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Identification of Therapeutic Targets and Prognostic Biomarkers Among Matrix Metalloproteinases in the Ovarian Cancer Microenvironment

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Key words
Ovarian cancer, Biomarker, Prognosis, Bioinformatics analysis, Tumor microenvironment, matrix metalloproteinases family

ABSTRACT

Ovarian cancer (OC) is one of the most common malignances with an ever-increasing incidence and high mortality. Cross-talk between cancer cells and interstitial cells exerts significant effects on ovarian and tumor development and is modulated in part by MMPs. MMPs in the tumor microenvironment can modulate immune cell trafficking and regulate tumor cell activities, thus exerting anti-tumor immunological effects and affecting patient outcomes; however, the expression and prognostic values of MMPs in ovarian cancer have not been clarified. Oncomine, NCBI, GEPIA, cBio Portal, Gene MANIA, DAVID 6.8, Meta scape, TRRUST, Linked Omics and TIMER were utilized in this study. The transcriptional levels of MMP1/7/9/10/11/14 in OC tissues were significantly elevated while the transcriptional levels of MMP2/16/23A/23B/28 were significantly reduced. A significant correlation was found between the expression of MMP7/9/12/15/25/27 and the pathological stage of OC patients. OC patients with low transcriptional levels of MMP1/4/12/17 were associated with a significantly better prognosis. The functions of differentially expressed MMPs are primarily related to the matrix metalloproteinases signaling pathway, cell surface receptor receptor interactions, and immune response signaling pathway. Our data suggest that JUN, STAT3, ETV4, ETS1, RELA and NFKB1 are key transcription factors for MMPs, and the SRC family of tyrosine kinases (LCK, LYN, and FYN), threonine kinase (ATR and ATM), CSNK2A1,CDK2 and EGFR are MMPs targets.The various miRNA targets of differentially expressed MMPs. We found significant correlations among the expression of MMPs and the infiltration of six types of immune cells (B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells). Our results may provide novel insights for the selection of immunotherapeutic targets and prognostic biomarkers for OC.

INTRODUCTION

Ovarian cancer (OC) is the most lethal human gynecological tumour and the incidence is on the increase in recent years accounts for approximately 5% of cancer deaths among women (Siegel *et al*., 2020; Torre *et al*., 2018). When diagnosed at an early stage, its prognosis is good and the five-year relative survival rate exceeds 90%. However, a lack of early detection, more than two-thirds of ovarian cancer patients are unfortunately diagnosed at advanced stages, which involve extensive peritoneal dissemination with massive ascites and acquired chemo-resistance during the treatment course (Tan *et al*., 2006). Indeed, patients with massive peritoneal dissemination throughout the abdominal cavity are usually incurable in more than 20% of the cases. Although great progress has been made on varieties of novel drugs, including immune checkpoint inhibitors, molecular target drugs and pharmacologic al inhibitors have been developed and applied for clinical trials, there is still no effective treatment for advanced-stage ovarian cancer and the survival remains very poor (Tewari *et al*., 2019;

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Abbreviations
OC, Ovarian cancer; TCGA, The Cancer Genome Atlas; PPI, NCBI, The National Coalition Building Institute; protein, protein interaction; GO, Gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological processes; CC, cellular components; MF, molecular function; GSEA, gene set enrichment analysis.

Hamanishi *et al*., 2015; Pujade-Lauraine *et al*., 2017). To overcome this lethal disease, the discovery of other novel therapeutic approaches is necessary.

Considering its high degree of lethality, there is an urgent need to identify the underlying cause of the disease and novel therapeutic targets. Therefore, in order to timely and effectively diagnose and treat ovarian cancer, as well as to improve the prognosis and survival rate of patients, it is essential to identify ovarian cancer-specific tumor markers with high sensitivity. Matrix metalloproteinases (MMPs) can be promising molecular targets, since recent evidence has revealed that ovarian cancer-derived MMPs are involved in the process of ovarian cancer progression, epithelial transformation, especially in peritoneal dissemination and the formation of pre-metastatic niche (Karam and Dorigo, 2012; Zhang and Chen, 2017).

MMPs are one important group of proteolytic enzymes and are capable of degrading components of the extracellular matrix and basement membrane (Zeng *et al*., 2020). Various studies have provided highly interesting observations regarding the biological role of MMPs in invasion and migration of ovarian cancer cells (Cai *et al*., 2007; Al-Alem *et al*., 2015). Others have identified factors that regulate the expression and function of MMPs in ovarian cancer models. Many researchers have suggested that MMPs have remarkably high expression in ovarian cancer tissues. MMPs are considered to be related to the occurrence, development, invasion and metastasis of ovarian cancers (Deryugina *et al*., 2006; Ray *et al*., 1995). The investigation of MMP mechanism in ovarian cancer will facilitate the development of effective anti-tumor drugs, and thereby improve the survival rate of patients with ovarian cancer. Herein, the latest research focous on therapeutic targets and prognostic biomarkers among MMPs in the ovarian cancer microenvironment.

Previous studies have characterized a general expression profile and the function of some MMPs in OC, but identifying suitable MMPs as therapeutic targets and prognostic biomarkers for OC is still a tremendous problem that urgently needs attention.With the rapid development of second generation gene sequencing technology and the establishment of various databases, comprehensive analysis of MMPs has become possible. In this study, we conducted an in-depth and comprehensive bioinformatics analysis of the expression of MMPs in OC and evaluated their potential as therapeutic targets and prognostic biomarkers based on several large public databases, thus providing additional data to help clinicians select appropriate therapeutic drugs and more accurately prognose long-term outcome in patients with OC.

MATERIALS AND METHODS

Oncomine

ONCOMINE (www.oncomine.org) is a translational bioinformatics service that provides powerful, genome-wide expression analysis (Rhodes *et al*., 2004). Data were extracted to evaluate the expression of MMPs in OC. In this study, a p 0.05, a fold change of 2, and a gene rank in the top 10% were set as the significance thresholds. Students t test was used to analyze the difference in the expression of MMPs in OC.

GEPIA

GEPIA (http://gepia.cancer-pku.cn/index.html) is an analysis tool containing RNA sequence expression data of 9736 tumors and 8587 normal tissue samples, which was developed at Peking University (Tang *et al*., 2017). In this study, we performed a differential mRNA expression analysis of tumor pathological stage analysis, and correlative prognostic analysis of MMPs with the Single Gene Analysis module of GEPIA. Multiple gene comparison analysis of MMPs was performed with the multiple gene comparison module of GEPIA, using the "OC" dataset. The p value cutoff was 0.05. Student's t test was used to generate a p value for expression or pathological stage analysis. Prognostic analysis was performed using a Kaplan–Meier curve.

GEO data collection and processing

Data were from NCBI GEO database (https://www.ncbi.nlm.nih.gov/GEO),We selected two gene chips of OC from GEO database, including GSE66957 and GSE4122. The selection criteria were as follows: (1) inclusion of normal and OC tissue samples; (2) expression profiling by array as the experiment type; (3) Homo sapiens; Among them, GSE66957 contained 12 normal tissue samples and 57 OC samples. The GSE4122 dataset contained 14 normal human ovary, 18 benign human ovary and 32 malignant, three borderline sample was not available and was excluded. The matrix files and platform annotation document of three microarray datasets were downloaded. We identified MMPs using a paired t-test with a random variance model, which is an improvement over the standard separatet-test when it is applied sample Microarray experiments (Wright and Simon, 2003).

cBio portal

cBioPortal (www.cbioportal.org), a comprehensive web resource,can visualize and analyze multidimensional cancer genomics data (Gao *et al*., 2013). Based on TCGA database, genetic alterations, coexpression, and the network module of MMPs was obtained from cBioPortal.

Five hundred twelve renal clear cell carcinoma samples (TCGA, provisional) were analyzed. Mrna expression z scores (RNA Seq V2 RSEM) were obtained using a z score threshold of ±2.0. Protein expression z scores (RPPA) were obtained using a z score threshold of ±2.0.

GeneMANIA

GeneMANIA (http://www.genemania.org) is a user-friendly website that provides information for protein and genetic interactions, pathways, co-expression, co-localization, and protein domain similarity of submitted genes (Warde-Farley *et al*., 2010).

String

STRING (https://string-db.org/) aims to collect, score, and integrate all publicly available sources of protein–protein interaction (PPI) data, and to complement these with computational predictions of potential functions (Szklarczyk *et al*., 2019). We conducted a PPI network analysis of differentially expressed MMPs to explore the interactions among them with String.

David 6.8

DAVID 6.8 (https://david.ncifcrf.gov/home.jsp) is a comprehensive, functional annotation website. That helps investigators better clarify the biological function of submitted genes (Huang da *et al*., 2009). In our study, the gene ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes(KEGG) pathway enrichment analysis of MMPs and closely related neighbor genes were isolated from DAVID 6.8 and visualized with R project using a ggplot2 package and a $p < 0.05$. Biological processes (BP), cellular components (CC), and molecular function (MF) were included in the GO enrichment analysis.

Metascape

Metascape (http://metascape.org) is a reliable, intuitive tool for gene annotation, and gene list enrichment analysis (Zhou *et al*., 2019). Based on the functional annotation of gene/protein lists, Metascape can facilitate data-driven decisions. In this study, the Express Analysis module was used to further verify the enrichment of MMPs and closely related neighbor genes.

TRRUST

TRRUST (https://www.grnpedia.org/trrust/) is a reliable, intuitive tool for human, and mouse transcriptional regulatory networks. Containing 8444 transcription factor (TF)-target regulatory relationships of 800 human TFs, the TRRUST database can provide information on how these interactions are regulated (Han *et al*., 2018).

Timer

TIMER (https://cistrome.shinyapps.io/timer/) is a reliable,intuitive tool that provides systematic evaluations of the infiltration of different immune cells and their clinical impact (Li *et al*., 2017). In our study, Gene module was used to evaluate the correlation between MMPs level and the infiltration of immune cells. Survival module was used to evaluate the correlation among clinical outcome and the infiltration of immune cells and MMPs expression.

Linked omics

Linked Omics (http://www.linkedomics.org/) is a publicly available portal tool that provides comprehensive multi-omics data analysis across 32 TCGA cancer types (Vasaikar *et al*., 2018). We used the LinkInterpreter module to derive biological insights into kinase target enrichment, miRNA target enrichment, and transcription factor target enrichment of MMPs. Gene Set Enrichment Analysis (GSEA) was used to perform analyses with a minimum number of genes (size) of 3 and a simulation of 500, within the KIRC dataset. Results were analyzed statistically using the Spearman correlation test. The p value cutoff was 0.05.

Statistical analysis

GraphPad prism 7.0 (GraphPad Software, USA)were used for Statistical analysis and graphic representation. The significance of the difference between the control and experimental groups was determined using Student's t-tests.

RESULTS

MMPs differentiated expression in OC

We first delved into the twenty five MMPs' transcriptional expression levels in OC via ONCOMINE dataset (Fig. 1, Table I). The results showed that the transcriptional levels of MMP1, MMP7, MMP9, MMP10, MMP11, MMP14 were significantly elevated while MMP2, MMP16, MMP23A, MMP23B and MMP28 was obviously reduced in OC vs normal ovarian tissue. The high expression levels of MMP1 (fold change=2.598, p=4.89E−11, TCGA) in ovarian serous cystadenocarcinoma and in ovarian mucinous adenocarcinoma (fold change= 2.322, p= 0.000194, Hendrix ovarian) VS normal. The data were consistent with the research dataset of Welsh Ovarian who demonstrated that MMP2 was remarkably decreased in ovarian serous surface papillary carcinoma compared with the normal tissue (fold change=-8.525, p=3.68E−5), and the research dataset of Yoshihara Ovarian Data set who demonstrated that MMP2 was remarkably decreased in ovarian serous adenocarcinoma compared with the normal tissue(fold change=-7.841, p=8.72E−8). MMP7 in

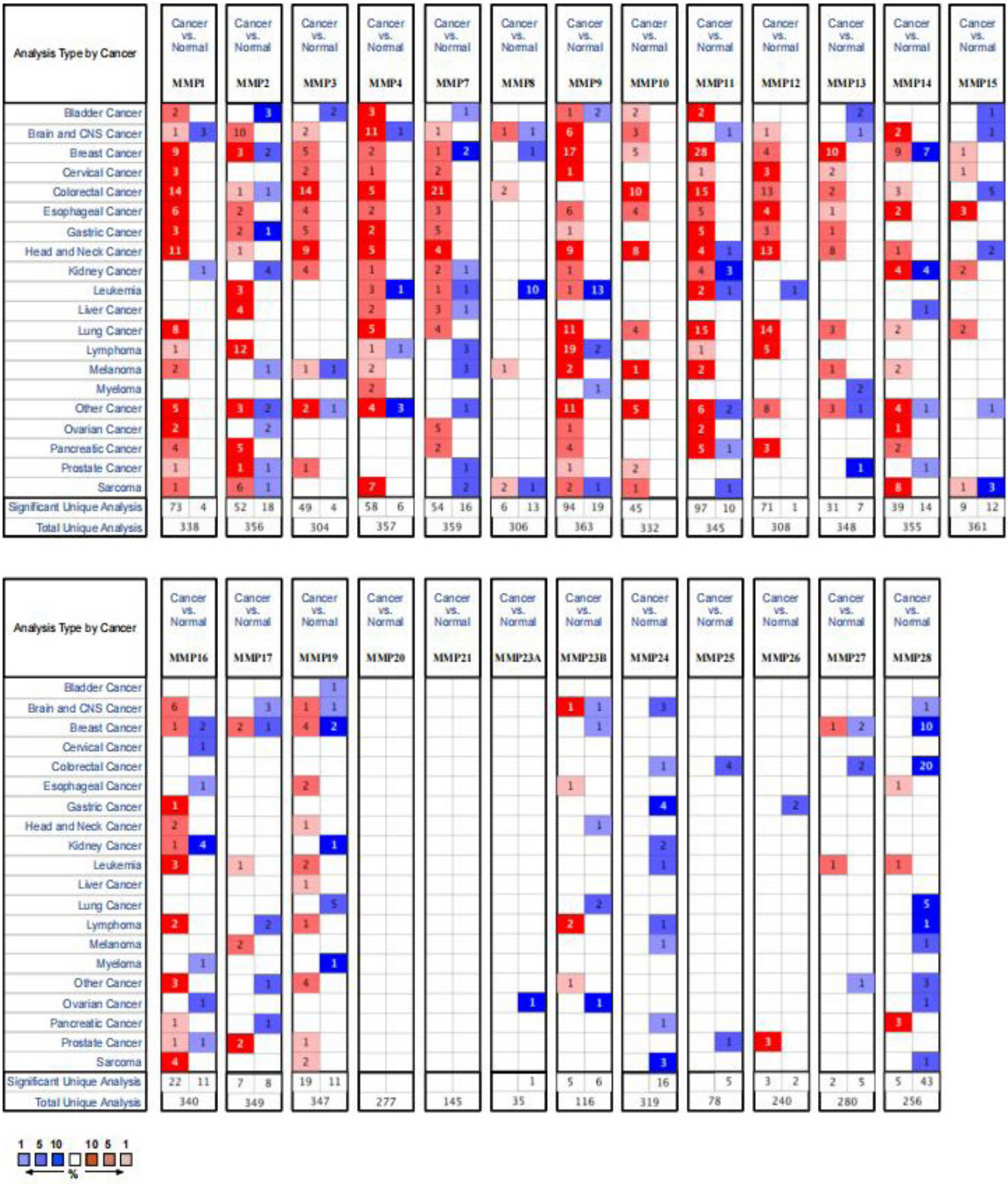


Fig. 1. Expression of MMPs in cancer vs. normal tissues from the oncomine database. The figure shows the numbers of datasets with statistically significant mRNA over-expression (red) or downregulated expression (blue) of MMP protein family.

Table I. The mRNA levels of MMPs in different types of OC tissues and normal ovarian tissues at transcriptome level (ONCOMINE).

TLR	Type	Fold change	P-value	t-test	References	PMID
MMP1	Ovarian serous cystadenocarcinoma	2.598	4.89E-11	11.852	TCGA ovarian	
MMP1	Ovarian mucinous adenocarcinoma	2.322	0.000194	4.697	Hendrix ovarian	PMID: 16452189
MMP2	Ovarian serous surface papillary carcinoma	-8.525	0.0000368	-4.595	Welsh ovarian	PMID: 11158614
MMP2	Ovarian serous adenocarcinoma	-7.841	8.72E-08	-6.181	Yoshihara ovarian	PMID: 19486012
MMP7	Ovarian serous surface papillary carcinoma	5.884	0.000000628	6.193	Welsh ovarian	PMID: 11158614
MMP7	Ovarian serous adenocarcinoma	8.884	0.005	3.913	Adib ovarian	PMID: 14760385
MMP7	Ovarian clear cell adenocarcinoma	4.502	0.002	3.85	Welsh ovarian	PMID: 15161682
MMP7	Ovarian serous adenocarcinoma	7.73	0.000164	5.053	Welsh ovarian	PMID: 15161682
MMP7	Ovarian endometrioid adenocarcinoma	7.356	0.003	3.396	Welsh ovarian	PMID: 15161682
MMP9	Ovarian endometrioid adenocarcinoma	2.388	0.002	3.566	Lu ovarian	PMID: 15161682
MMP11	Ovarian carcinoma	4.481	1.23E-41	19.695	Bonome ovarian	PMID: 18593951
MMP11	Ovarian serous cystadenocarcinoma	3.636	1.08E-13	17.561	TCGA ovarian	
MMP14	Ovarian carcinoma	2.196	4.28E-29	29.212	Bonome ovarian	PMID: 18593951
MMP16	Ovarian serous adenocarcinoma	-12.062	1.09E-09	-8.012	Yoshihara ovarian	PMID: 19486012
MMP23B	Ovarian serous adenocarcinoma	-12.159	5.1E-17	-12.964	Yoshihara ovarian	PMID: 19486012
MMP28	Ovarian serous adenocarcinoma	-14.094	6.04E-15	-10.964	Yoshihara ovarian	PMID: 19486012

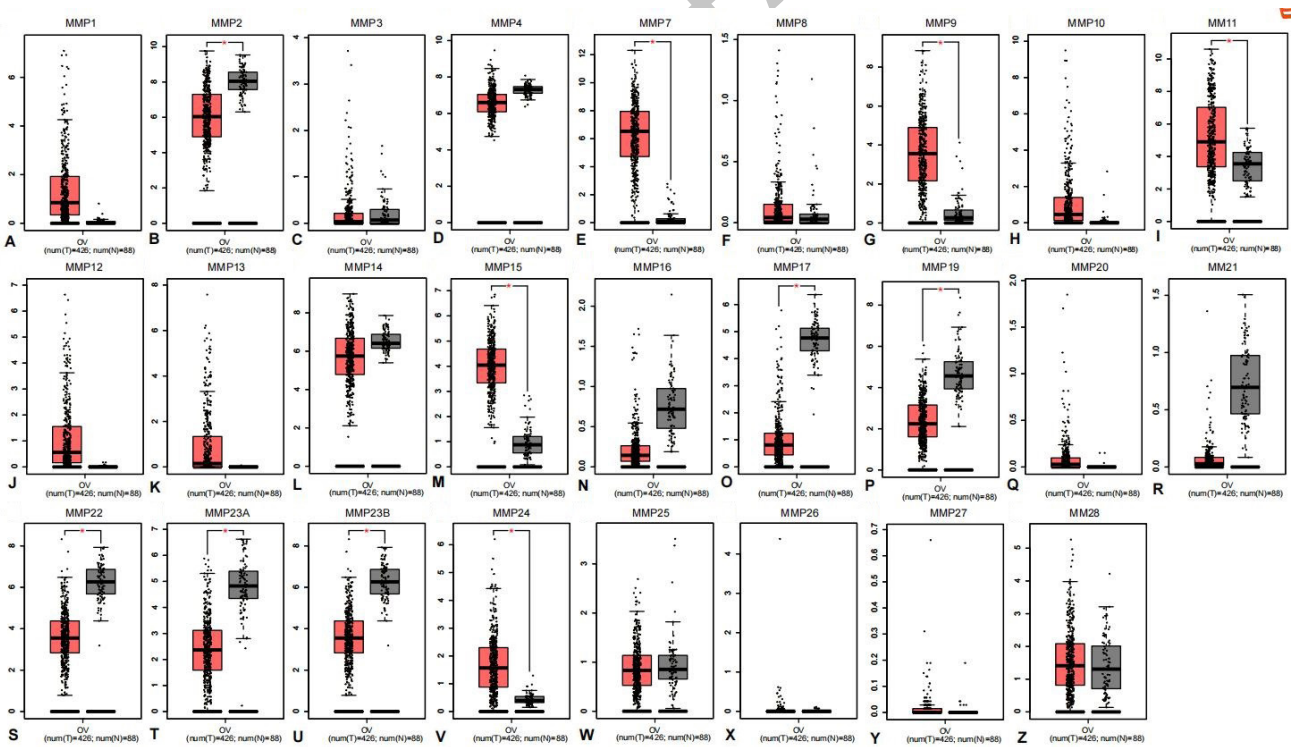


Fig. 2. Expression of MMPs in ovarian cancer and normal tissues analyzed using GEPIA. (A) MMP1, (B) MMP2, (C)MMP3, (D) MMP4, (E) MMP7, (F) MMP8, (G) MMP9, (H) MMP10, (I) MMP11, (J) MMP12, (K) MMP13, (L) MMP14 (M) MMP15, (N) MMP16, (O) MMP17, (P) MMP19, (Q) MMP20, (R) MMP21, (S) MMP22, (T) MMP23A, (U) MMP23B, (V) MMP24, (W) MMP25, (X) MMP26, (Y) MMP27 and (Z) MMP28. In the box plots, the thick line in the middle represents the median, and the upper and lower limits of the box represent the third and first quartile, respectively. The top and bottom of the error bars represent the maximum and minimum values of data, respectively; outliers were considered to Log2FC Cutoff: 1, p-value: 0.01.

ovarian serous surface papillary carcinoma vs. nomal (fold change=5.884,p=6.28E−07, Welsh ovarian) and in ovarian serous adenocarcinoma vs. normal (fold change=8.884, p=0.005, Adib ovarian ovarian), MMP7 in ovarian clear cell adenocarcinoma (fold change= 4.502,p=0.002), in ovarian serous adenocarcinoma (fold change= 7.73, p= 0.000164), in ovarian endometrioid adenocarcinoma (fold change=7.356, p=0.003) were proved by Lu and Yarbrough (2015). The results of MMP9 (fold change= 2.388, p= 0.002) were supported by Lu ovarian. Bonome Ovarian demonstrated that MMP11 (fold change= 4.481, 1.23E-41) and MMP14 (fold change= 2.196, p= 4.28E−29) was obviously increased in ovarian carcinoma. The same results of MMP11 (fold change= 3.636, p= 1.08E−13) in ovarian carcinoma were supported by TCGA data set. The expression of MMP16 (fold change= -12.062, 1.09 E−9), MMP23B (fold change=-12.159, 5.1E−17), MMP28 (fold change= -14.094, 6.04E−15) in Ovarian Serous Adenocarcinoma was decreased compare with normal.

We also found that 11 genes were differentially expressed in OC compared with normal tissue via GEPIA dataset (upregulation of MMP7, MMP9, MMP11,MMP15 and MMP24; downregulation of MMP2, MMP17, MMP19, MMP22, MMP23A and MMP23B) (Fig. 2). We also analyzed the differences in transcription levels of MMPs in OC and normal samples via GSE66957 dataset (**Supplementary Fig. 1**). We found that the transcription levels of MMP1, MMP7, MMP9, MMP12, MMP14, MMP15, MMP19, MMP20 and MMP23A, 23B were remarkably upregulated in OC samples when compared to normal samples. However, the transcription levels of MMP17, MMP21 and MMP24 were lower in OC samples than in the normal samples. We analyzed the differences in transcription levels of MMPs in OC, benign and normal samples via GSE4122 dataset (**Supplementary Fig. 2**), we found that the transcription levels of MMP7 and MMP15 were remarkably upregulated in OC samples when compared to normal samples, however, the transcription levels of MMP2, MMP17 and MMP23A B were lower in OC samples than in the normal samples. We also found that transcription levels of MMP2, MMP17 and MMP23A B were remarkably upregulated in benign tissue compared to malignant samples, while MMP7 and MMP9 were remarkably downregulated in benign tissue compared to malignant samples.

We also compared the relative expression levels of MMPs in OC tissues and found that among all MMPs we evaluated, the relative expression of MMP2, MMP4 (ILF3), MMP7, MMP11, MMP14 and MMP23B was the relative higher than others (Fig. 3). To identify additional MMPs associated with tumorigenesis, progression, and clinical outcome in OC, we evaluated all the MMPs that were differentially expressed in OC tumors vs normal tissues The relationship among the mRNA levels of distinct MMPs and tumor stages of OC was also explored via GEPIA. The results showed that the MMP7, MMP9, MMP12, MMP15, MMP25 and MMP27 groups varied significantly (Fig. 4).

Fig. 3. The relative level of MMPs in OC.

The prognostic value of MMPs in patients with OC

To evaluate the value of differentially expressed MMPs in the progression of OC, we assessed the correlation between differentially expressed MMPs and clinical outcome using GEPIA. Disease-free survival curves are presented in (Fig. 5). OC patients with low transcriptional levels of MMP1 (p= 0.017), MMP4:ILF3 (p= 0.03), MMP12 (p= 0.023) and were significantly associated with longer overall survival, OC patients with high transcriptional levels of MMP17 (p= 0.0094) and were significantly associated with longer overall survival. The value of differentially expressed MMPs in the overall survival of OC patients was also evaluated. We found that OC patients with low transcriptional levels of MMP25 (p= 0.018), were significantly associated with longer disease-free survival (Fig. 6).

Genetic alteration, co-expression, and interaction analyses of MMPs in patients with OC

We performed a comprehensive analysis of the molecular characteristics of differentially expressed MMPs. Provisional datasets of TCGA were utilized to analyze the genetic alterations of differentially expressed MMPs. As a result, MMP1, MMP2, MMP3, MMP4 (ILF3), MMP8, MMP9, MMP10, MMP11, MMP12, MMP13, MMP14, MMP15, MMP16, MMP17, MMP19, MMP20, MMP21, MMP23B, MMP23A, MMP24, MMP25, MMP26, MMP27 and MMP28 were altered in 9, 4, 12, 13, 11, 11, 11, 9, 2, 8, 8, 4, 4, 10, 3, 5, 9, 5, 8, 3, 8, 6, 0.5, 8 and 6% of the queried OC samples, respectively (Fig. 7A). Enhanced mRNA expression was the most common change in these samples.

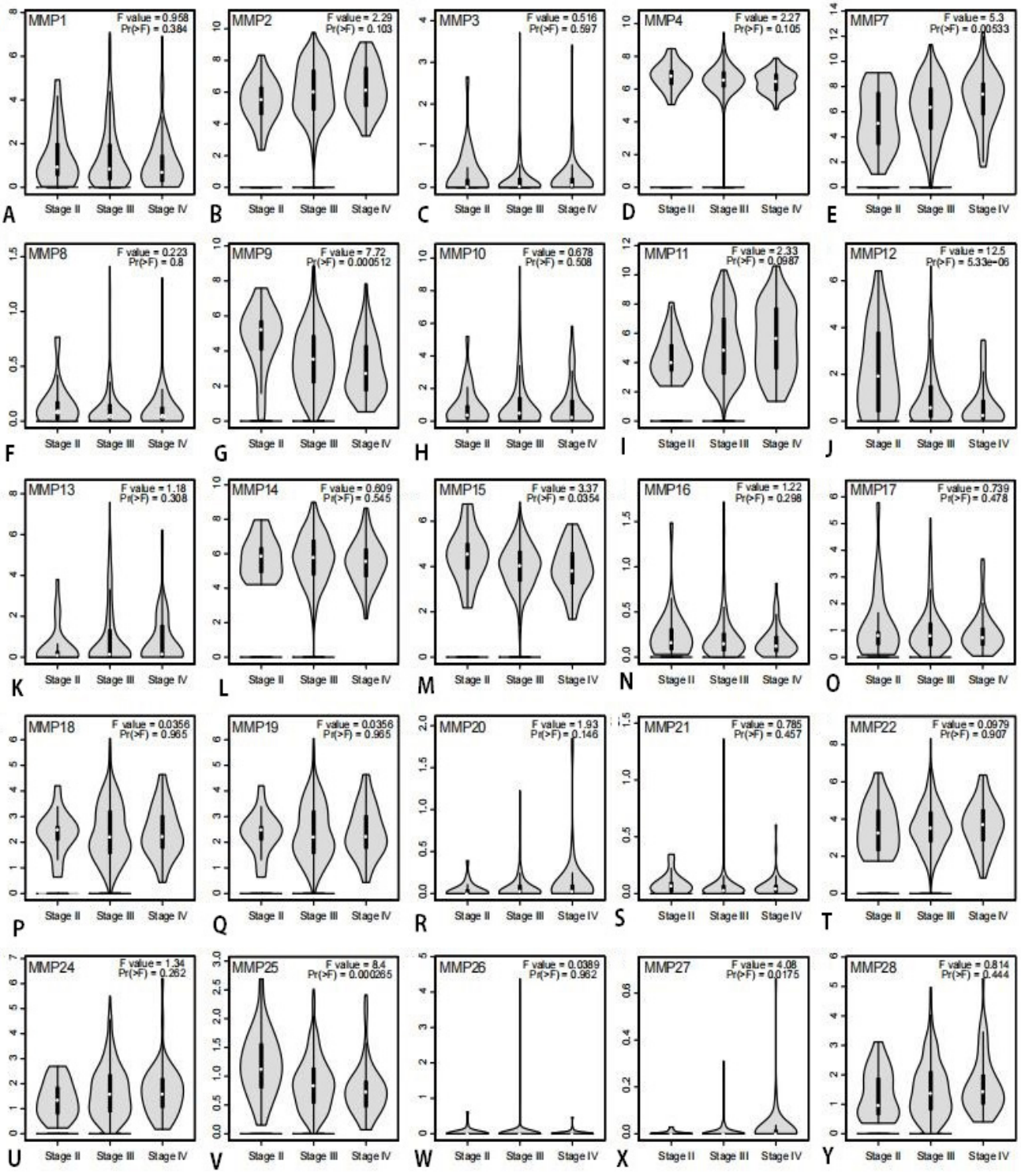


Fig. 4. Association of mRNA expression of MMPs and tumor stages in patients with ovarian cancer analyzed using GEPIA. (A-D) MMP1-4, (E-T) MMP7-22 and (U-Y) MMP24-28. In the violin plots, the white dots represent the median; the black bars represent the 95% confidence intervals; the black lines represent the interquartile range; and the width of the red shapes represent the density of distribution. F-value, the statistical value of F test; Pr (>F), P-value.

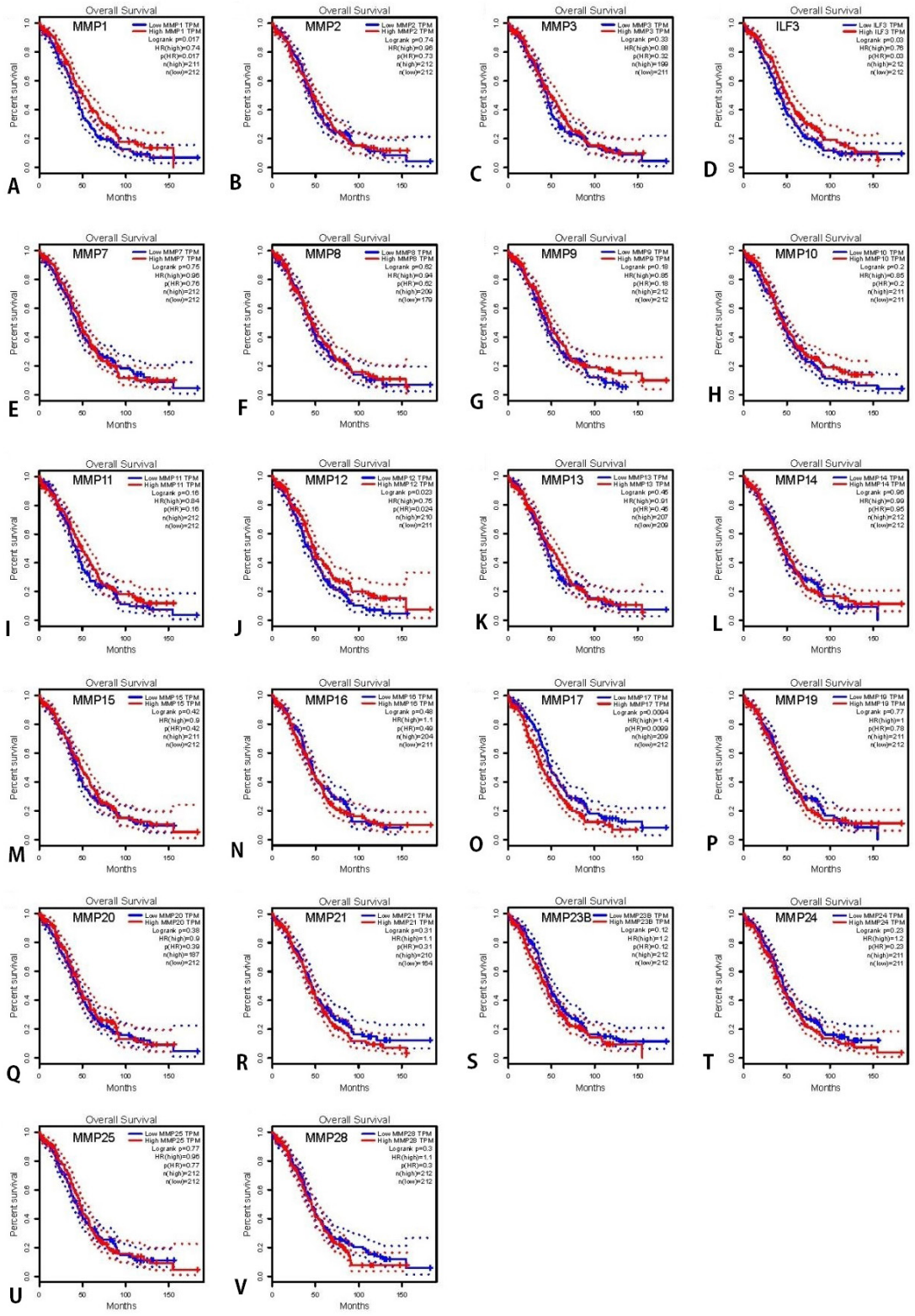


Fig. 5. OS of OC patients with high and low mRNA expression of MMP, analyzed using the Kaplan-Meier Plotter tool. (A-V)OS curves of MMP1-28 plotted for all patients . The threshold of p-value of < 0.05.

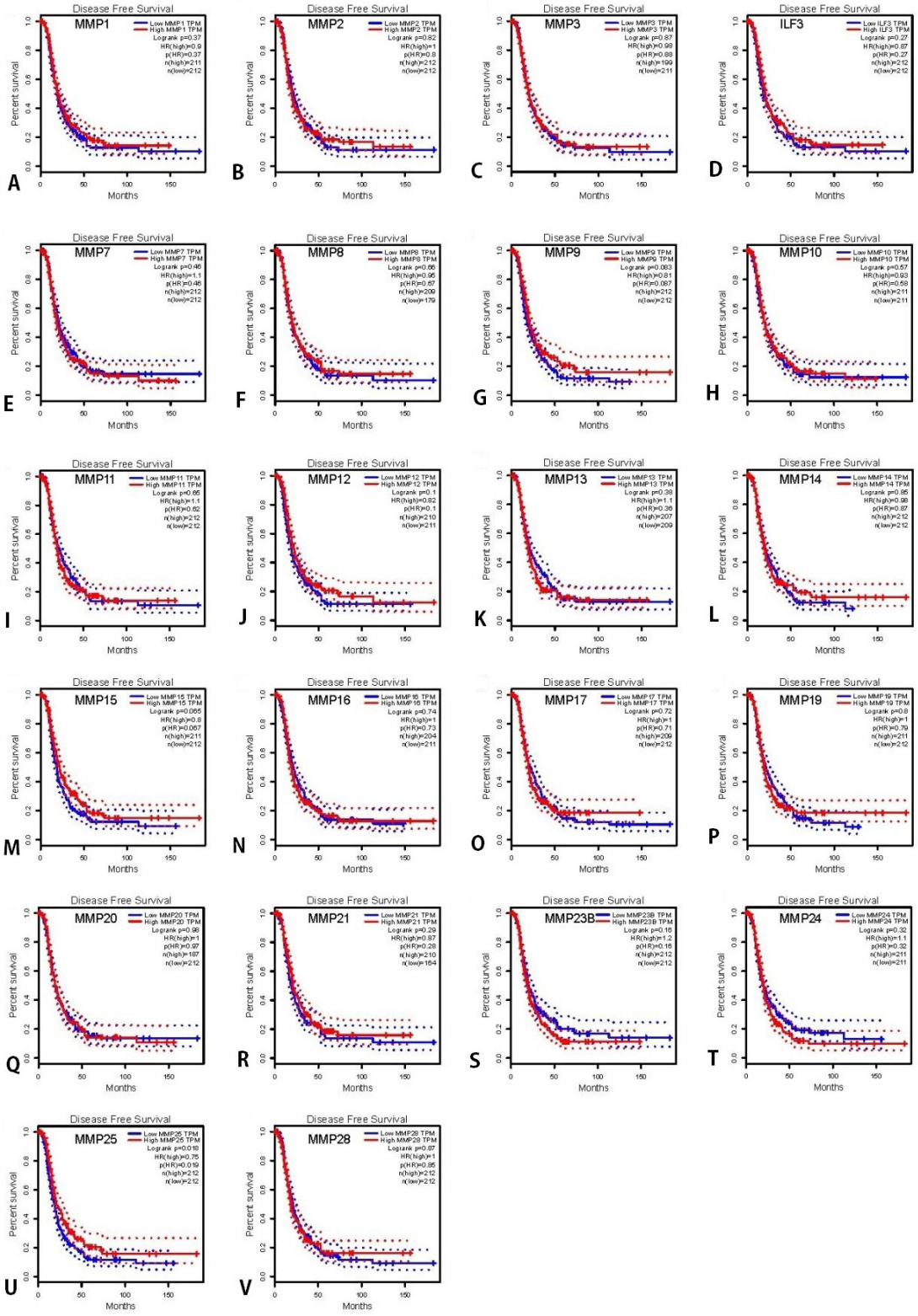


Fig. 6. DFS of OC patients with high and low mRNA expression of MMP, analyzed using the Kaplan-Meier Plotter tool. (A-V) PFS curves of MMP1-28 plotted for all patients. The threshold of p-value of < 0.05.

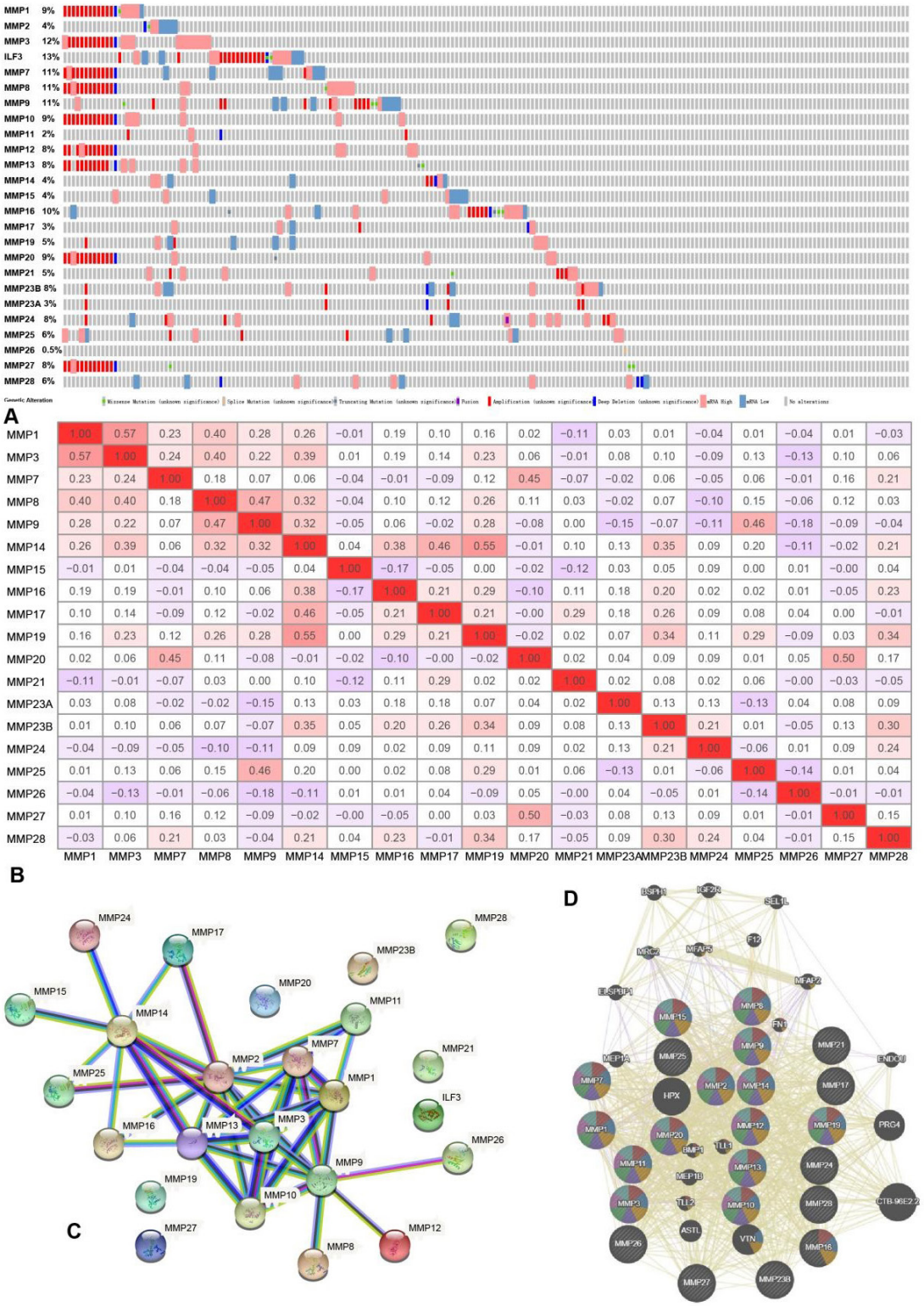


Fig. 7. The genetic alteration, co-expression, and PPI network analyses of MMPs. (A) Genetic alterations in MMPs in OC using cBioPortal. (B) Correlation heat map of MMPs in OC using TCGA database. (C) PPI network of MMPs using STRING. (D) Physical interaction network of MMPs using GeneMANIA.

We next explored the potential co-expression of the differentially expressed MMPs. There was a moderate to high correlation among the expression of MMP1, MMP3, MMP7, MMP8, MMP9, MMP14, MMP16 and MMP19 (Fig. 7B), a high correlation among MMP3, MMP17, MMP25 and MMP26 (Fig. 8B), and a high to moderate correlation among MMP7, MMP8, MMP20, MMP27 and MMP28 (Fig. 7B). A high correlation among MMP8, MMP17 and MMP25 (Fig. 8B). A high correlation MMP14, MMP23B and MMP28. A high correlation among MMP15, MMP16 and MMP21 (Fig. 7B). A high correlation among MMP16, MMP23A and MMP23B (Fig. 8B). A low to moderate correlation among MMP23B, MMP24, MMP25 (Fig. 8B).

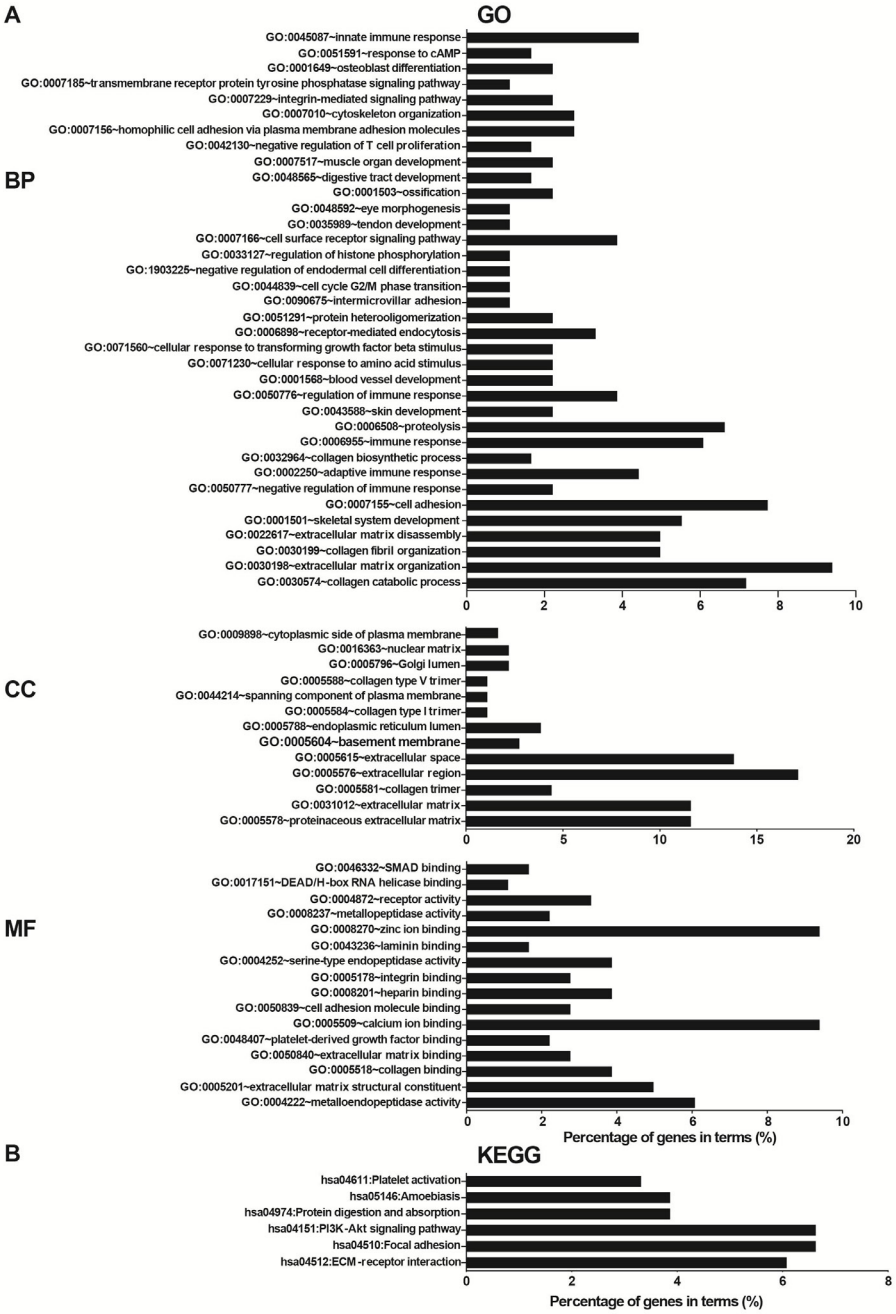


Fig. 8. Functional enrichment analysis of MMPs in OC (DAVID 6.8), (A) gene ontology (GO) and (B) Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis results.

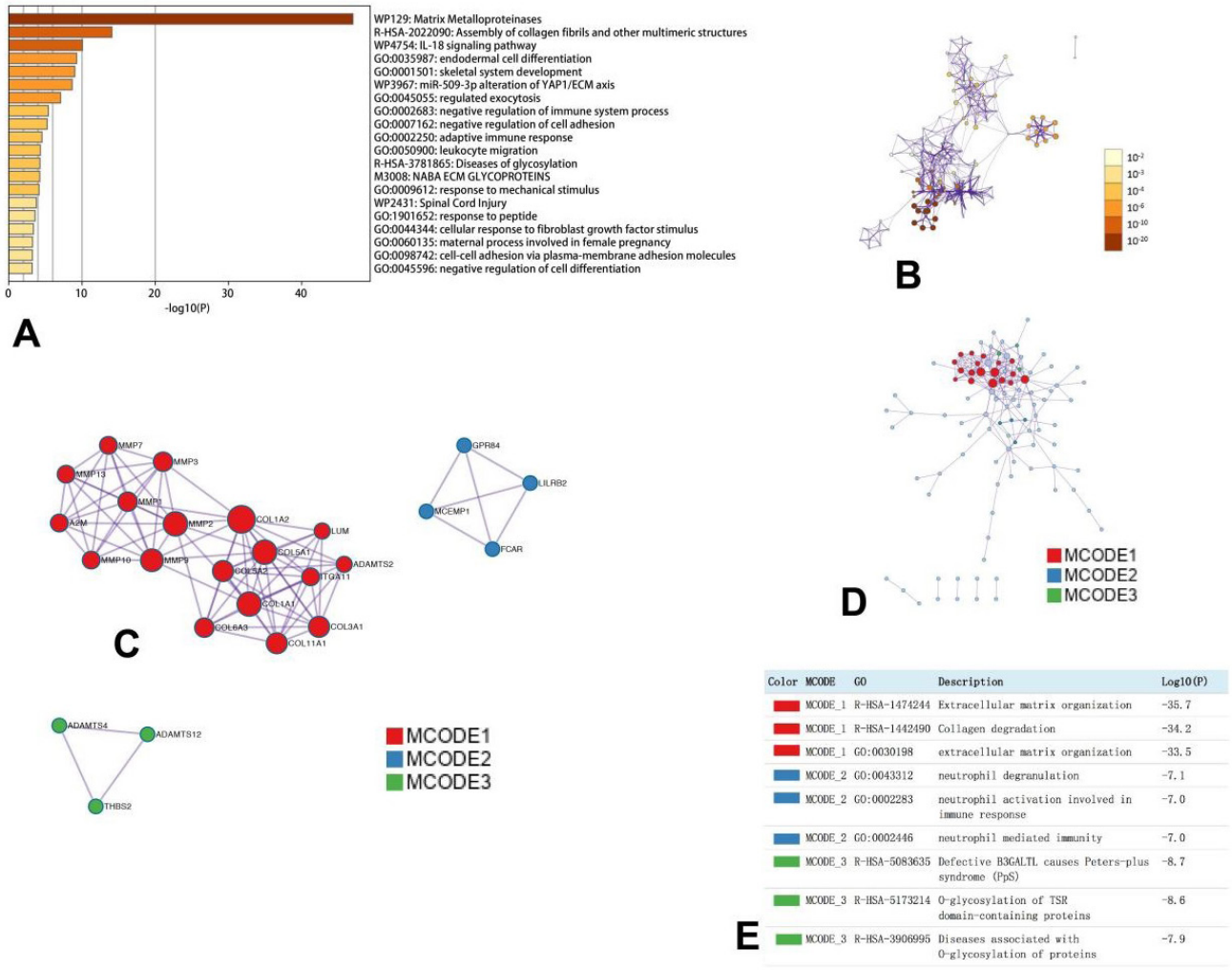
Moreover, we conducted a PPI network analysis of differentially expressed MMPs with STRING to explore the potential interactions among them. As expected, several nodes of 24 and several edges of 39 were obtained in the PPI network (Fig. 7C). The function of these differentially expressed MMPs was associated with the matrix metalloproteinases signaling pathway and the inflammatory response. Results of genemania also revealed that the functions of differential expressed MMPs were primarily related to extracellular matrix disassembly, extracellular matrix organization, extracellular structure organization, collagen catabolic process, multicellular organismal catabolic process, collagen metabolic process and multicellular organismal macromolecule metabolic process (Fig. 7D).

Functional enrichment analysis of MMPs in OC patients

GEPIA, DAVID 6.8 and Metascape were utilized to analyze the functions, pathway enrichment, and their neighboring genes of differentially expressed MMPs in OC (Figs. 8, 9). We delved into the top 10 associated genes of each differentiated MMPs via GEPIA dataset (Table II).

Table II. Key regulated factor of MMPs in OC (TRRUST).

Key TF	Description	Regulated gene	P- value	FDR
JUN	Jun proto-oncogene	MMP1, MMP12, MMP13, MMP2, MMP20, MMP3, MMP7, MMP9	3.62E-12	8.7E-11
STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)	MMP1, MMP10, MMP14, MMP2, MMP3, MMP7, MMP9	1.82E-10	2.19E-09
ETV4	ets variant 4	MMP1, MMP14, MMP2, MMP7	9.99E-09	7.99E-08
ETS1	V-ets erythroblastosis virus E26 oncogene homolog 1 (avian)	MMP1, MMP10, MMP13, MMP9, MMP3	2.81E-08	0.000000163
RELA	V-rel reticuloendotheliosis viral oncogene homolog A (avian)	MMP1, MMP12, MMP13, MMP14, MMP2, MMP3, MMP9	3.39E-08	0.000000163
ETS2	V-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	MMP1, MMP2, MMP3, MMP9	4.87E-08	0.000000195
MAZ	MYC-associated zinc finger protein (purine-binding transcription factor)	MMP1, MMP14, MMP9	7.67E-08	0.000000263
FOS	FBJ murine osteosarcoma viral oncogene homolog	MMP1, MMP3, MMP7, MMP9	0.000000525	0.00000158
NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	MMP1, MMP13, MMP14, MMP2, MMP3, MMP9	0.000000978	0.00000261
NFKBIA	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	MMP1, MMP3, MMP9	0.00000132	0.00000316
SRF	Serum response factor (c-fos serum response element-binding transcription factor)	MMP14, MMP2, MMP9	0.00000311	0.00000679
NCOA3	Nuclear receptor coactivator 3	MMP10, MMP7	0.0000272	0.0000543
KLF8	Kruppel-like factor 8	MMP14, MMP9	0.0000362	0.0000668
SNAI2	Snail homolog 2 (Drosophila)	MMP17, MMP9	0.000117	0.000201
SP1	Sp1 transcription factor	MMP11, MMP14, MMP2, MMP8, MMP28, MMP9	0.000177	0.000284
RUNX2	Runt-related transcription factor 2	MMP13, MMP2	0.000219	0.000329
CTNNB1	Catenin (cadherin-associated protein), beta 1, 88kDa	MMP14, MMP7	0.000296	0.000417
YBX1	Y box binding protein 1	MMP13, MMP2	0.000553	0.000738
TWIST1	Twist basic helix-loop-helix transcription factor 1	MMP1, MMP2	0.000754	0.000953
PPARG	Peroxisome proliferator-activated receptor gamma	MMP1, MMP9	0.00266	0.00319
HDAC1	Histone deacetylase 1	MMP28, MMP9	0.00307	0.00335
TFAP2A	Transcription factor AP-2 alpha (activating enhancer binding protein 2 alpha)	MMP2, MMP9	0.00307	0.00335
STAT1	Signal transducer and activator of transcription 1, 91kDa	MMP13, MMP9	0.00427	0.00446
TP53	Tumor protein p53	MMP1, MMP2	0.0155	0.0155



Color	MCODE	GO	Description	Log10(P)
	MCODE_1	R-HSA-1474244	Extracellular matrix organization	-35.7
	MCODE_1	R-HSA-1442490	Collagen degradation	-34.2
	MCODE_1	GO:0030198	extracellular matrix organization	-33.5
	MCODE_2	GO:0043312	neutrophil degranulation	-7.1
	MCODE_2	GO:0002283	neutrophil activation involved in immune response	-7.0
	MCODE_2	GO:0002446	neutrophil mediated immunity	-7.0
	MCODE_3	R-HSA-5083635	Defective B3GALTL causes Peters-plus syndrome (PpS)	-8.7
	MCODE_3	R-HSA-5173214	O-glycosylation of TSR domain-containing proteins	-8.6
	MCODE_3	R-HSA-3906995	Diseases associated with O-glycosylation of proteins	-7.9

Fig. 9. The enrichment analysis of MMPs and neighboring genes in OC (Metascape). (A) Heatmap of Gene Ontology (GO) enriched analysis (B) Interaction Network of GO enriched analysis, colored by cluster ID, color by p-value. (C, D). Protein, protein interaction network and MCODE, components identified in different expressed MMPs.

DAVID 6.8 and Metascape were utilized to analyze the functions of differentially expressed MMPs and their neighboring genes. Figure 9A shows the significant enriched GO items using DAVID 6.8. Among the enriched functions in the BP category (P<0.05), extracellular matrix organization, cell adhesion, proteolysis, immune response, collagen fibril organization, regulation of immune response, cell surface receptor signaling pathway and etc., were associated with the tumorigenesis and progression of OC. The extracellular region, proteinaceous extracellular matrix, extracellular region, basement membrane, extracellular space, endoplasmic reticulum lumen, cytoplasmic side of plasma membrane, nuclear matrix, Golgi lumen and etc. were the significant enriched items in the CC category (Fig. 8A). In the molecular function MF category (Fig. 8A), the differentially expressed MMPs and their neighboring genes were mainly enriched in metalloendopeptidase activity, extracellular matrix structural constituent, zinc ion binding and receptor activity. KEGG pathway analyses were also performed. As expected, among the significant KEGG pathways, ECM-receptor interaction, Amoebiasis, Focal adhesion, PI3K-Akt signaling pathway, protein digestion and absorption were significantly associated with the tumorigenesis and progression of OC (Fig. 8B).

Moreover, to better understand the relationship between MMPs and OC, we then performed a Metascape protein-protein interaction (PPI) enrichment analysis. The PPI network and MCODE components are shown in Figure 9. Data showed that the biological functions of MMPs

Table III. The kinase target networks of MMPs in OC (Linked Omics).

MMPS	Enriched kinases target	Description	Leading edge num	p value
MMP1	Kinase_AURKB	aurora kinase B	87	0
	Kinase_CSNK2A1	casein kinase 2 alpha 1	255	0
MMP2	Kinase_ATR	ATR serine/threonine kinase	66	0
	Kinase_ATM	ATM serine/threonine kinase	123	0
MMP3	Kinase_CDK2	cyclin dependent kinase 2	278	0
	Kinase_GRK3	G protein-coupled receptor kinase 3	282	0
MMP7	Kinase_LCK	LCK proto-oncogene, Src family tyrosine kinase	43	0
	Kinase_CDK2	Cyclin dependent kinase 2	278	0
MMP8	Kinase_LYN	LYN proto-oncogene, Src family tyrosine kinase	50	0
	Kinase_SYK	spleen associated tyrosine kinase	35	0
MMP9	Kinase_LCK	LCK proto-oncogene, Src family tyrosine kinase	43	0
	Kinase_LYN	LYN proto-oncogene, Src family tyrosine kinase	50	0
MMP10	Kinase_BCR	BCR, RhoGEF and GTPase activating protein	12	0
	Kinase_IKBKB	inhibitor of nuclear factor kappa B kinase subunit beta	28	0
MMP11	Kinase_ATM	ATM serine/threonine kinase	123	0
	Kinase_ATR	ATR serine/threonine kinase	66	0
MMP12	Kinase_LCK	LCK proto-oncogene, Src family tyrosine kinase	43	0
	Kinase_LYN	LYN proto-oncogene, Src family tyrosine kinase	50	0
MMP13	Kinase_ATR	ATR serine/threonine kinase	66	0
	Kinase_FYN	FYN proto-oncogene, Src family tyrosine kinase	66	0
MMP14	Kinase_SRC	SRC proto-oncogene, non-receptor tyrosine kinase	209	0
	Kinase_ATR	ATR serine/threonine kinase	66	0
MMP17	Kinase_RIPK2	receptor interacting serine/threonine kinase 2	11	0
	Kinase_PRKCG	protein kinase C gamma	49	0
MMP19	Kinase_ATR	ATR serine/threonine kinase	66	0
	Kinase_PRKG1	protein kinase, cGMP-dependent, type I	30	0
MMP20	Kinase_RPS6KA4	ribosomal protein S6 kinase A4	19	0
	Kinase_CSNK2A1	casein kinase 2 alpha 1	255	0
MMP21	Kinase_LCK	LCK proto-oncogene, Src family tyrosine kinase	43	0
	Kinase_TGFBR2	transforming growth factor beta receptor 2	6	0.002778
MMP23A	Kinase_LCK	LCK proto-oncogene, Src family tyrosine kinase	43	0
	Kinase_PDGFRB	platelet derived growth factor receptor beta	15	0
MMP23B	Kinase_ATR	ATR serine/threonine kinase	66	0
	Kinase_IKBKE	inhibitor of nuclear factor kappa B kinase subunit epsilon	15	0
MMP24	Kinase_CSNK2A1	casein kinase 2 alpha 1	255	0
	Kinase_RIPK2	receptor interacting serine/threonine kinase 2	11	0.004651
MMP25	Kinase_LYN	LYN proto-oncogene, Src family tyrosine kinase	50	0
	Kinase_SYK	spleen associated tyrosine kinase	35	0
MMP26	Kinase_GRK3	G protein-coupled receptor kinase 3	282	0
	Kinase_LCK	LCK proto-oncogene, Src family tyrosine kinase	43	0
MMP27	Kinase_RPS6KA4	ribosomal protein S6 kinase A4	19	0
	Kinase_EGFR	epidermal growth factor receptor	46	0
MMP28	Kinase_ATM	ATM serine/threonine kinase	123	0
	Kinase_CHEK1	checkpoint kinase 1	130	0

are mainly enriched in negative regulation of cell differentiation, cell-cell adhesion via plasma-membrane adhesion molecules, maternal process involved in female pregnancy, cellular response to fibroblast growth factor stimulus, response to peptide, Spinal Cord Injury, response to mechanical stimulus, Diseases of glycosylation, leukocyte migration, adaptive immune response, negative regulation of cell adhesion, negative regulation of immune system process, regulated exocytosis,miR-509-3p alteration of YAP1/ECM axis, skeletal system development, endodermal cell differentiation, IL-18 signaling pathway, assembly of collagen fibrils and other multimeric structures and Matrix Metalloproteinases binding in OC.

Transcription factor targets, kinase targets, and miRNA targets of MMPs in patients with OC

Due to the significant difference in the expression of MMPs in OC vs. normal tissue, we then analyzed possible transcription factor targets using the TRRUST (Table III), the kinase targets and miRNA targets of differentially expressed MMPs from Linked Omics database (Tables IV, V).

Table IV. The miRNA target networks of MMPs in OC (Linked Omics).

MMPS	Enriched miRNA target	Leading edge num	p value
MMP1	GTACAGG, MIR-486	5	0
	GCACCTT, MIR-18A, MIR-18B	17	0
MMP2	ACTGTGA, MIR-27A, MIR-27B	74	0
	GTGCCAA, MIR-96	62	0
MMP3	ATATGCA, MIR-448	64	0
	CTCCAAG, MIR-432	15	0
MMP7	CACTGTG, MIR-128A, MIR-128B	105	0
	CTGAGCC, MIR-24	56	0
MMP8	AGTCTAG, MIR-151	4	0
	AATGTGA, MIR-23A, MIR-23B	85	0
MMP9	GTGCCAT, MIR-183	43	0
	CACGTTT, MIR-302A	14	0.00806
MMP10	AAGCACA, MIR-218	88	0
	ACCAAAG, MIR-9	136	0
MMP11	AGTCTAG, MIR-151	4	0
	CTCCAAG, MIR-432	12	0
MMP12	GTTATAT, MIR-410	22	0.00677
	AGCATTA, MIR-155	29	0.00838
MMP13	AATGTGA, MIR-23A, MIR-23B	77	0
	ACCAAAG, MIR-9	117	0
MMP15	ACTACCT, MIR-196A, MIR-196B	32	0
	AGTCTAG, MIR-151	4	0
MMP16	TACGGGT, MIR-99A, MIR-100, MIR-99B	12	0
	ACTACCT, MIR-196A, MIR-196B	39	0
MMP17	AAGCACA, MIR-218	134	0
	ACCAAAG, MIR-9	116	0
MMP19	CCAGGGG, MIR-331	34	0
	TATTATA, MIR-374	94	0
MMP20	AAAGACA, MIR-511	37	0
	GACAATC, MIR-219	34	0.00227
MMP21	ACTACCT, MIR-196A, MIR-196B	53	0
	CTACCTC, LET-7A, LET-7B, LET-7C, LET-7D, LET-7E, LET-7F, MIR-98, LET-7G, LET-7I	146	0
MMP23A	AAAGGGA, MIR-204, MIR-211	97	0
	AAGCACA, MIR-218	136	0
MMP23B	CCCAGAG, MIR-326	49	0
	GTGCCAA, MIR-96	93	0
MMP24	CATGTAA, MIR-496	29	0
	CCAGGGG, MIR-331	25	0
MMP25	TCTAGAG, MIR-517	11	0
	GTGCAAT, MIR-25, MIR-32, MIR-92, MIR-363, MIR-367	95	0.00801
MMP26	TGCACTT, MIR-519C, MIR-519B, MIR-519A	104	0
	TGTTTAC, MIR-30A-5P, MIR-30C, MIR-30D, MIR-30B,MIR-30E-5P	135	0
MMP27	ACACTGG, MIR-199A, MIR-199B	52	0
	ACATATC, MIR-190	27	0
MMP28	GTGCAAT, MIR-25, MIR-32, MIR-92, MIR-363, MIR-367	64	0
	ATATGCA, MIR-448	51	0

Table V. The cox proportional hazard model of MMPs and six tumor-infiltrating immune cells in OC (TIMER).

	Coef	HR	95% CI_l	95%CI_u	p value	sig
B_cell	-2.798	0.061	0.000	98.212	0.458	
CD8_Tcell	-3.762	0.023	0.000	1.468	0.075	
CD4_Tcell	-15.339	0.000	0.000	0.000	0.000	***
Macrophage	8.874	7140.89	11.881	4291831.843	0.007	**
Neutrophil	7.674	2151.10	0.114	40483661.95	0.126	
Dendritic	-0.234	0.791	0.003	189.334	0.933	
MMP1	-0.049	0.952	0.807	1.123	0.562	
MMP2	0.011	1.011	0.828	1.235	0.914	
MMP3	-0.111	0.895	0.676	1.185	0.438	
MMP4 (ILF3)	-0.126	0.882	0.648	1.200	0.423	
MMP7	0.017	1.017	0.951	1.088	0.618	
MMP8	0.004	1.004	0.660	1.527	0.984	
MMP9	-0.035	0.966	0.798	1.169	0.722	
MMP10	-0.094	0.910	0.825	1.003	0.058	
MMP11	-0.197	0.821	0.663	1.018	0.072	
MMP12	-0.017	0.984	0.858	1.127	0.811	
MMP13	0.119	1.127	0.965	1.316	0.132	
MMP14	0.230	1.258	0.969	1.633	0.084	
MMP15	-0.196	0.822	0.621	1.087	0.169	
MMP16	0.055	1.057	0.804	1.390	0.692	
MMP17	0.660	1.936	0.979	3.827	0.058	
MMP19	0.168	1.183	0.767	1.824	0.447	
MMP20	0.148	1.160	0.796	1.689	0.440	
MMP21	-0.083	0.920	0.503	1.682	0.786	
MMP23B	-0.159	0.853	0.583	1.247	0.411	
MMP24	-0.160	0.852	0.647	1.124	0.257	
MMP25	-0.761	0.467	0.228	0.956	0.037	*
MMP26	0.188	1.207	0.750	1.943	0.438	
MMP27	0.324	1.383	0.742	2.577	0.308	
MMP28	0.078	1.081	0.729	1.601	0.699	

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

We found that main transcription factors (JUN, STAT3, ETV4, ETS1, TP53 and so on) were associated with the regulation of MMPs (Table III). AURKB and CSNK2A1 were the top two targets in the MMP1 target network. ATM and ATR were the top two targets of MMP2, MMP11. The targets of MMP3 were CDK2 and GRK3. The targets of MMP7 were LCK and CDK2. The targets of MMP7 were LCK and CDK2. LYN, SYK were the top two kinase targets in the MMP8 and MMP25, respectively. LYN,LCK were the targets of MMP9, MMP12. BCR and IKBKB were the top two targets of MMP10. Components of the MMP13 and MMP14 kinase-target networks were mainly associated with ATR and FYN, as well as SRC and ATR. RIPK2 and PRKCG were primarily associated with MMP17. ATR and PRKG1 were the top two targets in the MMP 19 kinase-target network. RPS6KA4 and CSNK2A1, LCK and TGFBR2 were the top two targets in the MMP20 and MMP21 kinase-target networks, respectively. PDGFRB and LCK were suggested as the targets for the MMP23A kinase-target network. ATR and IKBKE were primarily related to MMP23B. CSNK2A1 and RIPK2, and GRK3 and LCK were the top two targets in the MMP25 and MMP26 kinase-target networks, respectively. RPS6KA4 and EGFR,ATM and CHEK1 were suggested as targets for the MMP27, MMP28 kinase-target network. Similarly, we explored the enriched miRNA targets from Linked Omics database (results presented in Table IV). The top two enriched miRNA targets were GTACAGG, MIR-486 and GCACCTT, MIR-18A, MIR-18B in MMP1. ACTGTGA, MIR-27A, MIR-27B and GTGCCAA, MIR-96 were mainly enriched in MMP2. As the table describes, ATATGCA, MIR-448 and CTCCAAG, MIR-432 were enriched in MMP3, while CACTGTG, MIR-128A, MIR-128B and CTGAGCC, MIR-24 were enriched in MMP7. The enriched miRNA targets of MMP8, MMP9, MMP10, MMP11, MMP12, MMP13, MMP15, MMP16, MMP17, MMP20, MMP21, MMP23A, MMP23B, MMP24, MMP25, MMP26, MMP27 and MMP 28 are elaborated in Table V.

Immune cell infiltration of MMPs in patients with OC

MMPs are involved in inflammatory responses and immune cell infiltration , thus affffecting the clinical outcome of OC patients. Therefore, we embarked on a comprehensive exploration of the correlation between differentially expressed MMPs and immune cell infiltration using the TIMER database. Immune cells are the main cells of TME and infiltration of immune cells plays a pivotal role in tumor progression. Terefore, we further explored the correlation of differentially expressed MMPs and immune cells infiltration using TIMER database. Among these MMPs, we found that B cells' infiltration was negatively correlated with MMP1 (Cor=-0.148, p=1.13e−03), MMP2 (Cor=-0.126, p=5.56e−03), MMP3 (Cor=-0.18, p=7.41e−05), MMP8 (Cor=-0.113, p=1.3e−02), MMP10 (Cor=-0.113, p=1.34e−02), and MMP11 (Cor=-0.116, p=1.10e−02), MMP12 (Cor=-0.123, p=6.85e−03), MMP13 (Cor=-0.121, p=8.09e−03), MMP13 (Cor=-0.175, p=1.14e−04), MMP16 (Cor=-0.226, p=3.25e-09), MMP17 (Cor=-0.098, p=3.19e-02), MMP19 (Cor=-0.196, p=1.59e−05), MMP21 (Cor=-

0.114, *p*=1.23e-02), MMP23B (Cor=-0.191, *p*=2.50e-05), MMP24 (Cor=-0.105, *p*=2.10e-2), MMP26 (Cor=-0.09, *p*=4.85e-02), MMP27 (Cor=-0.116, *p*=1.13e−02) and MMP28 (Cor =-0.111, *p* =1.52e−2) while their infiltration was positively correlated with MMP7 (Cor =0.091, *p* =4.70e−02) (Fig. 10A-X). CD8+ T cells had a negative correlation with MMP4:ILF4(Cor =-0.174, *p* =1.28e−04), MMP14(Cor=-0.139, *p*=2.32e−03), MMP15 (Cor=-0.168, *p*=2.22e−04), MMP16 (Cor =-0.156, *p* =5.85e−04), MMP16 (Cor =-0.156, *p*=5.85e−04), MMP21 (Cor =-0.09, *p* = 4.76e−02) and MMP24 (Cor= -0.119, *p*=8.90e−03) (Fig. 11A-X). CD4+ T cells' infiltration existed in almost all differentiated MMPs, include positively correlated with MMP1 (Cor = 0.133, *p* =3.45e−03) , MMP9 (Cor = 0.36, *p* = 3.73e − 16), MMP12 (Cor = 0.179, *p* = 7.76e−05), MMP19 (Cor = 0.123, *p* = 7.09e−03) (Fig. 10A-X).

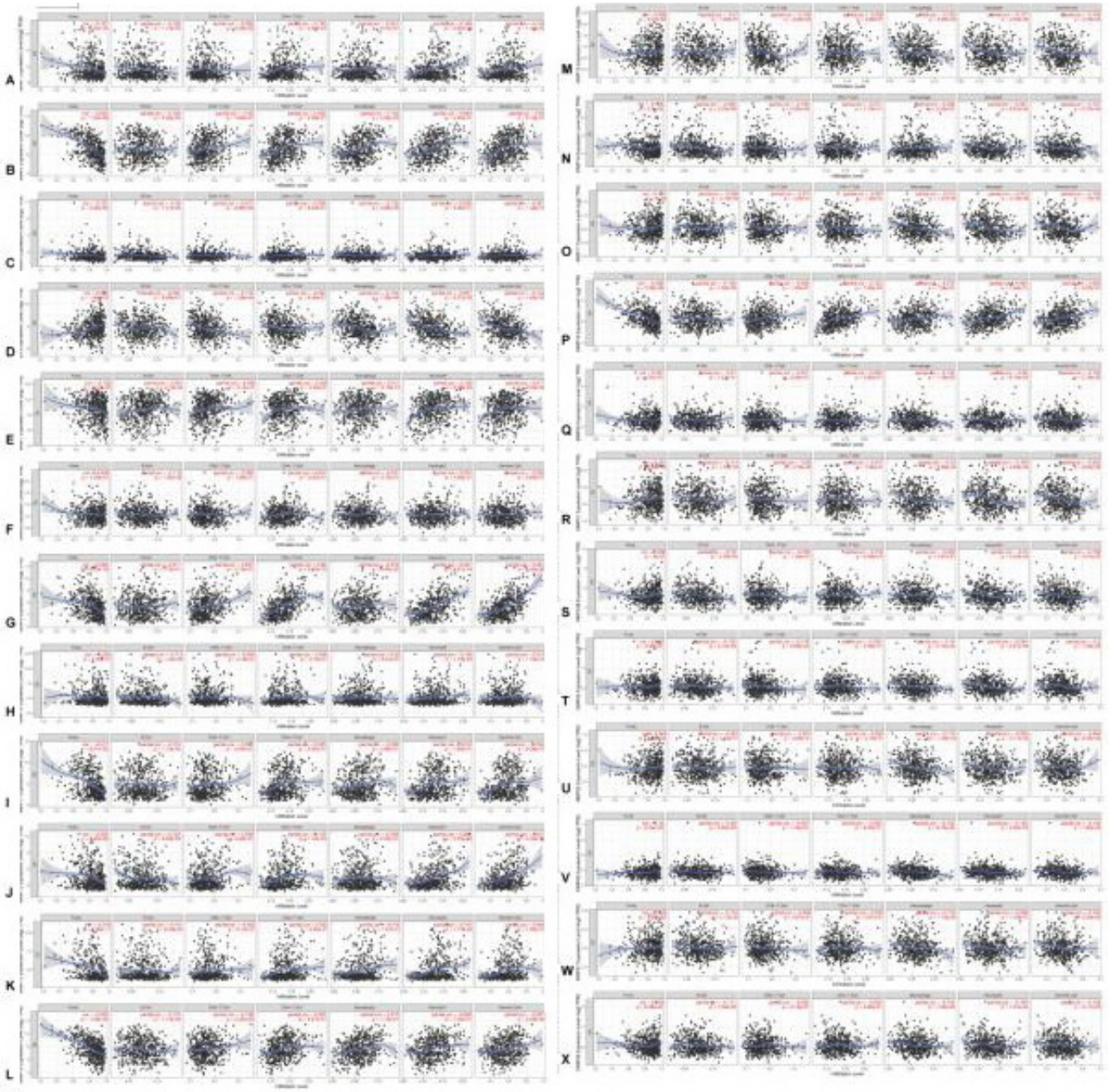


Fig. 10. The correlation between MMPs and immune cell infiltration in OC. A comprehensive analysis of the correlation between MMPs and six immune cell infiltrations (B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells) was performed using TIMER web server.

Fig. 11. The infiltration of immune cells caused by gene copy number alteration of differentially expressed MCMs (TIMER). **P*<0.05, ***P*<0.01 and ****P*<0.001.

The level of macrophages infiltration was negatively associated with MMP3 (Cor= -0.132, *p*= 3.68e-03), MMP4 (ILF=3) (Cor= -0.154, *p*= 7.38e−04), MMP15 (Cor= -0.156, *p*= 5.90e−04), MMP17 (Cor= -0.215, *p*= 1.97e−06), MMP20 (Cor= -0.123, *p*= 7.19e−03), MMP21 (Cor= -0.12, *p*= 8.58e−03), MMP24 (Cor= -0.14, *p*= 2.13e-03), MMP25 (Cor= -0.176, *p*= 1.08e−04), MMP26 (Cor = -0.152, *p*= 8.10e−04) and MMP28 (Cor= -0.154, *p*= 7.02e−04), while it was positively associated with MMP2 (Cor= 0.102, *p*= 2.52e−02) (Fig. 10A-X). The infiltration of neutrophils was positively correlated with MMP1 (Cor = 0.166, p =2.62e − 04), MMP7 (Cor = 0.126, *p*=5.58e−03), MMP9 (Cor= 0.341, *p*=1.61e − 14), MMP10 (Cor = 0.148, p =1.18e − 03), MMP12 (Cor = 0.284, *p*= 2.23e−10) and MMP13 (Cor= 0.116, *p*=1.13e − 02) in OC; while negatively correlated with MMP4 (ILF3) (Cor =-0.201, *p*=9.37e − 06), MMP15 (Cor = -0.137, p =2.55e − 03), MMP16 (Cor= -0.201, *p*= 8.81e−06), MMP17 (Cor=-0.212, *p* =2.78e−06), MMP21 (Cor= -0.165, p= 2.89e−04), MMP23B (Cor= -0.16, *p*=4.44e−04), MMP24 (Cor= -0.244, *p*= 6.51e−08), MMP25 (Cor= -0.095, *p*= 3.71e − 02), MMP26 (Cor= -0.133, *p*= 3.62e−03) and MMP28 (Cor= -0.169, *p*= 1.91e−04) (Fig. 11A-X). MMP1 (Cor = -0.145, *p*= 1.45e − 03), MMP9 (Cor= 0.361, *p*= 2.43e−16) and MMP12 (Cor= 0.247, *p*= 4.30e−08) were positively associated with the infiltration of dendritic cells, while negatively correlated with MMP15 (Cor= -0.159, *p*= 4.70e−04), MMP16 (Cor= -0.167, *p*= 2.37e−04), MMP17 (Cor= -0.195, *p*= 1.74e−05), MMP18 (Cor= -0.195, *p*= 1.74e−05), MMP20 (Cor= -0.105, *p* =2.15e−02), MMP21 (Cor= -0.202, *p*= 8.00e − 06), MMP23B (Cor= -0.188, *p*= 3.41e − 05), MMP24 (Cor= -0.2, *p*= 1.05e−05), MMP26 (Cor= -0.142, *p* =1.86e − 03), MMP27 (Cor = -0.106, *p*= 2.08e − 02) and MMP28 (Cor = -0.129, *p*= 4.71e − 03) (Fig. 10A-X). We also evaluated correlation of differentially expressed MMPs and immune cell infiltration. The Cox proportional hazard model was used, and we corrected for the following confounding factors: B cells, CD8_T cell, CD4+ T cells, macrophages, Dendritic, neutrophils, MMP1, MMP2, MMP3, MMP4 (ILF3), MMP7, MMP8, MMP9, MMP10, MMP11, MMP12, MMP13, MMP14, MMP15, MMP16, MMMP17, MMP19, MMP20, MMP21, MMP23B, MMP24, MMP25, MMP26, MMP27 and MMP28.CD4_Tcell (p= 0.000), Macrophage (p= 0.007) and MMP25 expression (p= 0.037) were significantly associated with the clinical outcome of OC patients (Supplementary Table 1). The module "SCAN" of TIMER was used to delve into the infiltration of immune cells caused by gene copy f differentiated MMPs. Our results proved that the alteration of gene copy number, to some extent, could influence the infiltration of immune cells (Fig. 11).

DISCUSSION

Recently, more and more studies have pointed out that the family of human MMPs are critical regulators of the tumor microenvironment, cancer cell proliferation, and metastatic process by facilitating extracellular matrix (ECM) degradation (Kapoor *et al*., 2016; Cui *et al*., 2017; Winer *et al*., 2018). Some studies MMPs are frequently expressed in ovarian cancer, and play an important role in in epithelial transformation, ovarian tumorigenesis and intraperitoneal metastasis, accumulating evidence has demonstrated a significant role for MMPs in tumorigenesis, tumor cell proliferation and apoptosis, and tumor metastasis. However, the exact functions of different MMPs in OC is not yet well-characterized (Cui *et al*., 2017). In this experiment, we identified the expression patterns, prognostic values, and potential functions of different MMPs in OC.

We first explored the expression of MMPs and their correlation with the pathological stage in OC. We found that 11 genes were differentially expressed in OC compared with normal tissue via ONCOMINE dataset (upregulation of MMP1, MMP7, MMP9, MMP10, MMP11 and MMP14; downregulation of MMP2, MMP16, MMP23A, MMP23B and MMP28). We also found that 11 genes were differentially expressed in OC compared with normal tissue via GEPIA dataset (upregulation of MMP7, MMP9, MMP11, MMP15 and MMP24; downregulation of MMP2, MMP17, MMP19, MMP22, MMP23A and MMP23B). MMP1, MMP7, MMP9, MMP12, MMP14, MMP15, MMP20 and MMP23A, 23B in GSE66957 also upreglataled in OC compared with normal tissue, MMP17, MMP21 and MMP24 also down regulated in OC compared with normal tissue (Supplementary Fig. 1). we found MMP2, MMP17 and MMP23A B in GSE4122 were lower in OC samples, however, MMP7 and MMP15 in GSE4122 were upregulated (Supplementary Fig. 2). Moreover, we found that the expression of MMP7, MMP9, MMP12, MMP15, MMP25 and MMP27 increased as the tumors progressed. In a word, nearly all families of MMPs different expressed significantly in ovarian cancer tissue, or tumor progression in various database. Amer Karam and Oliver Dorigo summarized that MMP1, MMP2, MMP3, MMP7 MMP9 and MMP14 was significantly overexpressed or down erexpressed and their activity is regulated in OC (7). Zhang and Chen (2017) only made a summary of MMP1, MMP2, MMP7, MMP9, MMP10, MMP13 and MMP14 in tumor cell adhesion during different levels of ovarian cancer progression (Winer *et al*., 2018).

OC patients with low expression of MMP1, MMP4:ILF3 and MMP12, high expression of MMP17 were significantly associated with better overall survival.

OC patients with low transcriptional levels of MMP25 were significantly associated with longer disease-free survival. These different data demonstrate that differentially expressed MMPs may play a significant role in OC. Vos *et al*. (2016) found that the disexpression of MMP-14 and MMP-2 expression in ovarian cancer with data on long-term follow-up. However, previous studies that showed the expression level and prognostic value of MMPs in various cancers are limited.

Since multiple MMPs were significantly differentially expressed in OC, we explored their molecular characteristics in OC. There were frequent genetic alterations in the MMPs differentially expressed in OC. Elevated mRNA expression was the most alteration. Tumorigenesis and the progression of OC are complex and multi-faceted, and genetic alteration plays an important role in this process (Yen *et al*., 2019). We found a low to high correlation among the differentially expressed MMPs, suggesting that these MMPs play a synergistic role in the tumorigenesis and progression of OC.

We then focused on the function of differentially expressed MMPs using GO enrichment analysis and KEGG pathway enrichment analysis. As expected, we found that the functions of these genes are primarily related to the extracellular matrix organization, cell adhesion, proteolysis, collagen fibril organization, platelet-derived growth factor binding, regulation of immune response, cell surface receptor signaling pathway. Previous studies have demonstrated that matrix metalloproteinases signaling pathways play key roles in the cell migration, proliferation, angiogenesis and contraction of various cancers (Van Doren, 2015). ECM-receptor interaction, YAP1/ECM axis, cellular response to fibroblast growth factor stimulus, collagen fibril organization ,PI3K-Akt signaling pathway, plays a significant role in biological processes associated with tumorigenesis, including cancer cell proliferation, angiogenesis, metastasis, invasivenes and drug resistance (Ediriweera *et al*., 2019; Norouzi-Barough *et al*., 2018). These data suggest that the MMPs, which are differentially expressed in OC, are potential drug therapeutic targets. As MMPs and their tissue inhibitors (TIMPs) may be potentially used in molecular targeting of cancers, accumulating researches have focused on ECM remodeling associated with follicular development in OC (Goldman and Shalev, 2004).

We found that main transcription factors (JUN, STAT3, ETV4, ETS1, TP53 and so on) were associated with the regulation of MMPs (Table III). AURKB and CSNK2A1 were the top two targets in the MMP1 target network. ATM and ATR were the top two targets of MMP2, MMP11. The targets of MMP3 were CDK2 and GRK3. The targets of MMP7 were LCK and CDK2. The targets of MMP7 were LCK and CDK2. LYN, SYK were the top two kinase targets in the MMP8 and MMP25, respectively. LYN,LCK were the targets of MMP9, MMP12. BCR and IKBKB were the top two targets of MMP10. Components of the MMP13 and MMP14 kinase-target networks were mainly associated with ATR and FYN, as well as SRC and ATR. RIPK2 and PRKCG were primarily associated with MMP17. ATR and PRKG1 were the top two targets in the MMP 19 kinase-target network. RPS6KA4 and CSNK2A1, LCK and TGFBR2 were the top two targets in the MMP20 and MMP21 kinase-target networks, respectively. PDGFRB and LCK were suggested as the targets for the MMP23A kinase-target network. ATR and IKBKE were primarily related to MMP23B. CSNK2A1 and RIPK2, and GRK3 and LCK were the top two targets in the MMP25 and MMP26 kinase-target networks, respectively. RPS6KA4 and EGFR,ATM and CHEK1 were suggested as targets for the MMP27, MMP28 kinase-target network. Similarly, we explored the enriched miRNA targets from LinkedOmics database (results presented in Table IV). The top two enriched miRNA targets were GTACAGG, MIR-486 and GCACCTT, MIR-18A, MIR-18B in MMP1. ACTGTGA, MIR-27A, MIR-27B and GTGCCAA, MIR-96 were mainly enriched in MMP2. As the table describes, ATATGCA, MIR-448 and CTCCAAG, MIR-432 were enriched in MMP3, while CACTGTG, MIR-128A, MIR-128B and CTGAGCC, MIR-24 were enriched in MMP7. The enriched miRNA targets of MMP8, MMP9, MMP10, MMP11, MMP12, MMP13, MMP15, MMP16, MMP17, MMP20, MMP21, MMP23A, MMP23B, MMP24, MMP25, MMP26, MMP27 and MMP 28 are elaborated in Table V.

We also sought to characterize the transcription factor targets, kinase and miRNA targets of the differentially expressed MMPs, and found that JUN, STAT1, STAT3, ETV4, ETS1, RELA, NFKB1, MAZ, SP1, TP53 and so on may be key transcription factors in there gulation of MMPs. The transcriptional regulator STAT3 has key roles in vertebrate development and mature tissue function including control of inflammation and immunity (Hillmer *et al*., 2016). STAT1 is postulated to regulate several immune-mediated diseases by inducing proinflammatory subsets (Kang *et al*., 2019). RELA phosphorylation is involved in disease progression, notably inflammatory diseases and cancer, by regulating NF-κB signaling (Lu and Yarbrugh, 2015). NFKB1, a suppressor of inflammation and cancer, plays an inhibitory role in the tumorigenesis and progression of a variety of cancers by reducing the abnormal activation of the NF-κB signaling pathway (Cartwright *et al*., 2016; Concetti and Wilson, 2018). Some research has showed that Sp1 is overexpressed in cancer cells and contributes to the formation of cancer

(Hsu *et al*., 2012). Inhibiting the expression level of Sp1 can significantly inhibit the proliferation (Bang *et al*., 2016). Our results may provide additional data about the complicated relation among OC, MMPs, and various signaling pathway in tumor development and progression.

We then investigated the kinase targets of differentially expressed CXC chemokines; the kinase targets were mainly in ATR, ATM, LCK, FYN, LYN,SRC, CDK2, and CSNK2A1. These kinases are involved in genomic stability, DNA damage, cell cycle progression, and epithelial–mesenchymal transition (Greer *et al*., 2017; Boggon and Eck, 2004; Fukumoto *et al*., 2014; Al-Ramadi *et al*., 1998; Kouhkan *et al*., 2016). The result suggested that MIR-486, MIR-18A B, MIR-27, MIR96, MIR-9, MIR-432, MIR-155, MIR-410, etc., may be targets for differential expression of MMPs. These microRNAs influence cancer formation and progress by modulating cancer cell migration, invasion, and apoptosis. (Rupaimoole and Slack, 2017; Hayes *et al*., 2014; Lange *et al*., 2019; Fei *et al*., 2019; Zhang *et al*., 2019; Zhou *et al*., 2018). The results of our observation indicated that these microRNAs were the important regulator of MMPs in OC. The results may provide us with potential therapeutic targets in OC.

Recently emerging data suggested that immune cell infiltration plays a key role in tumor progression. Bindea *et al*. (2013) MMPs can mediate the migration and localization of immune cells (Gomez-Lopez *et al*., 2014). Increasing evidence suggests that communication between immune cell infiltration and tumor progression and recurrence, and plays a key role in both disease clinical outcome and response to immunotherapy (Bindea *et al*., 2013; Liu *et al*., 2017). CD4+ T cells may be involved in the recognition of tumor antigens, and the activation of M1 macrophages may mediate the inhibition of tumor growth. CD4+ T-cell help and cytotoxic activity of CD4+ and CD8+ T-cells play an important role in eliminating infected cells and cancer cells (Lidenge *et al*., 2020; Lin *et al*., 2019).

Our research delved into infiltration of six immune cells (B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells) that were correlated with differentiated MMPs. The result indicated that MMPs could be immune regulators in OC. More and more clinical trials are needing to validate potential biomarkers of MMPs. Thus, it is likely that MMPs not only as being diagnostic biomarkers but also affect the immune condition in OC.

We found a significant positive correlation between the expression of MMPs and the infiltration of the six immune cell types (CD8+T cells, CD4+T cells, B cells, macrophages, dendritic cells and neutrophils), which indicating that MMPS may not only act as prognostic markers, but also reflect immune status in OC. More and more clinical trials are needing to validate potential biomarkers of MMPs. All in all, our research took advantages of public databases to systematically delve into data of MMPs in OC.

All in all, our research took advantages of public databases to systematically delve into data of MMPs in OC. There are some limitations in our research. Analysis on the transcriptional level, kinase regulators, microRNA can reflect some aspects of MMPs regulating immune status, but not global changes, for example lcnRNA, CircRNA and protein phosphorylation. Our results may need to be verified by indepth experiments *in vivo* and *in vitro*. Multiple clinical trials are needed to validate potential biomarkers of MMPs. We hope our results can provide novel insights for our researchers and these potential targets could be applied in clinic someday.

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Availability of data and materials

The data and materials of this experiment are available.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20231205030220

Statement of conflict interest

The authors have declared no conflict of interest.

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Supplementary Material

Identification of Therapeutic Targets and Prognostic Biomarkers Among Matrix Metalloproteinases in the Ovarian Cancer Microenvironment

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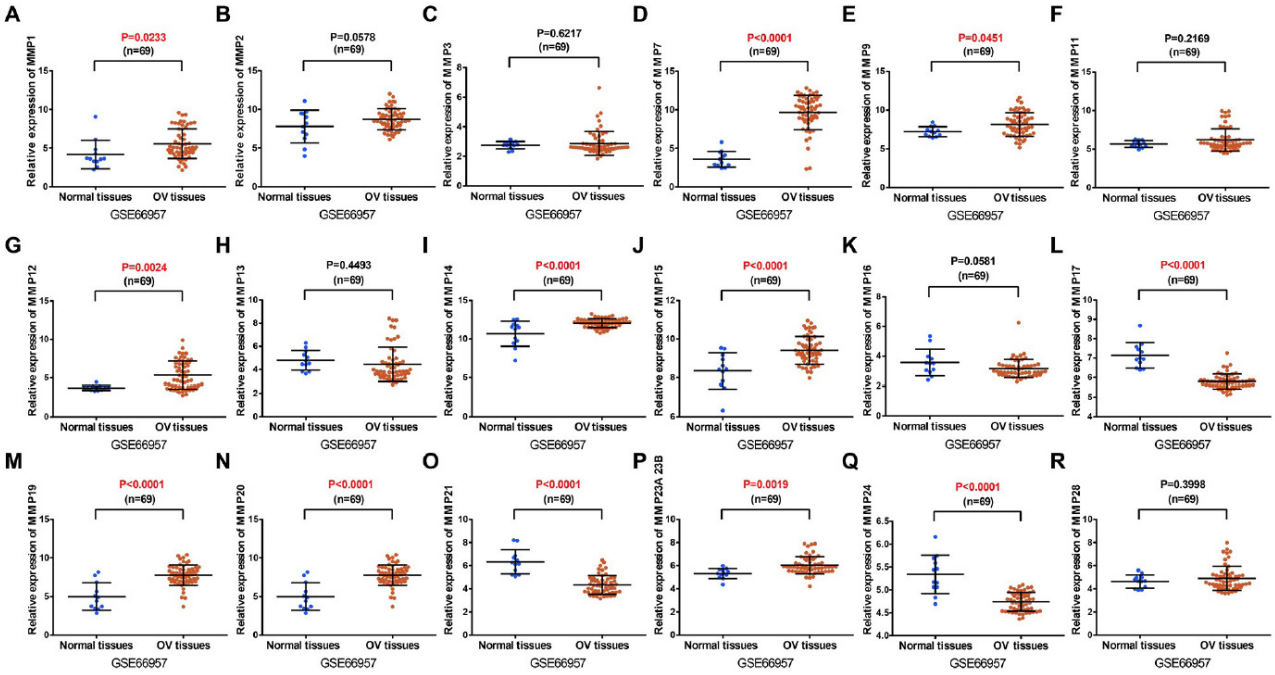

Supplementary Fig. 1. 1Differences in transcription levels of MMPs in OC and normal samples via GSE66957 dataset

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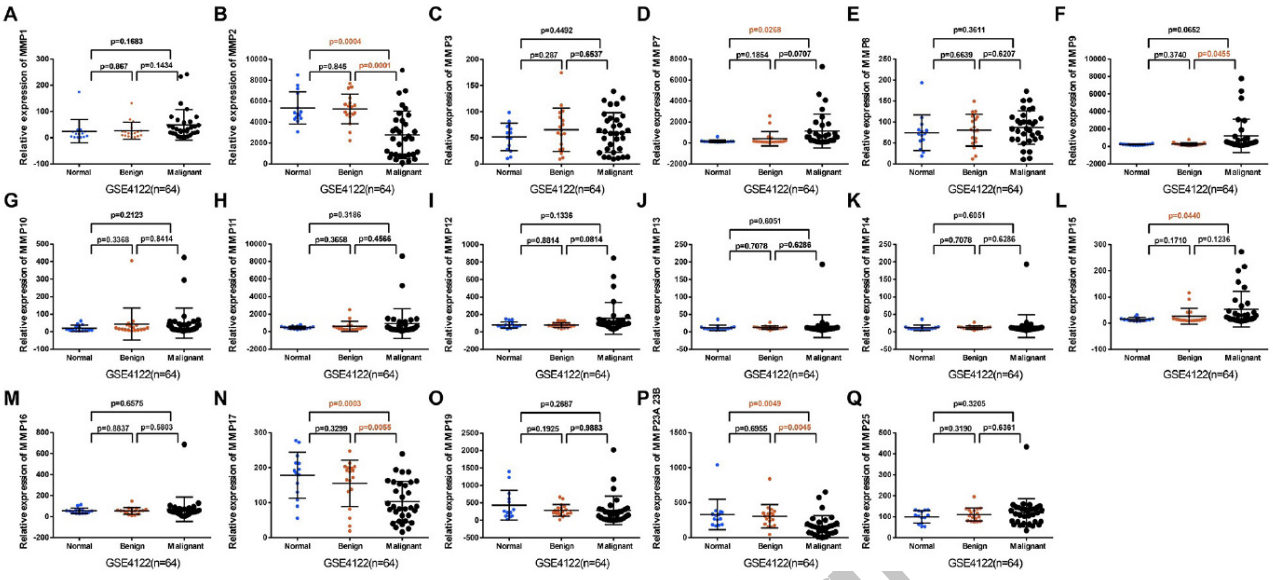
0030-9923/2024/0001-0001 $ 9.00/0

Supplementary Table 1. The cox proportional hazard model of MMPs and six tumor-infiltrating immune cells in OV (TIMER).

	coef	HR	95%CI_l	95%CI_u	p-value	sig
B_cell	-2.798	0.061	0.000	98.212	0.458	
CD8_Tcell	-3.762	0.023	0.000	1.468	0.075	
CD4_Tcell	-15.339	0.000	0.000	0.000	0.000	***
Macrophage	8.874	7140.897	11.881	4291831.843	0.007	**
Neutrophil	7.674	2151.101	0.114	40483661.954	0.126	
Dendritic	-0.234	0.791	0.003	189.334	0.933	
MMP1	-0.049	0.952	0.807	1.123	0.562	
MMP2	0.011	1.011	0.828	1.235	0.914	
MMP3	-0.111	0.895	0.676	1.185	0.438	
MMP4(ILF3)	-0.126	0.882	0.648	1.200	0.423	
MMP7	0.017	1.017	0.951	1.088	0.618	
MMP8	0.004	1.004	0.660	1.527	0.984	
MMP9	-0.035	0.966	0.798	1.169	0.722	
MMP10	-0.094	0.910	0.825	1.003	0.058	
MMP11	-0.197	0.821	0.663	1.018	0.072	
MMP12	-0.017	0.984	0.858	1.127	0.811	
MMP13	0.119	1.127	0.965	1.316	0.132	
MMP14	0.230	1.258	0.969	1.633	0.084	
MMP15	-0.196	0.822	0.621	1.087	0.169	
MMP16	0.055	1.057	0.804	1.390	0.692	
MMP17	0.660	1.936	0.979	3.827	0.058	
MMP19	0.168	1.183	0.767	1.824	0.447	
MMP20	0.148	1.160	0.796	1.689	0.440	
MMP21	-0.083	0.920	0.503	1.682	0.786	
MMP23B	-0.159	0.853	0.583	1.247	0.411	
MMP24	-0.160	0.852	0.647	1.124	0.257	
MMP25	-0.761	0.467	0.228	0.956	0.037	*
MMP26	0.188	1.207	0.750	1.943	0.438	
MMP27	0.324	1.383	0.742	2.577	0.308	
MMP28	0.078	1.081	0.729	1.601	0.699	

*P < 0.05, **P < 0.01, ***P < 0.001.



Supplementary Fig. 2. Differences in transcription levels of MMPs in OC, benign and normal samples via GSE4122 dataset.