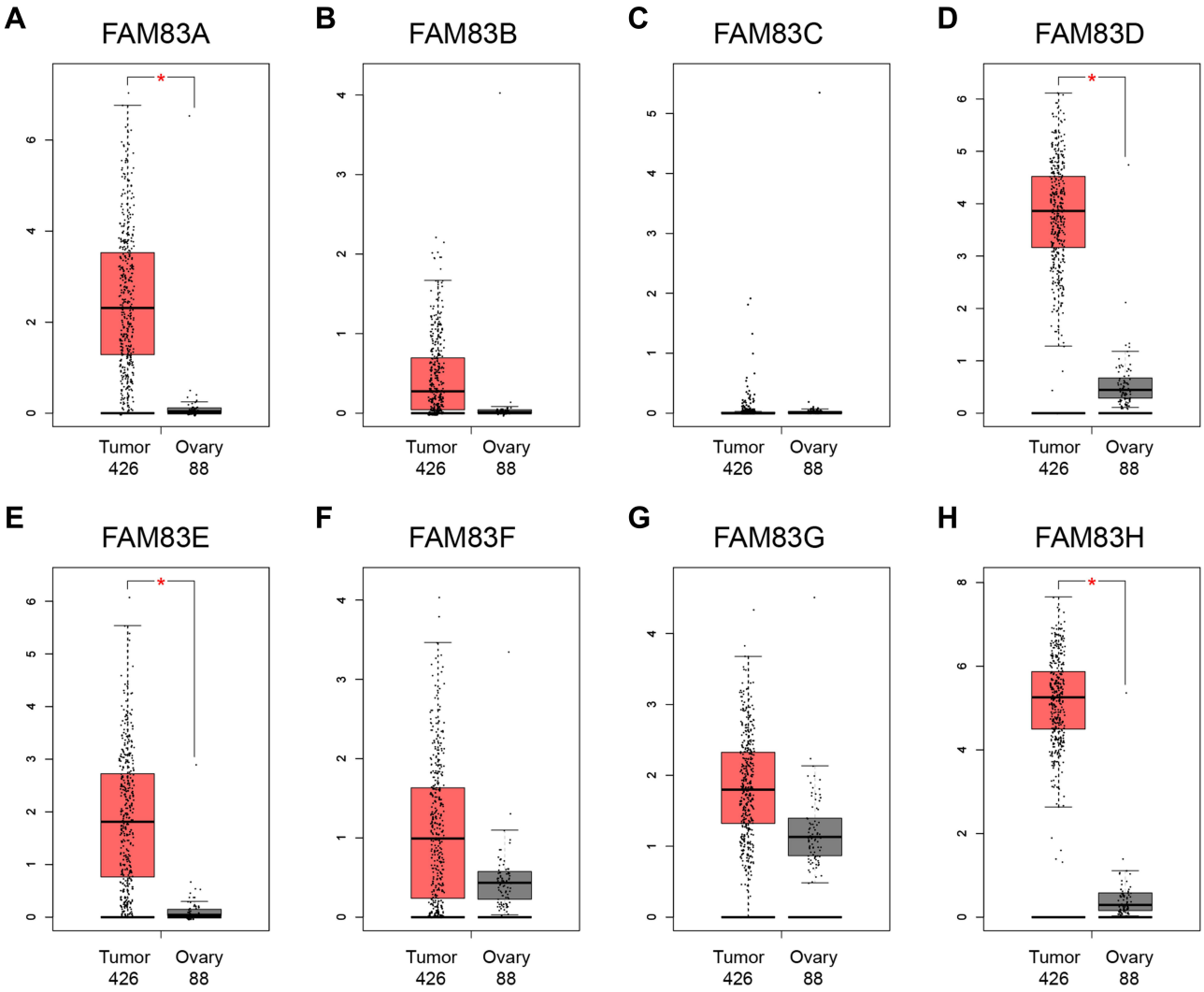

Figure 2 The mRNA expression levels of *FAM83* family members in 426 ovarian cancer tissues and 88 normal ovary tissues (GEPIA) (**A**–**H**).
Notes: *Indicates a statistically significant difference between the two groups; Student's *t*-test was applied to identify the *FAM83* genes expression difference between ovarian cancer and normal ovary tissue.

Genetic Alterations of FAM83 Family Members in OC

To investigate the genetic alterations of *FAM83* genes in OC, we selected 1680 ovarian serous cystadenocarcinoma cases from three datasets (606 cases from TCGA Firehose Legacy; 585 cases from TCGA PanCancer Atlas; and 489 cases from TCGA Nature 2011) for further analysis in the cBioPortal database. First, the genetic alteration rates of *FAM83* genes in the three datasets were 46.83%, 39.26% and 38.87%, respectively (Figure 3A). Second, we found that all eight *FAM83* genes showed different levels of genetic alterations. *FAM83A/H* showed the highest

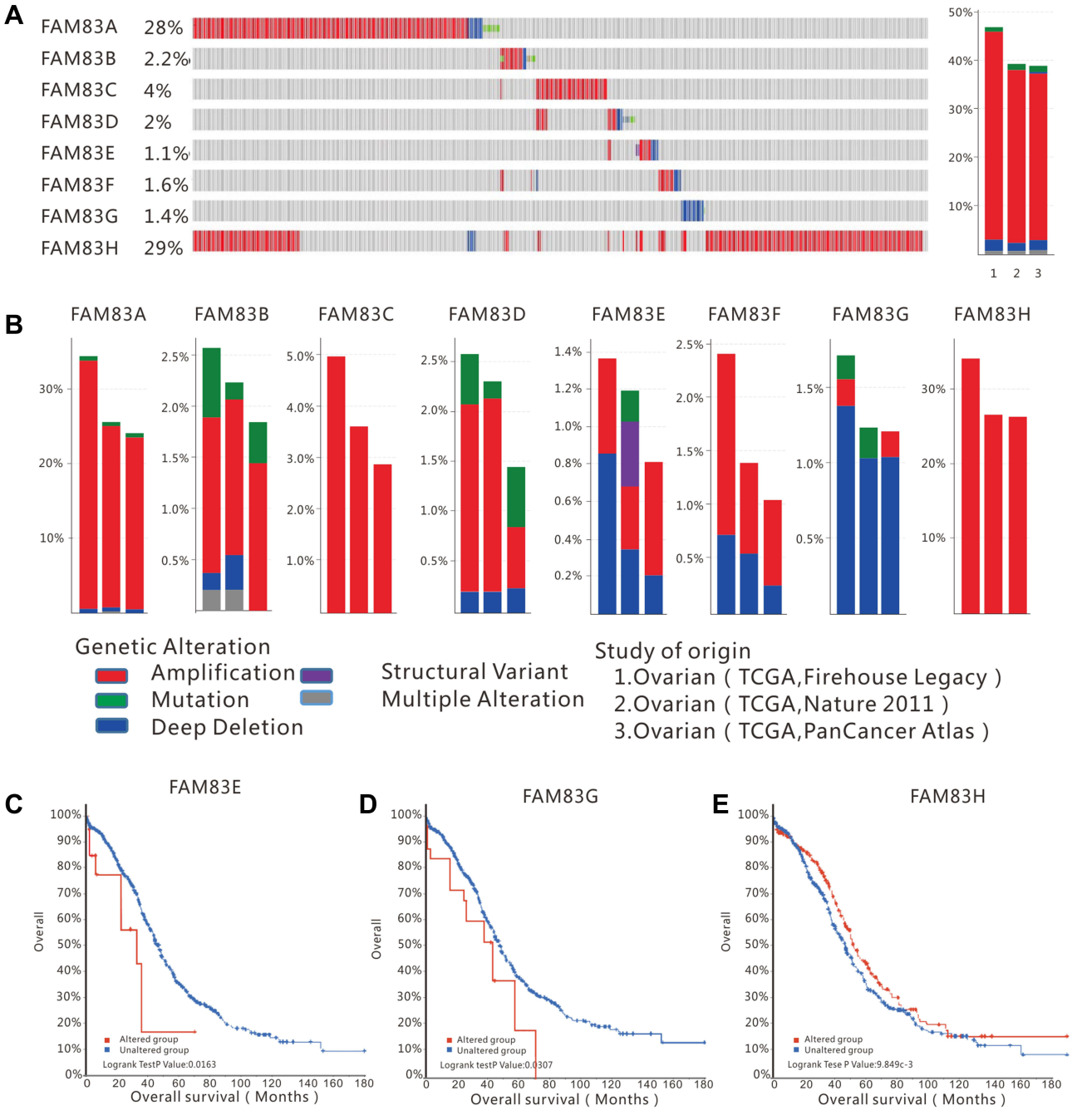

Figure 3 *FAM83* family genes alterations in ovarian cancer (cBioportal).

Notes: (**A**) Left: OncoPrint visual summary of alterations in *FAM83s*; right: summary of genetic alteration frequency in *FAM83* genes. (**B**) Analysis of genetic alteration frequency in *FAM83* family members in three datasets (TCGA Firehouse Legacy, TCGA Nature 2011 and TCGA PanCancer Atlas). The alterations included amplification, mutations, deep deletions, structural and multiple alterations. Kaplan–Meier analysis for overall survival in patients with or without *FAM83* gens alterations, (**C**) *FAM83E*; (**D**) *FAM83G* and (**E**) *FAM83H*.

incidence of gene alterations among all *FAM83* members (28% and 29%, respectively), while *FAM83E/F/G* presented fewer genetic changes. In terms of genetic variation types, the eight *FAM83* genes exhibited five types of alteration, namely, amplification, mutation, deep deletion, multiple alteration and structural variation. As demonstrated in Figure 3B, amplification was the most frequent genetic alteration event in *FAM83* genes, especially *FAM83A/C/H*. Moreover, deep deletion was found to account for a considerable proportion of alterations in *FAM83E/F/G*. Of note, only *FAM83B* and *FAM83E* genes showed multiple alterations and structural variations.

Finally, the Kaplan–Meier method was applied to investigate the effect of genetic alterations of each *FAM83* gene on the prognosis of patients with OC. Our results revealed that patients with alterations in *FAM83E* and *FAM83G* had shorter OS than those without any alterations (Figure 3C and D). In contrast, patients with *FAM83H* alterations had a favorable prognosis (Figure 3E). However, genetic alterations in the other *FAM83* genes did not show a significant effect on prognosis (Figure S2). These results imply that genetic alterations of some *FAM83* genes might directly affect the survival outcomes of patients with OC.

Prognostic Value of FAM83 Genes in Patients with OC

Increased expression of core oncogenes often leads to a decrease in survival time in cancer patients. In this study, we used the Kaplan–Meier method to clarify the impact of *FAM83* gene expression levels on the survival of patients with OC. As shown in Figures 4 and 5, high expression of *FAM83D/H* were significantly correlated with poor OS and PFS, whereas high expression levels of *FAM83B/E* were significantly associated with favorable OS and PFS. No significant correlation was found between *FAM83C/F* expression and the survival time of patients with OC. Interestingly, *FAM83A* expression showed inconsistent associations with OS and PFS. Specifically, *FAM83A* upregulation had no effect on OS but was associated with shorter PFS. Survival analysis was not performed for *FAM83G* due to the lack of related survival data in the Kaplan–Meier plotter database.

Based on the overall results of Oncomine, GEPIA, cBioPortal and Kaplan–Meier plotter database analyses, *FAM83D/H* were selected for further analysis in diverse subgroups. We evaluated the prognostic value of *FAM83D/H* in different cancer histology subtypes, clinical stages, pathological grades and TP53 statuses. As shown in Table 2, increased *FAM83D* expression was correlated with poor OS in both the endometrioid and serous ovarian malignancy subgroups. In terms of the clinical stage, the OS of all grade patients showed a negative relationship with *FAM83D* expression. Additionally, *FAM83D* overexpression predicted short OS in the poor differentiation (Grade 3) subgroup. Similarly, the prognostic value of *FAM83H* was also found to be remarkably related to the above-mentioned clinical features.

Functional Enrichment Analysis of FAM83D and FAM83H in OC

Improved understanding of the biological functions of *FAM83D/H* may help to clarify their potential mechanisms in OC. To that end, we selected 353 genes and 491 genes that were closely related to *FAM83D* and *FAM83H*, respectively, according to Spearman correlation coefficients of > 0.35 or < -0.35, from the cBioPortal database. Then, we performed GO and KEGG function enrichment analyses of the associated genes in DAVID Bioinformatics Resources 6.8. *FAM83D/H* were found to be involved in multiple cancer-related signaling pathways and biological processes. As depicted in Figure 6A and B, genes relevant to *FAM83D* in OC were involved in cell cycle, DNA replication, the Fanconi anemia pathway, microRNAs in cancer, pathways in cancer, pyrimidine metabolism, the p53 signaling pathway, cell division, mitotic nuclear division, DNA repair, cell proliferation, and G1/S and G2/M transition of mitotic cell cycle. Figure 6C and D provided the significantly related pathways and biological processes of *FAM83H* co-expressed genes, including endocytosis, RNA transport, mRNA surveillance, cell cycle, basal transcription factors, transcription, DNA-templated, cell–cell adhesion, regulation of GTPase activity, cell division, mitotic nuclear division, protein SUMOylation, regulation of signal transduction by p53 class mediator and wound healing.

Co-Expression Analysis of FAM83D and FAM83H in OC

KEGG analysis revealed that *FAM83D/H* are closely associated with the cell cycle signaling pathway, so we further explored their co-expression relationships with the 34 cell-cycle related genes that are pre-defined in the cBioportal database. The detailed information of the 34 genes is provided in Table S2. The co-expression analysis showed

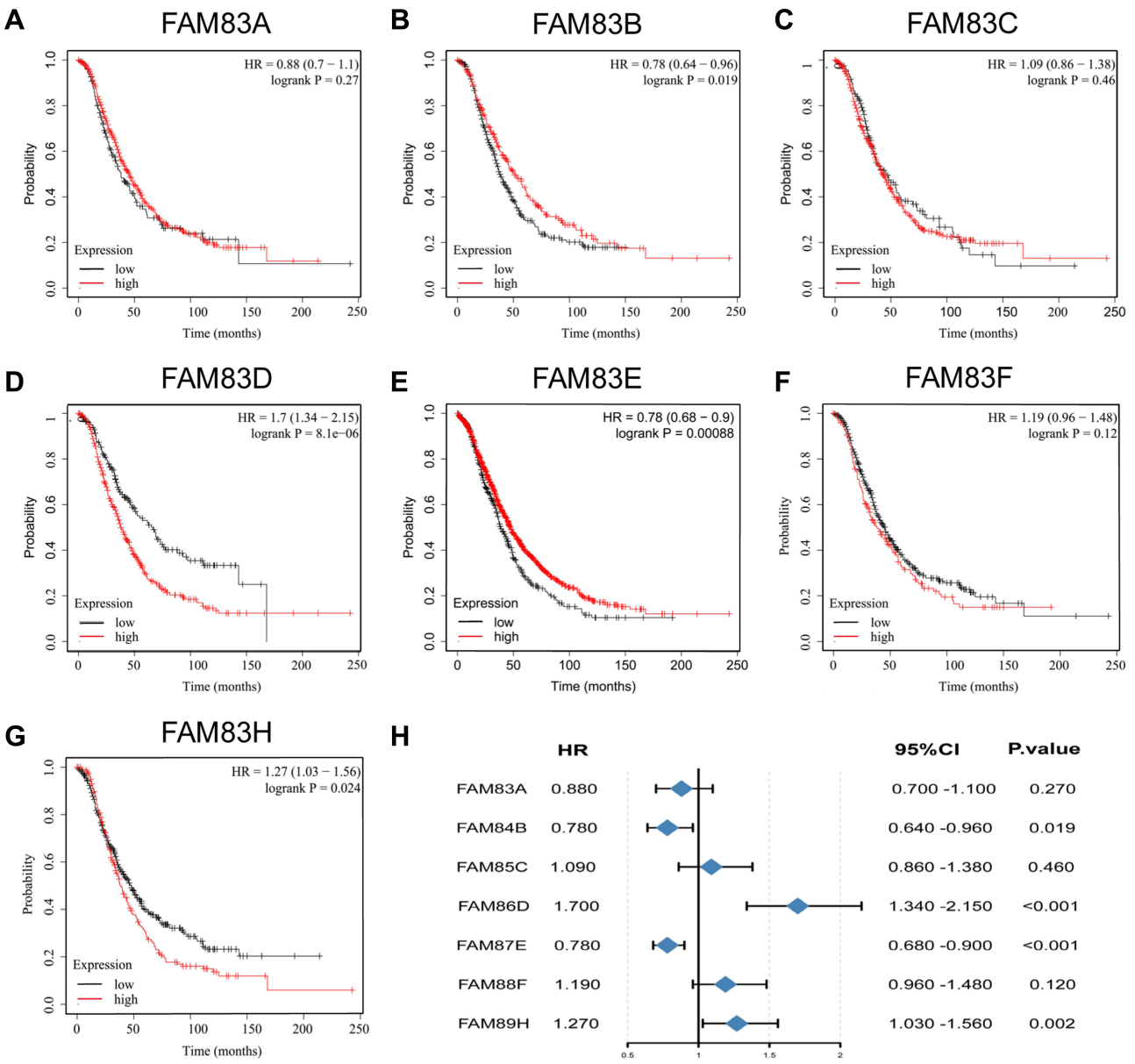

Figure 4 Prognostic value of *FAM83s* mRNA levels in patients with OC (OS in Kaplan–Meier plotter).
Notes: (**A**–**G**) Prognostic significance of each *FAM83* gene in OC. (**H**) Prognostic HRs of each *FAM83* gene in OC. The red and black lines represent high and low expression, respectively. The Log rank test was used to calculate the p-value.
Abbreviations: OC, ovarian cancer; OS, overall survival; HR, hazard ratio.

that *FAM83D* was positively associated with *E2F8, CDK1, CCNB1, RBL1, E2F1, E2F7, E2F2, ADC25A, CDK2, CCNE1, CDKN2A, CDK4, CCNA1* and *CDKN1B* and negatively associated with *MYC, CCND2, CDK6, RBL2* and *CCND1* (Figure 7A). In addition, *FAM83D* was significantly and positively correlated with the genes encoding MKI67 and PCNA, two well-known tumor cell proliferation factors (Spearman correlation coefficients = 0.45 and 0.6, respectively; Figure S3). Meanwhile, *FAM83H* was positively associated with *CCND1, MYC* and *JAK2* and negatively associated with *CCNB1, CDK2, CDKN1B, E2F6* and *CDK4* (Figure 7B).

FAM83D and FAM83H Related to Immune Infiltration in OC

The TIMER database was examined to estimate the association of *FAM83D/H* expression with immune cell infiltration. As shown in Figure 8A, *FAM83D* expression was negatively related to $CD8^+$ T cell infiltration ($r = -0.115$, $p = 1.2E^{-2}$), and positively related to $CD4^+$ T cell infiltration ($r = 0.1$, $p =$

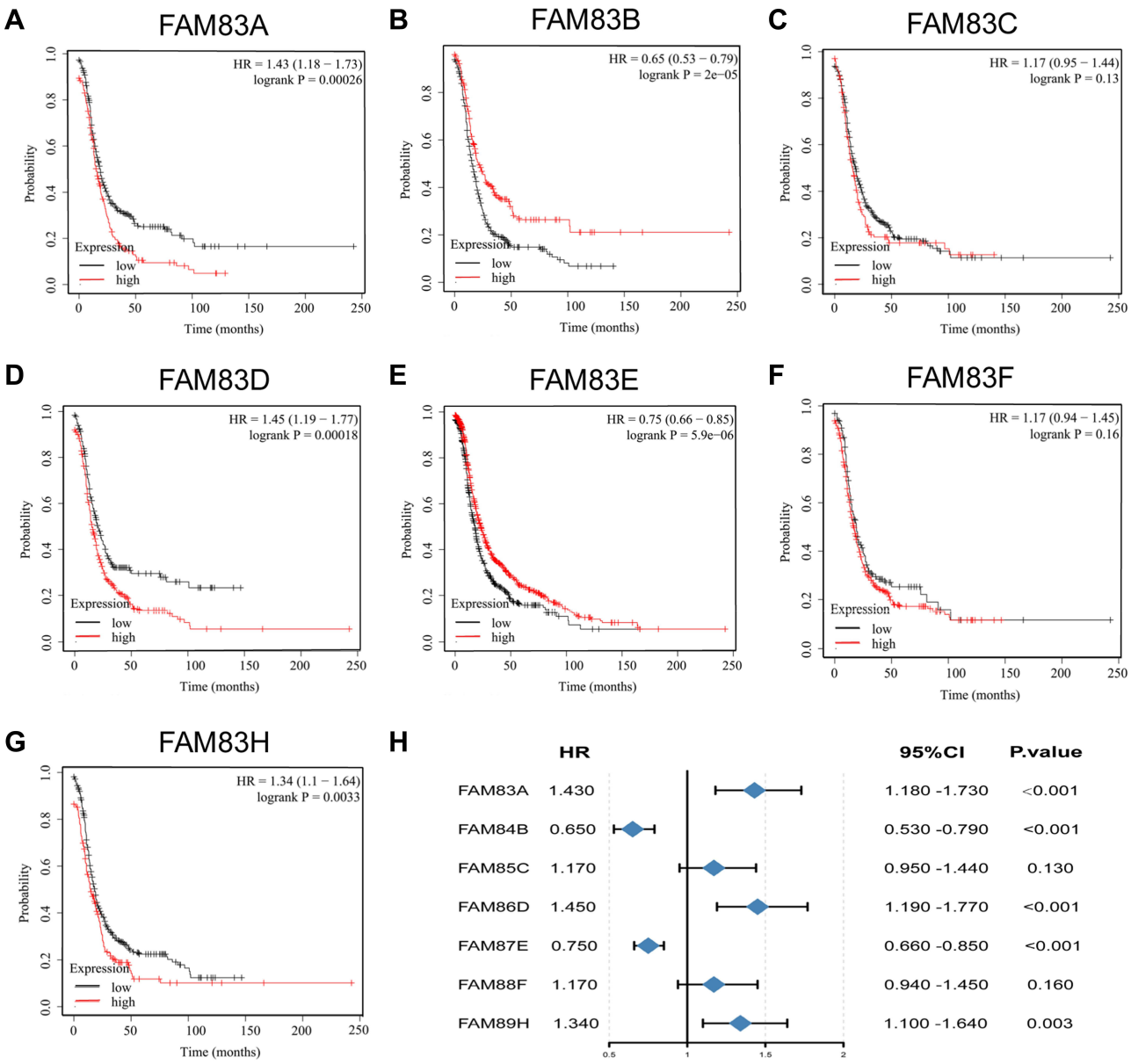

Figure 5 Prognostic value of *FAM83s* mRNA levels in patients with OC (PFS in Kaplan–Meier plotter).
Notes: (**A**–**G**) Prognostic significance of each *FAM83* gene in OC. (**H**) Prognostic HRs of each *FAM83* gene in OC. The red and black lines represent high and low expression, respectively. The Log rank test was used to calculate the p-value.
Abbreviations: OC, ovarian cancer; PFS, progression-free survival; HR, hazard ratio.

$2.88E^{-2}$) and macrophage infiltration (r = 0.127, p = $5.24E^{-3}$). No correlation was detected between *FAM83D* expression and tumor cell purity or B cell, neutrophil or dendritic cell infiltration (Figure 8B. Meanwhile, *FAM83H* expression was positively associated with macrophage (r = −0.272, p = $1.34E^{-9}$), neutrophil (r = −0.121, p = $7.93E^{-3}$) and dendritic cell (r = −0.139, p = $2.33E^{-3}$) infiltration Figure 7B (Figure 8B). Compared with normal diploids, arm-level gain of *FAM83D* were negatively associated with the immune infiltration of B cells and myeloid dendritic cells, but positively associated with macrophage in OC (Figure 8C). Unlike *FAM83D*, the high amplification of *FAM83H* is positively related to the infiltration of B cells and myeloid dendritic cells, and the arm-level deletion of *FAM83H* is negatively related to the infiltration of CD8+ T cell (Figure 8D).

IHC and Clinicopathological Analysis of FAM83H in OC

There was no relevant literature yet reporting *FAM83H* in OC; therefore, we performed IHC analysis and

Table 2 The Prognostic Value of *FAM83D* and *FAM83H* in Different Subtypes

Subtype	*FAM83D*		*FAM83H*	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**
Histology				
Endometrioid	1.7E9 (0-Ihf)	0.043*	3.23 (0.45–23.12)	0.22
Serous	1.41 (1.09–1.83)	0.0086*	1.33 (1.06–1.66)	0.014*
Stage				
1+2	10.76 (1.41–81.88)	0.0041*	2.90 (1.05–8.01)	0.031*
3+4	1.42 (1.13–1.78)	0.0023*	1.27 (0.98–1.66)	0.075
Grade				
1	2.51 (0.69–9.21	0.15	2.29 (0.71–7.36)	0.15
2	1.54 (0.99–2.39)	0.053	1.78 (1.08–2.92)	0.022*
3	1.38 (1.06–1.79)	0.015*	1.20 (0.92–1.55)	0.17
TP53				
Mutated	0.76 (0.51–1.12)	0.16	1.87 (1.27–2.75)	0.0012*
Wild type	2.24 (0.78–6.39)	0.12	2.28 (0.82–6.37)	0.1

Note: *p<0.05; Each subgroup was analyzed using the Kaplan–Meier method.
Abbreviation: HR, hazard ratio.

clinicopathological analysis to reveal the role of *FAM83H* in OC. The IHC results presented in Figures 9 and S4 show that *FAM83H* protein was mainly localized in the cytoplasm, and its staining intensity was markedly higher in OC tissue than in normal ovarian tissue (Figure 9A, $p < 0.001$). Figure 9B presents four typical *FAM83H* protein-stained sections with scores of 1+, 2+, 3+ and 4+, demonstrating a clear trend of increasing protein expression. As shown in Figure 9C, the FIGO stage was associated with *FAM83H* protein expression, which was significantly upregulated in patients with stage III–IV comparison with stage I–II. There was no significant relationship between the expression of *FAM83H* protein and the pathological subtype and pathological T stage of OC. However, it is obvious that the expression of *FAM83H* was significantly higher in clear cell subtype than that in mucinous subtype.

Discussion

Although an increasing number of studies have demonstrated the critical regulatory role of *FAM83* genes in various tumors, their prognostic value and functional mechanisms in OC have not yet been fully elucidated. In this study, we performed a systematic assessment of the biological significance of *FAM83* genes in OC using both the bioinformatics data from several authoritative databases and the clinical data of patients with OC from our hospital. To our knowledge, this is the first study to reveal the expression status and prognostic significance of *FAM83* genes in OC, providing a novel insight into the pathological mechanism of OC.

Apart from the conserved globular structural domain DUF1669 located at the N terminus, no other similar sequences were detected between the *FAM83* genes, which partly explains the functional differences between these family members.[8,12] *FAM83A* was first identified as a driver of tyrosine kinase inhibitor (TKI) resistance by genetic analysis and as a tumor-specific biomarker for lung cancer; it significantly accelerates the progression of lung cancer though the Wnt and Hippo Signaling pathways.[13] *FAM83B* overexpression was found to activate the PI3K/AKT pathway and reduce cell sensitivity to inhibitors of small molecules such as PI3K, AKT and mTOR, thus promoting tumor development and chemoresistance.[14] *FAM83D*, which encodes a mitotic spindle-associated protein, is a candidate oncogene for tumorigenesis. *FAM83D* knockdown has been reported to downregulate expression of the tumor suppressor gene *FBXW7*, which induces apoptosis and inhibits cell proliferation and clone formation in colorectal cancer cells.[24] *FAM83G* is involved in the regulation of the BMP signaling pathway, and its protein product is a substrate for type I BMP receptor kinase.[25,26] The function of *FAM83H* was first identified in calcium-deficient enamel hypoplasia and is closely related to the thickness, density and morphology of enamel in mice.[27] However, the potential biological functions of *FAM83C, FAM83E* and *FAM83F* have not yet been

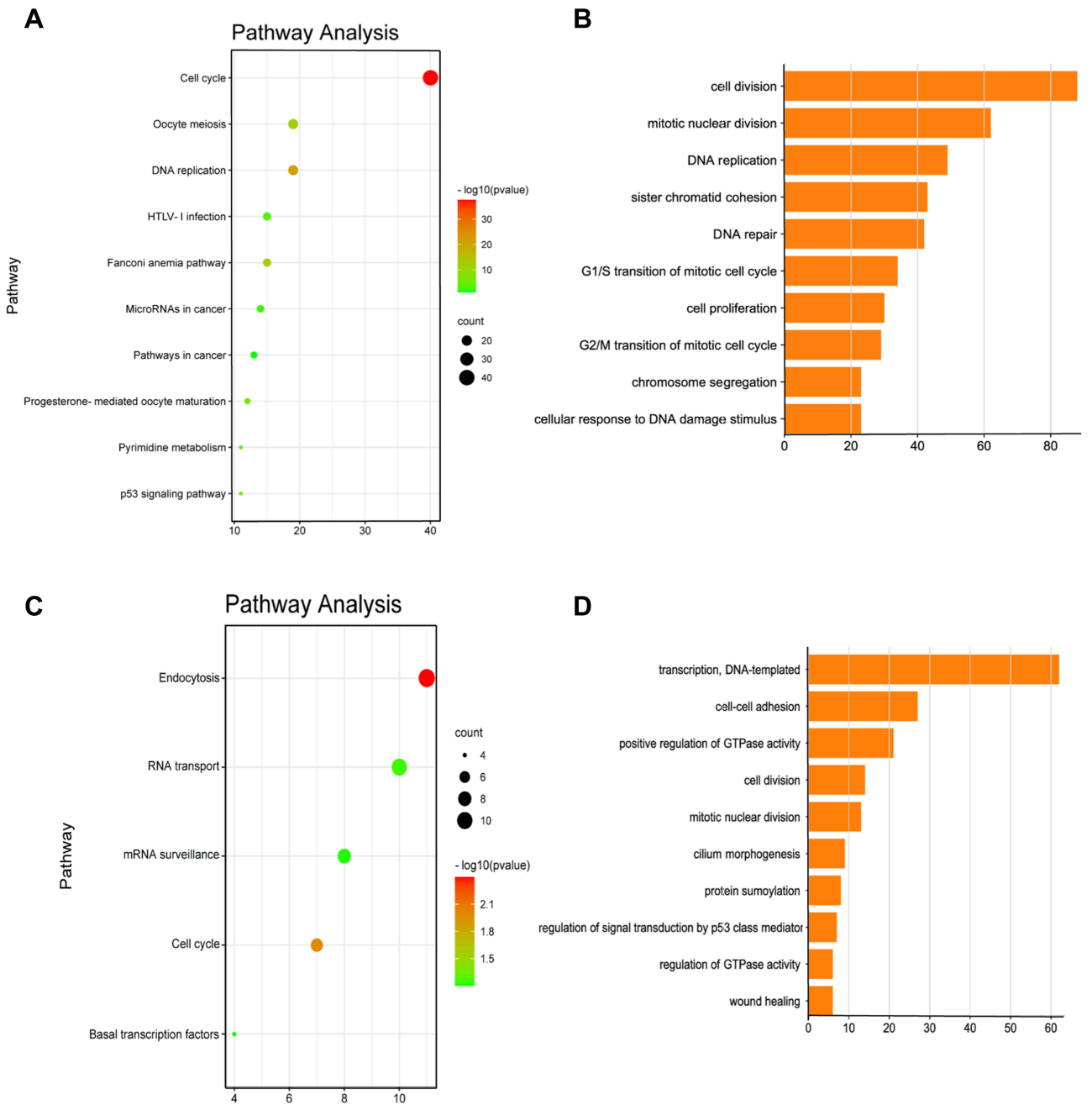

Figure 6 Functional enrichment analysis of *FAM83D* and *FAM83H* in ovarian cancer.
Notes: (**A**) Significant pathway enrichment of *FAM83D* co-expression genes. (**B**) Significant biological process enrichment of *FAM83D* co-expressed genes. (**C**) Significant pathway enrichment of *FAM83H* co-expression genes. (**D**) Significant biological process enrichment of *FAM83H* co-expressed genes; statistic method: Spearman correlation analysis.

reported. Given their crucial roles in tumor progression, a systematic investigation of the roles of FAM83 family genes in OC is warranted.

In this study, we first explored the similarities and differences in the transcriptome levels of *FAM83* family members in different tumor types by pan-cancer analysis. The results of Oncomine analysis demonstrated that aberrant expression of *FAM83* genes (especially *FAM83D, FAM83F* and *FAM83H*) was universal among various cancer types. Their upregulation and oncogenic effects have been revealed by previous researchers. For instance, Hu et al indicated that *FAM83A* expression was increased in non-small-cell lung cancer (NSCLC), which significantly promoted cancer cell proliferation and

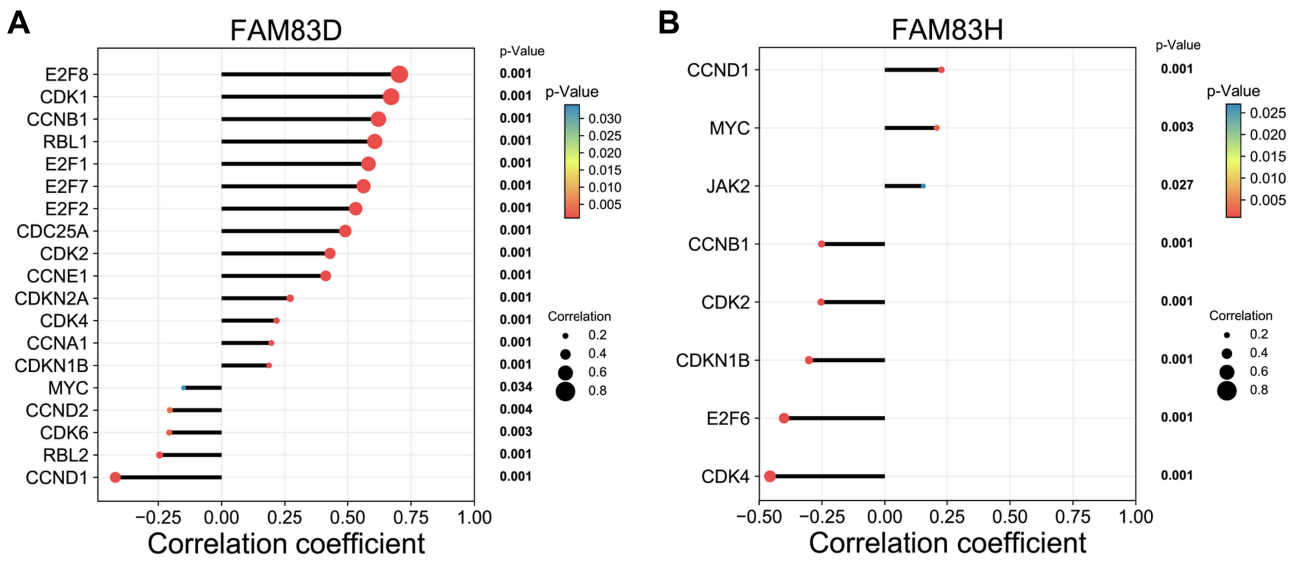

Figure 7 Co-expression analysis of *FAM83D/H* and cell cycle pathway-related genes.
Notes: (**A**) Cell cycle-related genes significantly associated with *FAM83D*. (**B**) Cell cycle-related genes significantly associated with *FAM83H*. Statistic method: Spearman correlation analysis.

protected against apoptosis via the ERK and PI3K/Akt/mTOR signaling pathways.[28] *FAM83B* expression was notably elevated in tumor tissue and cell lines of pancreatic ductal adenocarcinoma, and it increased as the advanced clinical stage and poor vital status.[29] Gan et al showed that *FAM83C* was upregulated in NSCLC compared with normal lung tissue, and that higher *FAM83C* expression was remarkably associated with shorter OS and PFS in patients with NSCLC.[30] *FAM83D*, which is currently a hot topic in cancer research, has been shown to be upregulated in hepatocellular cancer, gastric cancer, breast cancer and colorectal cancer.[24,31–33] Likewise, *FAM83F* and *FAM83H* have been reported to be highly expressed in papillary thyroid cancer and pancreatic cancer, respectively.[34,35] Of note, few studies have investigated the expression of *FAM83E* and *FAM83G* in tumors. In agreement with previous studies, our Oncomine analysis results suggested that *FAM83D, FAM83F* and *FAM83H* are highly expressed in OC. Notably, *FAM83* genes, specifically *FAM83D* and *FAM83H*, show different or even opposite expression status in different tumor types. As the human body is a vast and complex system, the roles of *FAM83* genes in tumorigenesis might differ according to the type of tissue or cancer.[36]

To further determine the expression of *FAM83* genes in OC, we compared their mRNA expression levels between 426 OC samples and 88 normal ovarian tissue sample in GEPIA. The results suggested that *FAM83A, FAM83D, FAM83E* and *FAM83H* were significantly upregulated in OC. Notably, *FAM83D* upregulation has been demonstrated in former experimental studies. For example, Zhu et al showed that *FAM83D* was highly expressed in OC and markedly enhanced the invasion and proliferation of cancer cells.[16] Moreover, Zhang et al identified *FAM83D* as an excellent biomarker to distinguish between invasive epithelial ovarian cancer and low malignant potential ovarian tumor owing to its aberrant expression and tendency to promote malignancy.[17] However, few studies have investigated the expression of *FAM83A, FAM83E* and *FAM83H* in OC, and this is the first study to identify overexpression of these genes through big data mining. Our findings may inform future research on the pathogenesis of OC.

Genetic alterations, such as CNVs and mutations, are the main drivers of gene-specific expression. To confirm the association of alterations of *FAM83* genes with OC, we conducted a genetic analysis in the cBioPortal database, which revealed that the overall alteration rate in *FAM83* genes ranged from 38.87% to 46.83% across three different OC datasets. The most common genetic changes in the *FAM83* family were the amplification of *FAM83A* and *FAM83H*. The high amplification rate of *FAM83A/H* and overexpression of *FAM83A/H* represented amazing consistency in OC. Moreover, the Kaplan–Meier survival curve indicated that the patients with *FAM83E* or *FAM83G* alteration had longer OS than patients without any alteration, while those with *FAM83H* alteration had shorter OS;

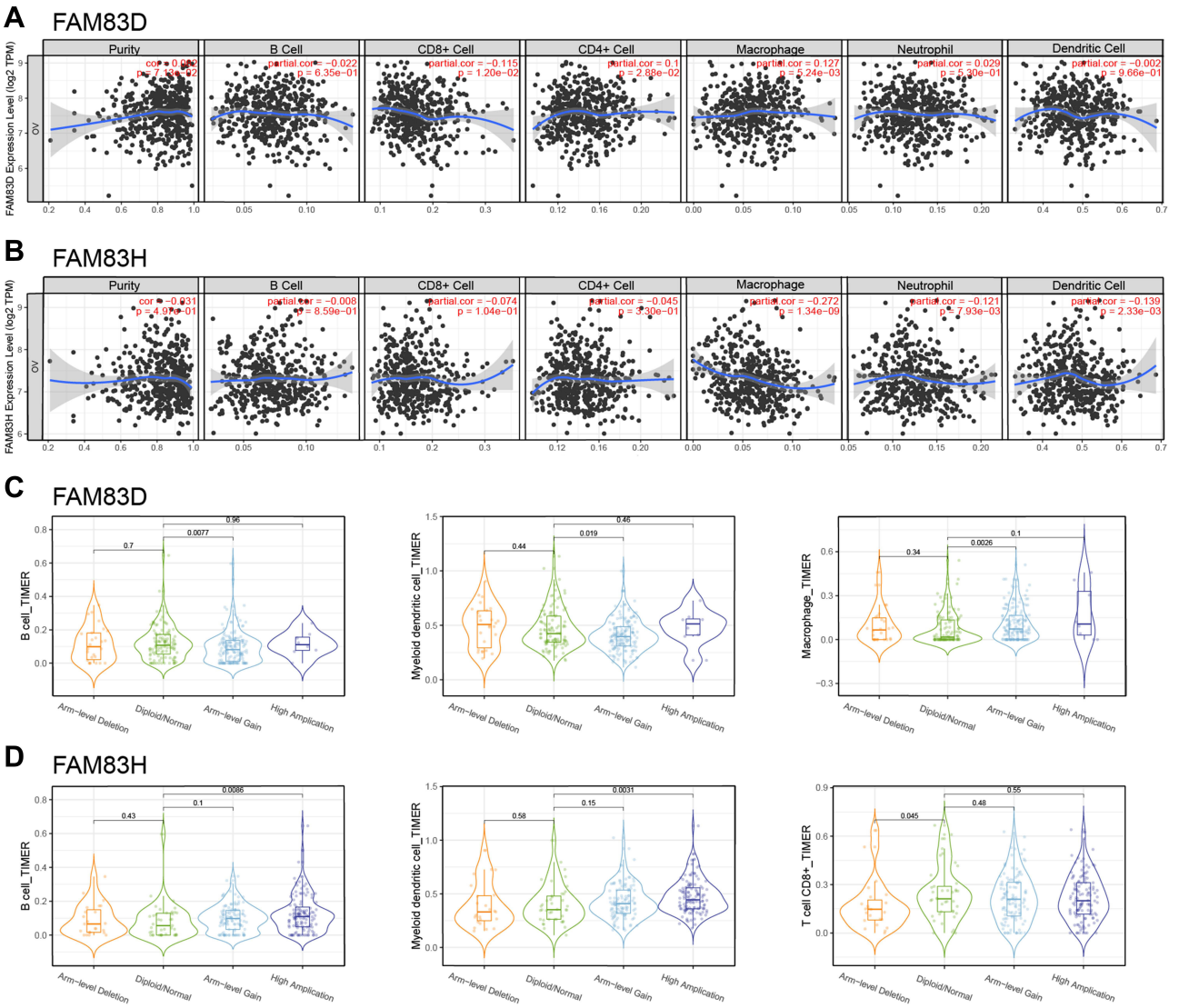

Figure 8 Correlation between *FAM83D/H* expression and immune infiltration.
Notes: (**A** and **B**) Relationship between *FAM83D/H* expression and six immune cell infiltration including B cell, CD8+ T cell, CD4+ T cell, Macrophage, Neutrophil and Dendritic cell. (**C** and **D**) The relationship between somatic copy number alterations of *FAM83D/H* and immune cell infiltration;

this suggests that alterations in these *FAM83* genes directly affect the prognosis of patients with OC.

Given the prevalence of abnormalities in *FAM83* expression at the transcriptome and genome levels, it is necessary to further explore the influence of abnormal expression of these genes on OC prognosis. Our Kaplan–Meier survival analysis showed that patients in the high *FAM83B/E* expression group had longer OS and PFS than those in the low *FAM83B/E* expression group, which suggests that *FAM83B* and *FAM83E* are protective factors that possibly function as tumor suppressor genes in OC. Mechanically, one study affirmed that *FAM83B* could inhibit the Wnt signaling pathway and thereby inhibit cisplatin resistance in OC, which further explains why patients with low *FAM83B* expression have a poor prognosis.[15] In contrast, *FAM83D* and *FAM83H* were found to be risk factors for poor prognosis and may thus function as oncogenes. The oncogenic role of *FAM83D* in OC has been demonstrated by previous studies.[16,17] However, the mechanism by which *FAM83E* and *FAM83H* promote or suppress the occurrence and development of OC has yet not been revealed, which warrants further investigation.

Taken together, the results of our Oncomine, GEPIA and Kaplan–Meier plotter database analyses (Table 3) indicated that the expression of *FAM83D* and *FAM83H* is significantly associated with OC prognosis. Hence, we had reason to believe that *FAM83D/H* may be key FAM83 family genes involved in promoting OC progression. We

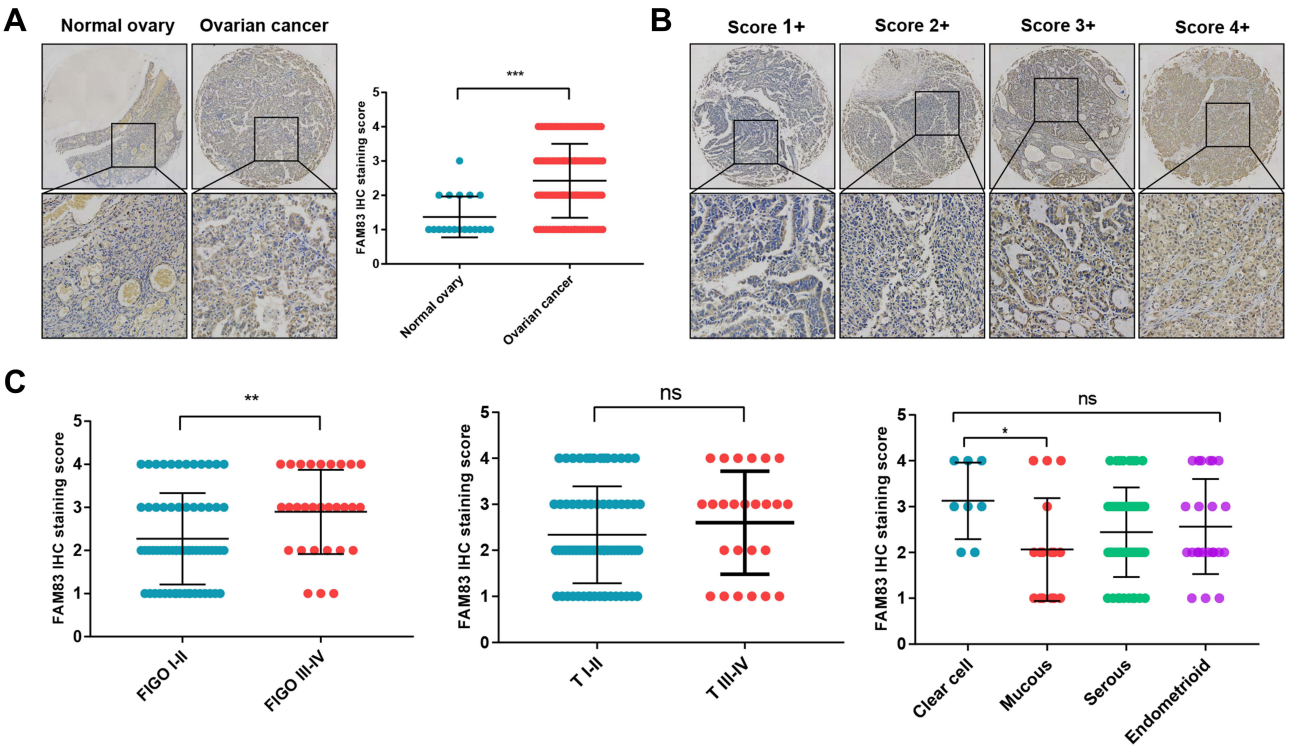

Figure 9 IHC analysis and clinicopathological analysis of *FAM83H* in OC.
Notes: (**A**) Immunohistochemical detection of *FAM83H* in OC tissues. (**B**) Four typical immunohistochemical sections with score 1, 2, 3 and 4, respectively. (**C**) Association between the expression of *FAM83H* and different clinical features in OC; Student's *t*-test (unpaired, two-tailed) or the Wilcoxon signed-rank test were used to analyze the relationships between *FAM83H* expression and different clinicopathological feature; *p<0.05; **p<0.01; ***p<0.001.
Abbreviations: OC, ovarian cancer; ns, not statistically significant.

therefore further explored the potential mechanisms of their involvement in the pathogenesis of OC. First, KEGG pathway analysis showed that *FAM83D/H* were closely related to the cell cycle pathway. Next, GO enrichment analysis indicated that multiple cell cycle-related biological processes were also closely associated with *FAM83D/H*. Further co-expression analysis showed that many cell cycle-related genes were also significantly associated with *FAM83D/H*. Thus, we speculated that *FAM8383D/H* is involved in the malignant progression of OC through the cell cycle signaling pathway. Likewise, a strong correlation between *FAM83D* and the cell cycle has been demonstrated in human lung adenocarcinoma and hepatocellular carcinoma.[31,37] In terms of the specific mechanism of *FAM83H* in the pathological progression of OC, previous studies have indicated that the oncogene *MYC* can enhance the proliferation of hepatocellular carcinoma cells by regulating the transcription of *FAM83H*.[38] In addition, *FAM83H* expression is closely associated with β-catenin activity, and immunoprecipitation experiments showed that *FAM83H* can interact directly with β-catenin.[39] Although few studies have reported the oncogenic function of *FAM83H* in OC to date, our results showed that the mRNA and protein expression of *FAM83H* in OC was higher than that in normal tissues and was positively associated with malignant clinical features. Our results, together with previous evidence, suggest

Table 3 Summary of Bioinformation Analysis of *FAM83* Genes in Ovarian Cancer

Gene	Expression		Prognosis (Kaplan–Meier Plotter)	
	Oncomine	GEPIA	OC (OS)	OC (PFS)
FAM83A		√		√
FAM83B			√	√
FAM83C				
FAM83D	√	√	√	√
FAM83E		√	√	√
FAM83F	√			
FAM83G				
FAM83H	√	√	√	√

Notes: The expression and prognosis of FAM83D and FAM83H in OC represents statistically significant in three databases (Oncomine, GEPIA and Kaplan–Meier plotter); √ Indicates that the result is statistically significant.
Abbreviations: OS, overall survival; PFS, progression-free survival.

that *FAM83D* and *FAM83H* may be promising biomarkers of OC.

A comprehensive evaluation of the type, number and distribution of immune infiltrating cells in the tumor microenvironment would enable us to gain a deeper understanding of the interaction between the immune system and OC and ultimately reveal the immune escape mechanism of this cancer.[40] As a special heterogeneous group, tumor-infiltrating lymphocytes (TILs) play a crucial role in tumor immunity. CD4+ T cells secrete large amounts of inflammatory cytokines, such as IFN-γ and TGF-β, and CD4+ regulatory T cells are essential in maintaining immune tolerance.[41] Chen et al demonstrated that TIGIT, a well-known immune checkpoint, could enhance CD4+ regulatory T cell responses and mediate immunosuppression in a murine OC model.[42] Meanwhile, the presence of CD8+ TILs was correlated with a favorable prognosis in patients with epithelial OC.[43] Interestingly, our results showed that CD4+ T cells were positively correlated with *FAM83D*, and CD8+ T cells were negatively correlated with *FAM83D*. Therefore, we speculated that the increase in the CD4+/CD8+ T cell ratio might be suggestive of dysregulation of the immune status of the organism caused by high expression of *FAM83D*. Obviously, there was a negative association between *FAM83H* expression and the infiltrated abundance of six types of immune cells. Immune and inflammatory cells are vital components of the tumor microenvironment, and their reduction and deletion are known to be closely associated with the proliferation, invasion, adhesion, angiogenesis, drug resistance and other malignant processes of tumors.[44] In general, we first explore the relationship between local immune status and *FAM83D* and *FAM83H* in ovarian cancer to provide a basis for immunotherapy in ovarian cancer.

In this work, we revealed close relationships between *FAM83* genes and OC. However, some unavoidable limitations of our analysis must be considered. For instance, raw data were processed and analyzed in different open-access databases, which may cause inconsistent results or statistical deviations. However, the incomparable strengths of public databases are that large amounts of valuable data can be acquired in a short time and that the integrated results generated are highly credible and applicable.[45] In the future, we plan to conduct in-depth investigation of the specific mechanisms of *FAM83* genes in OC pathogenesis.

Conclusion

In conclusion, this study provides the first systematic insight into the aberrant expression of *FAM83* family genes in OC and their differential effects on OC prognosis. Furthermore, IHC and clinicopathological analysis validated the overexpression of *FAM83H* in OC and revealed its significant association with the malignant progression of OC. Our results suggest that *FAM83D* and *FAM83H* might be promising prognostic biomarkers for OC and potential novel targets for OC immunotherapy.

Acknowledgments

This work was supported by the Supported by Science and Technology Tackling Project of Henan Province (No. 192102310067) and Henan International Joint Laboratory of Ovarian Malignant Tumor.

Disclosure

The authors report no conflicts of interest in this work.

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