Prognostic Significance of TM4SF1 and DDR1 Expression in Ovarian Cancer

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Abstract

Background: TM4SF1 and DDR1 are expressed in many cancers, but their expression in ovarian cancer and the relationship between their expression and patient prognosis are still unclear. The present study aimed to explore the expression of TM4SF1 and DDR1 as well as their relationship with the prognosis of ovarian cancer.

Methods: The Oncomine database and gene expression profile interactive analysis (GEPIA) were used to compare the different expression levels of TM4SF1 and DDR1 in ovarian cancer, and Kaplan–Meier plotter was used to analyse the relationship between gene expression and patient prognosis. The interacting proteins of TM4SF1 and DDR1 were analysed by STRING, which is an online protein interaction analysis tool, and enrichment analysis of Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways was conducted for these interacting proteins. Immunohistochemical staining was performed to detect the expression of TM4SF1 and DDR1 protein in ovarian cancer tissue and to analyse the relationship between the expression and prognosis.

Results: Database and clinical sample analyses showed that the expression levels of TM4SF1 and DDR1 were significantly higher in ovarian cancer and that TM4SF1 and DDR1 were coexpressed in some cases. STRING analysis found that the TM4SF1 and DDR1 proteins had an interaction relationship. The overall survival (OS) and progression-free survival (PFS) of ovarian cancer patients with TM4SF1 and DDR1 coexpression were significantly shorter than those of patients lacking TM4SF1 and DDR1 coexpression. Multivariate analysis showed that TM4SF1 and DDR1 protein coexpression was an independent prognostic factor.

Conclusions: TM4SF1 and DDR1 proteins were coexpressed in some ovarian cancer tissues and were considered adverse prognostic factors for ovarian cancer. Our findings suggested that there might be an interaction or mutual regulatory mechanism of TM4SF1 and DDR1.

Introduction

Ovarian cancer is one of the three common malignant tumours of the female reproductive system. The mortality rate of ovarian cancer ranks first among gynaecological malignancies, and the 5-year survival rate is only approximately 40% [1, 2]. Ovarian cancer cells are highly invasive, and widespread metastasis of the pelvis, abdominal cavity and retroperitoneal lymph nodes can occur in the early stage. Even if ovarian cancer patients receive standard treatment, approximately 70% of patients still experience recurrence, metastasis and drug resistance within 2 years, posing a serious threat to women's lives and health.

Transmembrane 4 L six family member 1 (TM4SF1) is a distant relative of the four-transmembrane protein superfamily. TM4SF1 is highly expressed in a variety of epithelial cancer tissues, and it regulates intracellular calcium levels, tyrosine phosphorylation and protein kinase C [3, 4]. In addition, TM4SF1 is involved in the formation of vascular endothelial pseudopodia and angiogenesis, and it plays an important role in the regulation of cell development, activation, growth and movement [5]. It has been reported that overexpression of TM4SF1 in a variety of epithelial cancer tissues is associated with poor prognosis [6–9]. Discoidin domain receptor 1 (DDR1) is a member of the discoid domain receptor in the receptor tyrosine kinase family, and abnormally activated DDR1 is related to the occurrence and development of tumours. Significantly higher expression of DDR1 exists in many types of malignant tumours [10], such as breast cancer, non-small-cell lung cancer, gastric cancer and colorectal cancer, and DDR1 is regarded as an adverse prognostic factor [11–14]. Similar to TM4SF1, DDR1 is critical in tumour cell survival, drug resistance, self-renewal, differentiation, adhesion and migration, and it participates in the regulation of tumour progression and metastasis [15].

According to previous studies, TM4SF1 and DDR1 interact with and activate resting cancer cells in distant organs to cause tumour recurrence and metastasis [16], but the relationship between the expression of TM4SF1 and DDR1 in ovarian cancer prognosis is still unclear. Therefore, the present study investigated the expression of TM4SF1 and DDR1 in ovarian cancer and their correlation with the prognosis of ovarian cancer.

Materials And Methods

Patients and tissue samples

Patients were treated at the Department of Gynaecological Oncology of Guangxi Medical University Affiliated Cancer Hospital from January 2013 to June 2017. All patients received ovarian tumour cytoreductive surgery and adjuvant chemotherapy. All cases were confirmed by pathological diagnosis, and complete clinicopathological parameters were obtained, including age, hospitalization time, operation time, operation method, degree of surgical resection, ascites, tumour pelvic metastasis, abdominal cavity metastasis, distant metastasis, histopathological type, degree of differentiation, tumour staging, adjuvant chemotherapy regimen, adjuvant chemotherapy frequency, recurrence and survival after treatment. The follow-up data were obtained by telephone or outpatient visits. The present study was approved by the Ethics Committee of Guangxi Medical University Affiliated Cancer Hospital.

Immunohistochemistry (IHC) staining

Paraffin-embedded ovarian cancer tissues were obtained from the Department of Pathology of Guangxi Medical University Affiliated Cancer Hospital. The tissues were cut into 4 μm thick paraffin sections and heated at 60°C for 20 min. The sections were then soaked in xylene and different concentrations of alcohol (95%, 70% and 50%) for dewaxing followed by soaking in distilled water for 5 min for hydration. The hydrated tissue sections were immersed in a container with 0.01 M citrate buffer (pH 6.0) and heated to 92–98 °C for 10–115 min for antigenic repair. According to the protocol, the two-step SP immunohistochemistry kit (Beijing Zhongshan Jinbridge) was used for the immunohistochemical detection of TM4SF1 and DDR1 expression in normal ovarian epithelium, benign ovarian tumour tissue and ovarian cancer tissue. The rabbit anti-human TM4SF1 antibody was purchased from Abcam (ab113504), and the rabbit anti-human DDR1 antibody was purchased from Cell Signaling Technology (#5583). The antibodies were diluted at 1:100 and then developed in 3,3'-diaminobenzidine solution. The immunostained tissue sections were independently observed by two experienced pathologists on an optical microscope (Olympus, Japan) and were scored according to methods previously reported in the literature [17].

GEPIA analysis

GEPIA is a newly developed interactive online analysis tool (http://gepia.cancer-pku. cn/), and we used GEPIA to analyse the RNA sequencing expression data of 9736 tumour tissues from The Cancer Genome Atlas (TCGA) and 8587 normal tissues from the Genotype-Tissue Expression (GTEx) database [18]. In the present study, GEPIA was used to determine the expression levels of the TM4SF1 and DDR1 genes in different cancer types, and the differential expression of the TM4SF1 and DDR1 genes in ovarian cancer tissue and normal ovarian tissue was compared. Survival analysis of OS and DFS was then conducted to compare TM4SF1 and DDR1 expression in ovarian cancer patients. The hazard ratio (HR) and P or Cox P values of the logarithmic rank test were calculated and displayed in the graph.

Oncomine analysis

Oncomine is currently the world's largest oncogene chip database and integrated data mining platform （https://www.oncomine.org）, collecting 715 gene expression datasets and 86,733 cancer tissue and normal tissue samples [19, 20]. First of all, we searched with "TM4SF1" and "DDR1" as keywords, and the results were displayed and set as: "P-value: 0.01", "Fold Change: 1.5" and "Gene Rank: all" to analyze the differential expressions of TM4SF1 and DDR1 in different types of tumors and corresponding normal tissues. Then, "TM4SF1" and "DDR1" were used as keywords, and "Ovarian Cancer", " Cancer vs. Normal " and "mRNA" data types were used as filter conditions, and the meta-analysis provided by Oncomine database was used to verify the differential expression of TM4SF1 and DDR1 genes in ovarian cancer tissue.

PPI network

STRING (version 11.0) is an online search tool for analysing protein–protein interactions and functional protein networks, and it contains confirmed and predicted direct and indirect protein–protein interaction biological data [21]. In the present study, TM4SF1 and DDR1 were used as query conditions, and the minimum required interaction score was set to above 0.4 of medium reliability. The top 50 proteins interacting directly with TM4SF1 and DDR1 were queried according to the interaction score.

Functional and pathway enrichment analyses

Using the R ClusterProfiler program package, TM4SF1, DDR1 and the top 50 filtered interacting proteins that directly interact with TM4SF1 and DDR1 were used to perform GO function and KEGG pathway enrichment analyses [22]. The GO functional enrichment analysis included the following three categories: biological process (BP), cell composition (CC) and molecular function (MF). A corrected P value of <0.05 was considered statistically significant.

Kaplan–Meier (KM) plotter database analysis

KM plotter is an online database that assesses the relationship of 54,000 genes in 21 cancer types with survival rates [23]. The relationships of TM4SF1 expression, DDR1 expression and combined TM4SF1 and DDR1 expression with OS and PFS in ovarian cancer were analysed.

Statistical analysis

Functional enrichment analysis was performed by R 3.63 software (https://www.r-project.org/). The clusterProfiler program package was used to perform GO and KEGG enrichment analyses, and enrichplot and ggplot2 program packages were used to visualize the enrichment analysis results.

Results

Expression of TM4SF1 and DDR1 in cancers

The biological functions and roles of TM4SF1 and DDR1 in cancer are still unclear. To reveal their roles in tumorigenesis and development, differentially expressed genes were identified. The results showed that TM4SF1 and DDR1 were highly expressed in a variety of common malignant tumours (Figure 1). GEPIA analysis showed that TM4SF1 and DDR1 were significantly higher in ovarian cancer than in normal ovaries (both P□0.05). A meta-analysis of the Oncomine database (including 10 analyses of 7 ovarian cancer datasets) also showed that TM4SF1 and DDR1 were significantly more highly expressed in ovarian cancer than in normal ovarian tissue (TM4SF1: P=0.004; DDR1: P=3.22E- 4) as shown in Figure 2.

Enrichment analysis of TM4SF1- and DDR1-interacting proteins

The top 50 proteins that directly interact with TM4SF1 and DDR1 were queried by the STRING online tool, and 28 and 25 proteins were found to interact with TM4SF1 and DDR1, respectively. Among these interacting proteins, both CYSTM1 and NT5M interacted with TM4SF1 and DDR1, and TM4SF1 also interacted with DDR1 (Figure 3).

GO enrichment analysis of the interacting proteins showed that they were mainly involved in the composition of ECM, collagen, integrin and their complexes as well as adhesion spots, endoplasmic reticulum and other intracellular and extracellular components. GO enrichment analysis also indicated that these interacting proteins had the molecular function of binding with integrin, cell adhesion molecules, growth factors, proteoglycans, neurotrophic factor receptors and γ -catenin. GO analysis also showed that they were mainly involved in cell-matrix adhesion and cell response to amino acid stimulation, and it indicated that they played biological functions by activating integrin and collagen-mediated signalling pathways (Figure 4A, Supplementary Table 1). KEGG enrichment analysis showed that these interacting proteins were mainly involved in adhesion spot-related signal transmission, ECM-receptor interaction, actin cytoskeleton regulation, small RNA signalling pathways related to cancer, proteoglycan signalling pathways related to cancer, the PI3K/Akt signalling pathway, neurotrophic factor signalling pathways and a variety of tumour-related pathways, such as small cell lung cancer-, chronic myeloid leukaemia-, thyroid cancer- and bladder cancer-related pathways (Figure 4B, Supplementary Table 2).

Expression of TM4SF1 and DDR1 proteins in ovarian cancer and the relation with clinicopathologic features

A total of 94 patients with epithelial ovarian cancer were included, and the patient age ranged from 28 to 83 years old with a median age of 51 years. A total of 56 patients received neoadjuvant chemotherapy. The results showed that 46 patients (48.94%) were positive for TM4SF1 protein, and 52 patients (55.32%) were positive for DDR1 protein. In addition, 26 patients (27.66%) were positive for both TM4SF1 and DDR1 protein. The staining of TM4SF1 and DDR1 proteins in ovarian cancer tissues is shown in Figure 5. Correlation analysis showed that only the positive expression of DDR1 protein was significantly different in different histological grades, FIGO stages and intraperitoneal metastases (both P□0.05, Table 1).

Relationship of TM4SF1 and DDR1 expression with the prognosis of ovarian cancer

Biological database analysis showed that ovarian cancer patients with high expression of TM4SF1 had significantly lower disease-free survival(DFS) or PFS than those with low expression (DFS: HR=1.3, P=0.046, n=424, Figure 6A; PFS: HR=1.17, P =0.019, N=1435, Figure 6E). In addition, the expression of DDR1 had no correlation with patient DFS or PFS, and the expression of TM4SF1 and DDR1 had no significant correlation with patient OS (Figure 6B-D, Figure 6F-H). However, KM plotter multigene analysis showed that higher expression of TM4SF1 and DDR1 was significantly associated with shorter PFS of patients with ovarian cancer (HR = 1.15, P =0.039, n=1435, Figure 6I) but not with OS (Figure 6J).

Analysis of clinical data showed that 94 patients had ovarian cancer with a median follow-up of 33 months, and there was no significant difference in the median OS between TM4SF1-positive and TM4SF1-negative patients (29 vs. 47 months, P>0.05). In contrast, the median OS of DDR1-positive patients was significantly shorter than that of DDR1-negative patients (31 vs. >73 months, P<0.05). The median OS of patients with TM4SF1 and DDR1 coexpression was significantly shorter than that of patients lacking TM4SF1 and DDR1 coexpression (21 vs. 49 months, P<0.05). In addition, the median PFS of TM4SF1-positive patients was significantly shorter than that of TM4SF1-negative patients (18 vs. 26 months, P<0.05). The median PFS of patients with TM4SF1 and DDR1

coexpression was significantly shorter than that of patients lacking TM4SF1 and DDR1 coexpression (12 vs. 26 months, P<0.05), while the expression of DDR1 was not related to the median PFS (P>0.05) (Figure 7). Thus, the TM4SF1 and DDR1 coexpression indicated that patients with ovarian cancer had shorter PFS and OS.

Analysis of prognostic factors in ovarian cancer

Regarding the clinicopathological factors, univariate analysis showed that FIGO stage, intraperitoneal invasion, DDR1 expression, TM4SF1 expression and coexpression of DDR1 and TM4SF1 were factors affecting the OS of patients with ovarian cancer, while FIGO stage, lymph node resection and coexpression of TM4SF1 and DDR1 were factors affecting the PFS of ovarian cancer patients (Table 2).

Cox multivariate analysis showed that TM4SF1 and DDR1 coexpression was the only independent risk factor affecting OS and PFS in ovarian cancer patients (FIG. 8A, B), suggesting that the expression of TM4SF1 and DDR1 may be synergistically involved in the development of ovarian cancer.

Discussion

Ovarian cancer is a highly malignant gynaecological tumour. Despite the development of treatment methods, the prognosis of patients is still far from ideal. However, the pathogenesis of ovarian cancer is still unclear, and few effective therapeutic targets are available. TM4SF1 and DDR1 proteins have been reported to be expressed in a variety of cancer tissues and are associated with poor prognosis in patients [6–9, 15], and database analysis has also reported similar results. However, the role of TM4SF1 and DDR1 in the occurrence, development and prognosis of ovarian cancer remains unclear.

Antitumour immunity induced by TM4SF1 inhibits the growth and migration of TM4SF1-positive cancer cells in vitro and in vivo [24]. Previous studies have found that the positive rate of TM4SF1 protein in ovarian cancer tissues, especially metastatic lymph nodes, is significantly higher than that in benign ovarian tumours and normal ovarian tissues. The expression status of TM4SF1 in ovarian cancer is significantly related to FIGO staging and tissue differentiation [25]. Similarly, our study also found that the positive rate of TM4SF1 protein in ovarian cancer tissues was higher, but it was slightly lower than that previously reported. This difference may be due to some cases with neoadjuvant chemotherapy and some stale specimens as well as different experimental conditions, sample sizes and operators. Among the related factors that affect the prognosis of patients, the PFS of TM4SF1-positive patients was significantly shorter than that of TM4SF1-negative patients but did not affect the OS of patients. Our study illuminated that the positive expression of TM4SF1 was not an independent risk factor for PFS and OS in ovarian cancer. In view of the difference in the positive rate of TM4SF1 compared to previous studies, the effect of neoadjuvant chemotherapy on the tissue expression of TM4SF1 requires further research to confirm.

DDR1 has different effects on the biological functions, disease progression and prognosis of different types of tumour cells, but increasing evidence has shown that it is involved in prometastatic and prosurvival signals [26]. Although DDR1 was not an independent risk factor affecting the prognosis of ovarian cancer, univariate analysis showed that it was significantly related to OS. Our study found that the positive rate of DDR1 in ovarian cancer tissues was 55.32%, while the positive expression rate in patients with advanced stage (stages III to IV), low differentiation (or high grade) and peritoneal metastasis was significantly higher than that in patients with early stage (stages I to II), well differentiated tissues and nonperitoneal metastasis. Advanced stage and high-grade cancer are both poor prognostic factors for ovarian cancer. DDR1 was highly expressed in these sample types in our study, suggesting that DDR1 overexpression is positively correlated with the severity of the disease and that high

pathological grade affects the OS of patients. Our results were consistent with previous results reported by Quan et al. [27], implying that DDR1 might play a role in promoting the survival and metastasis of ovarian cancer cells.

The immunohistochemical staining of ovarian cancer tissue sections showed that TM4SF1 and DDR1 proteins were mainly distributed in the cell membrane and that both were expressed in some cases. STRING analysis showed that there was an interaction between TM4SF1 and DDR. Moreover, the coexpression of M4SF1 and DDR1 was not only significantly related to PFS and OS in patients with ovarian cancer but was also an independent poor prognostic factor. Therefore, TM4SF1 and DDR1 might play important biological functions through interaction. Gao et al. [16] found that TM4SF1 interacts with DDR1 in breast cancer cells and that collagen I promotes their interaction and reactivation of dormant metastatic breast cancer cells in multiple organs, leading to breast cancer recurrence and multiple organ metastasis. Due to the limitation of immunostaining, whether TM4SF1 and DDR1 proteins are colocalized and interact with each other in ovarian cancer cells and whether a mutual regulatory mechanism between TM4SF1 and DDR exists remain to be further studied.

The present study reported the relationship of the combined expression of TM4SF1 and DDR1 with the prognosis of ovarian cancer patients. However, there were several limitations. First, there was not enough clinical samples, and the individual differences may have impacted the experimental results. Second, the tissue specimen quality, experimental operations and statistical errors may have affected the experimental results. Our research mainly focused on clinical studies, and further studies need to be conducted on the relevant regulatory mechanisms of TM4SF1 and DDR1 in ovarian cancer cells at the protein, cell function and animal levels.

Conclusion

The TM4SF1 and DDR1 proteins were coexpressed in some ovarian cancer tissues, which may be adverse prognostic factors for ovarian cancer patients, and there may be an interaction or mutual regulatory mechanism between TM4SF1 and DDR1.

Abbreviations

GEPIA: Gene Expression Profiling Interactive Analysis; GO: Gene Ontology; STRING: Search Tool for the Retrieval of Interacting Genes/Proteins; KEGG: Kyoto Encyclopaedia of Genes and Genomes; OS: Overall survival; PFS: progression-free survival ; TM4SF1:Transmembrane 4 L six family member 1; DDR1:Discoidin domain receptor 1; TCGA: The Cancer Genome Atlas; GTEx: The Genotype-Tissue Expression; DFS: Disease-free survival; KM: Kaplan–Meier; ECM: Extracellular matrix; PPI: Protein–protein interaction.

Declarations

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Authors' contributions

Zhijiong Huang and Hongyu Yao carried out the study concepts, literature research, clinical studies, data analysis, experimental studies, manuscript writing and editing; Zhijun Yang performed the study design, experimental instruction and manuscript modification. All authors have read and approved the submission of the manuscript.

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Availability of data and materials

The data that support the findings of this study are not publicly available due to their containing information that could compromise the privacy of research participants but are available on request from the corresponding author.

Ethics approval and consent to participate

All patients signed the written informed consent. All procedures were approved by the Ethics Committee of Guangxi Medical University Cancer Hospital and operated in keeping with the standards set out in the Announcement of Helsinki and Laboratory Guidelines of Research in China.

Consent for publication

Not applicable.

Competing interests

All authors have no conflicts of interest and declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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Tables

Table 1. Expression of TM4SF1 and DDR1 in ovarian cancer patients with different clinicopathologic features

Factors	N	TM4SF1(+) [n(%)]	P	DDR1(+) [n(%)]	P	TM4SF1(+)&DDR1(+) [n(%)]	P
age							
≤50 years	46	27（58.70）	0.064	26 （56.52）	0.818	16（34.78）	0.131
＞50 years	48	19（39.58）		26 （50.00）		10（20.83）	
histological grade							
G1-2	20	11（55.00）	0.541	6（30.00）	0.010	5（25.00）	0.764
G3	74	35（47.30）		46(62.16)		21（28.66）	
FIGO stage							
stage（）	12	5（41.67）	0.589	2（16.67）	0.004	2（16.67）	0.362
stage（）	82	41（50.00）		50(60.98)		24（29.37）	
pathological type							
serous carcinoma	82	37（45.12）	0.053	47(57.32)	0.605	22（26.83）	0.638
non-serous carcinoma	12	9（75.00）		5(41.67)		4（33.33）	
intraperitoneal metastasis							
yes	63	33（52.38）	0.341	42 （66.67）	0.002	20（31.75）	0.207
no	31	13（41.94）		10 （32.26）		6（19.35）	
Neoadjuvant chemotherapy							
yes	56	25（44.62）	0.312	32 （57.14）	0.666	14（25.00）	0.484
no	38	21（55.26）		20 （52.63）		12（31.58）	
lymphatic metastasis▲							
yes	25	13（52.00）	0.453	15 （60.00）	0.113	7（28.00）	0.282
no	31	13（52.00）		12 （38.70）		5（16.13）	

note：▲Comparisons were made in the 56 patients with resected lymph nodes.

Table 2 Univariate analysis of the correlation of clinicopathological factors with OS and PFS in ovarian cancer

parameter	OS			PFS		
	HR	95%CI	P	HR	95%CI	P
age	0.877	0.506–1.520	0.641	0.866	0.541–1.385	0.548
grade	1.146	0.573–2.292	0.700	1.387	0.742–2.592	0.305
stage	6.860	1.650–28.520	0.008	2.999	1.287–6.990	0.011
histological_type	0.877	0.374–2.057	0.763	1.574	0.680–3.640	0.289
abdominal_invasion	2.253	1.193–4.257	0.012	1.631	0.981–2.711	0.059
NACT	1.750	0.953–3.214	0.071	1.565	0.946–2.589	0.082
lymph_node_excision	0.617	0.355–1.072	0.087	0.628	0.390–1.010	0.055
TM4SF1	1.454	0.838–2.525	0.183	1.646	1.026–2.642	0.039
DDR1	2.324	1.271–4.249	0.006	1.555	0.962–2.513	0.072
TM4SF1&DDR1	2.559	1.450–4.517	0.001	2.572	1.566–4.224	0.000

NACT：neoadjuvant chemotherapy

Figures

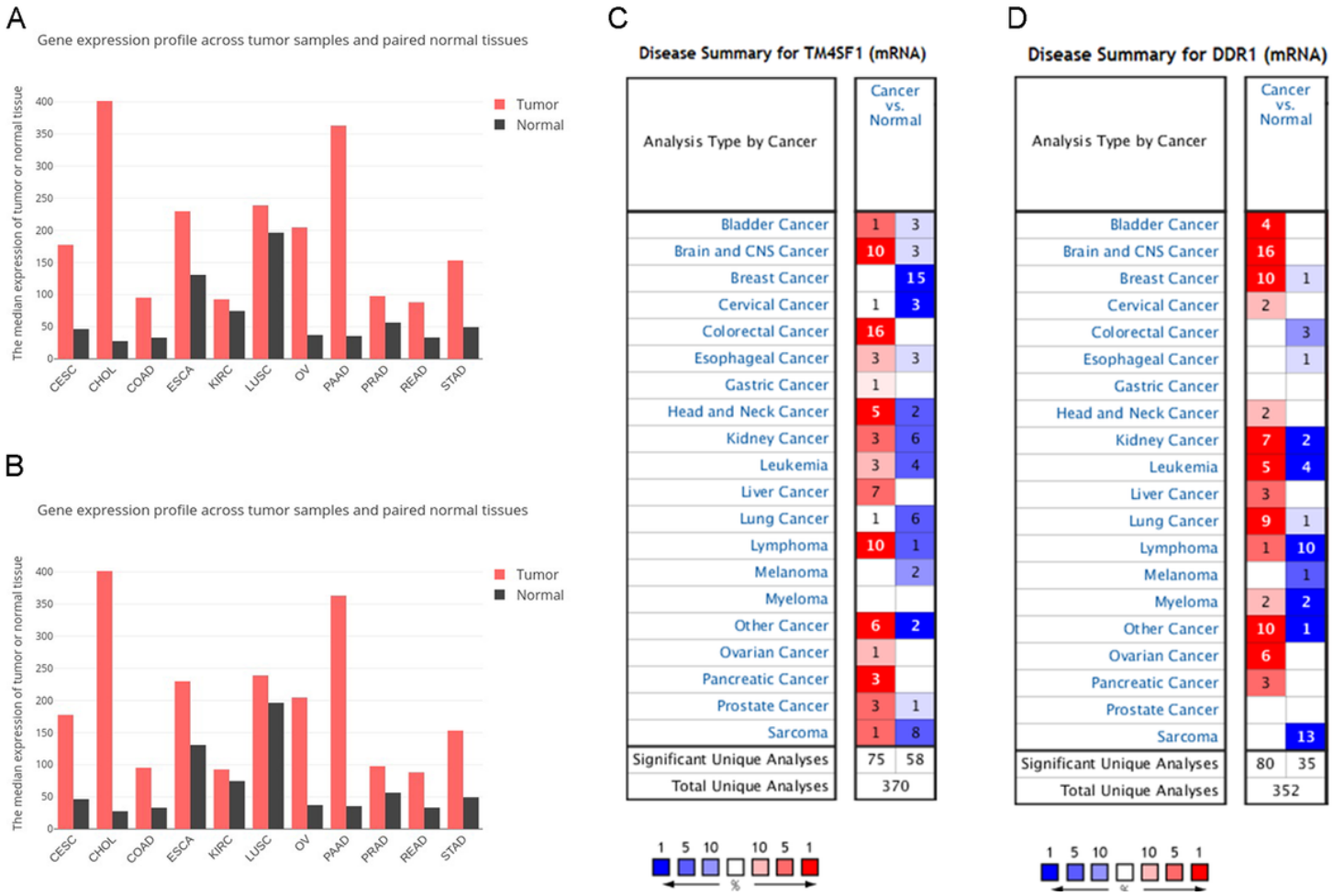

Figure 1

The biological functions and roles of TM4SF1 and DDR1 in cancer are still unclear. To reveal their roles in tumorigenesis and development, differentially expressed genes were identified. The results showed that TM4SF1 and DDR1 were highly expressed in a variety of common malignant tumours

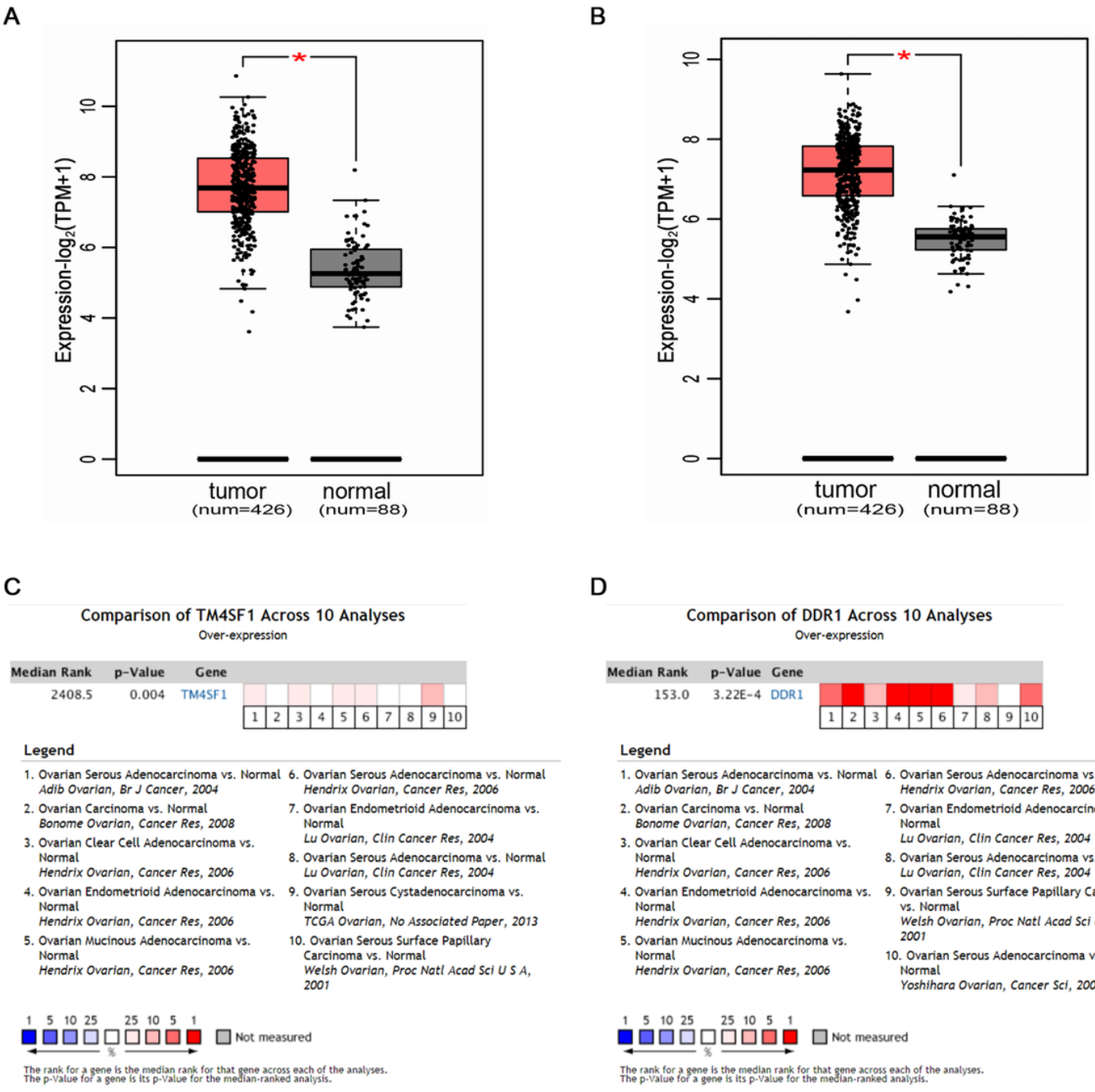

Figure 2

GEPIA analysis showed that TM4SF1 and DDR1 were significantly higher in ovarian cancer than in normal ovaries (both P□0.05). A meta-analysis of the Oncomine database (including 10 analyses of 7 ovarian cancer datasets) also showed that TM4SF1 and DDR1 were significantly more highly expressed in ovarian cancer than in normal ovarian tissue (TM4SF1: P=0.004; DDR1: P=3.22E- 4)

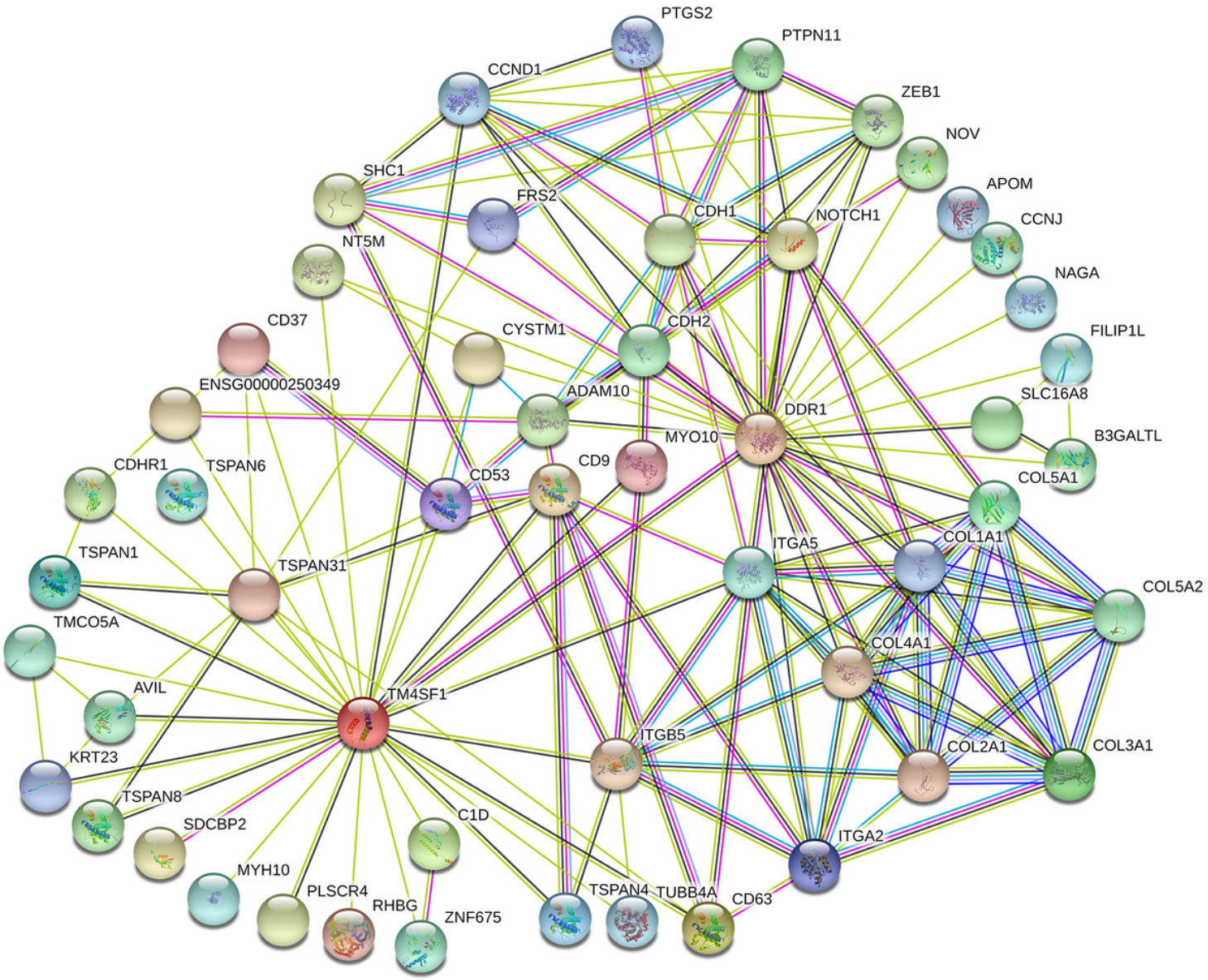

Figure 3

The top 50 proteins that directly interact with TM4SF1 and DDR1 were queried by the STRING online tool, and 28 and 25 proteins were found to interact with TM4SF1 and DDR1, respectively. Among these interacting proteins, both CYSTM1 and NT5M interacted with TM4SF1 and DDR1, and TM4SF1 also interacted with DDR1

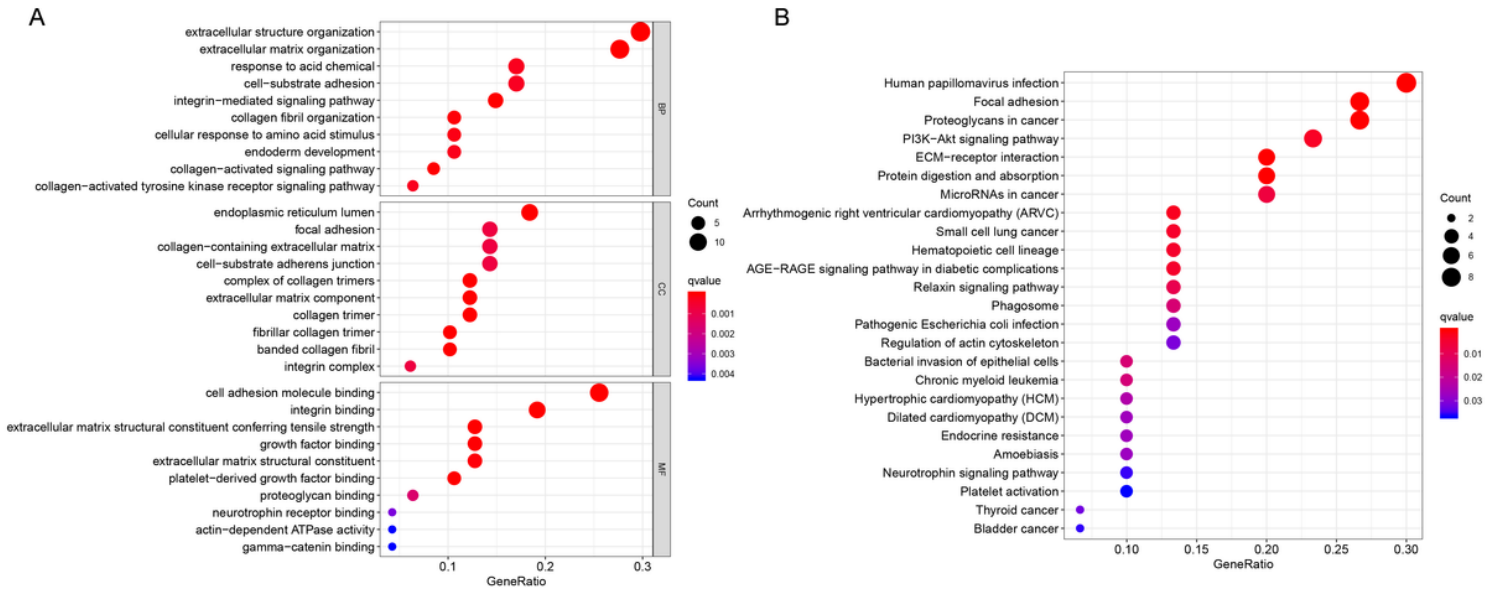

Figure 4

GO enrichment analysis of the interacting proteins showed that they were mainly involved in the composition of ECM, collagen, integrin and their complexes as well as adhesion spots, endoplasmic reticulum and other intracellular and extracellular components. GO enrichment analysis also indicated that these interacting proteins had the molecular function of binding with integrin, cell adhesion molecules, growth factors, proteoglycans, neurotrophic factor receptors and γ -catenin. GO analysis also showed that they were mainly involved in cell-matrix adhesion and cell response to amino acid stimulation, and it indicated that they played biological functions by activating integrin and collagen-mediated signalling pathways

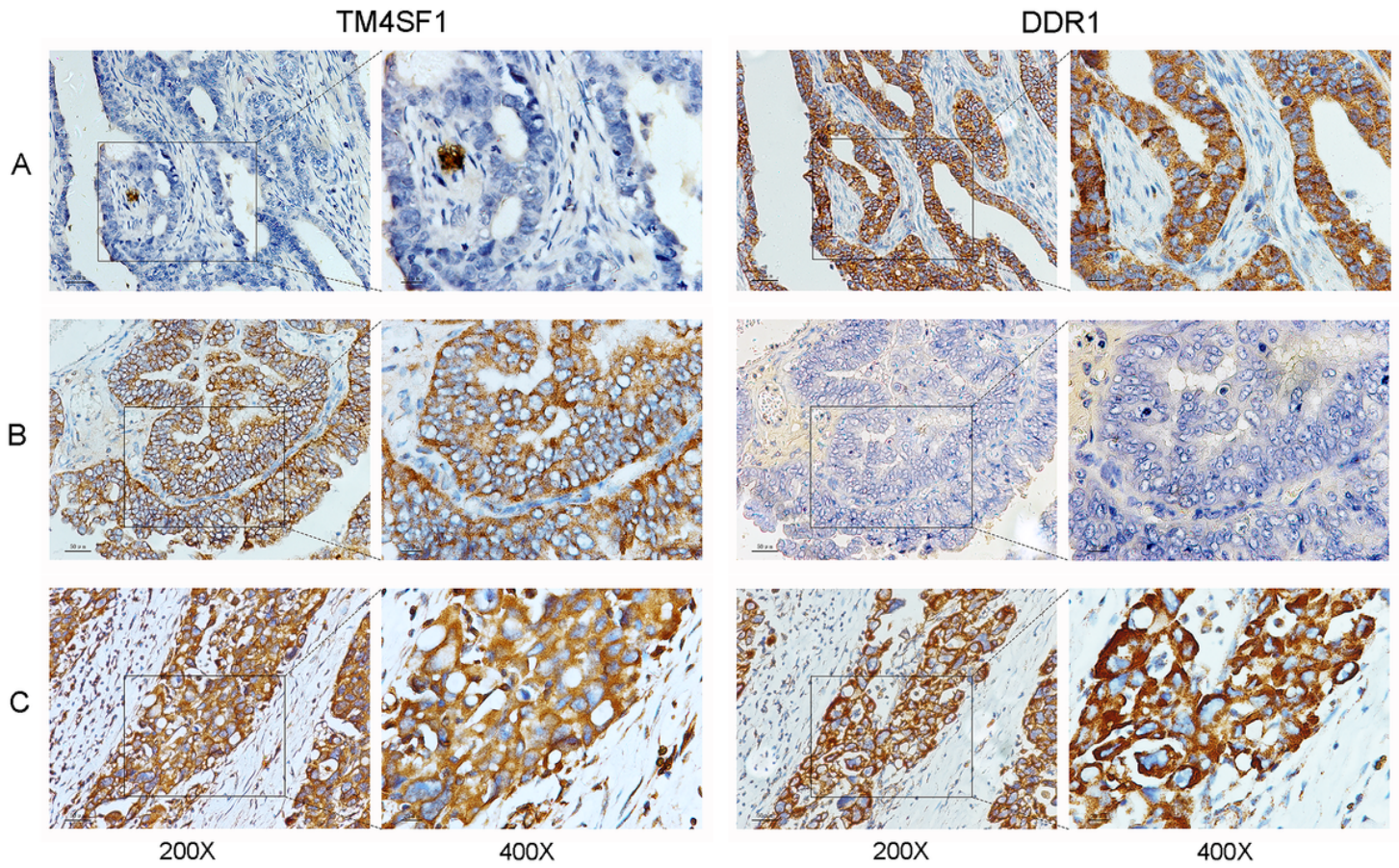

Figure 5

A total of 94 patients with epithelial ovarian cancer were included, and the patient age ranged from 28 to 83 years old with a median age of 51 years. A total of 56 patients received neoadjuvant chemotherapy. The results showed that 46 patients (48.94%) were positive for TM4SF1 protein, and 52 patients (55.32%) were positive for DDR1 protein. In addition, 26 patients (27.66%) were positive for both TM4SF1 and DDR1 protein. The staining of TM4SF1 and DDR1 proteins in ovarian cancer tissues

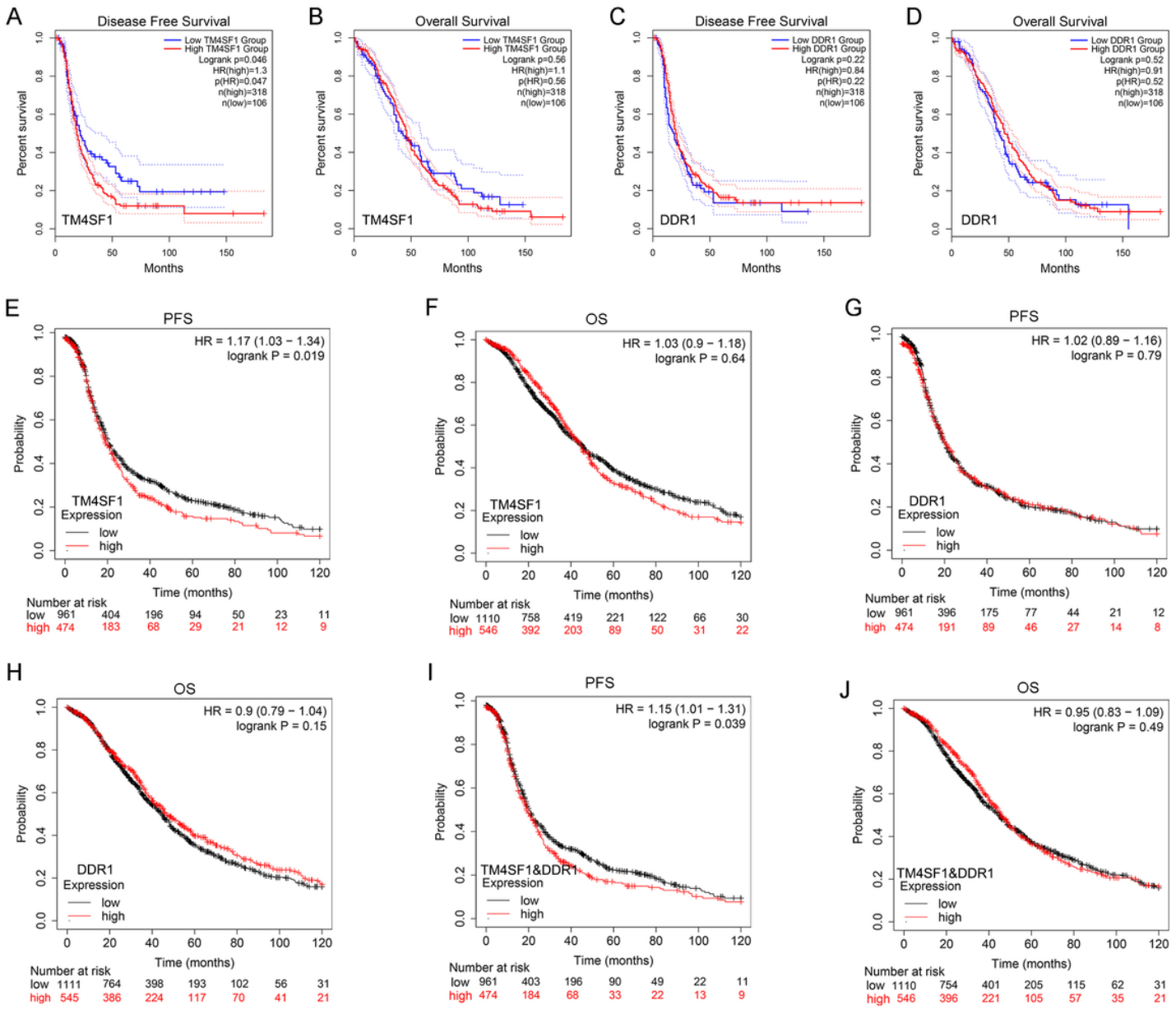

Figure 6

Biological database analysis showed that ovarian cancer patients with high expression of TM4SF1 had significantly lower disease-free survival(DFS) or PFS than those with low expression (DFS: HR=1.3, P=0.046, n=424, Figure 6A; PFS: HR=1.17, P =0.019, N=1435, Figure 6E). In addition, the expression of DDR1 had no correlation with patient DFS or PFS, and the expression of TM4SF1 and DDR1 had no significant correlation with patient OS (Figure 6B-D, Figure 6F-H). However, KM plotter multigene analysis showed that higher expression of TM4SF1 and DDR1 was significantly associated with shorter PFS of patients with ovarian cancer (HR = 1.15, P =0.039, n=1435, Figure 6I) but not with OS (Figure 6J).

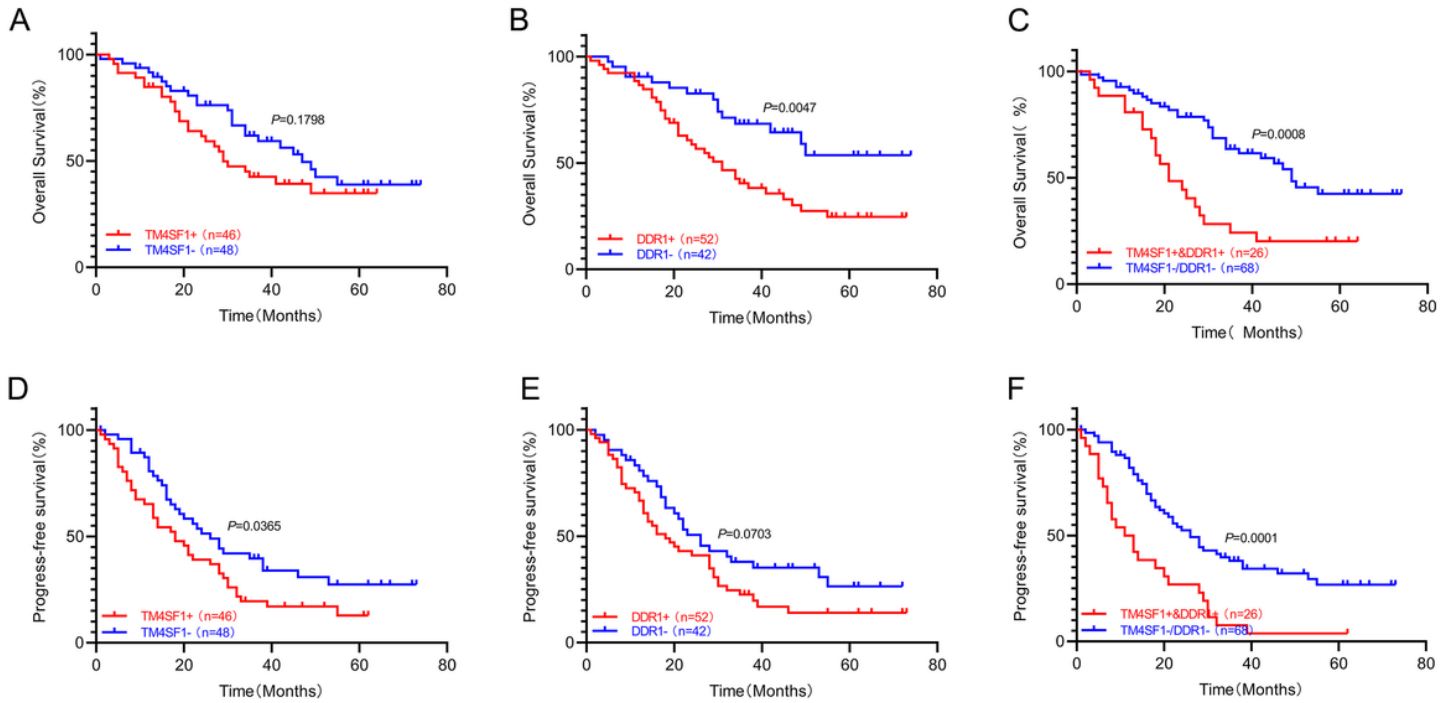

Figure 7

The median OS of patients with TM4SF1 and DDR1 coexpression was significantly shorter than that of patients lacking TM4SF1 and DDR1 coexpression (21 vs. 49 months, P<0.05). In addition, the median PFS of TM4SF1-positive patients was significantly shorter than that of TM4SF1-negative patients (18 vs. 26 months, P<0.05). The median PFS of patients with TM4SF1 and DDR1 coexpression was significantly shorter than that of patients lacking TM4SF1 and DDR1 coexpression (12 vs. 26 months, P<0.05), while the expression of DDR1 was not related to the median PFS (P>0.05)

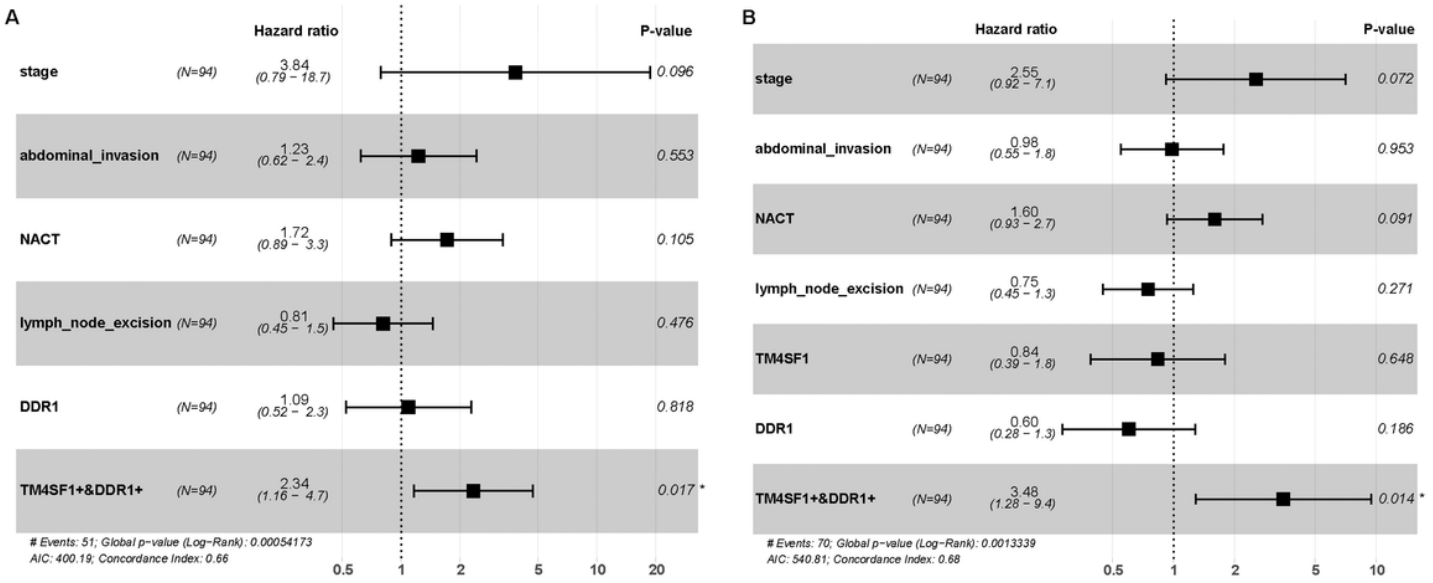

Figure 8

Cox multivariate analysis showed that TM4SF1 and DDR1 coexpression was the only independent risk factor affecting OS and PFS in ovarian cancer patients

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementary.docx