Analyzing the Impact of ATF3 in Tumorigenesis and Immune Cell Infiltration of Ovarian Tumor: A Bioinformatics Study

Xiujuan wu (whg_2018@henu.edu.cn)

School of Life Sciences Jiangsu Normal University: Jiangsu Normal University School of Life Sciences

https://orcid.org/0000-0002-3045-6370

Research Article

Keywords: ATF3, ovarian, Ontology, Tumor Immunoassay Resource

Posted Date: May 10th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-471861/v1

Abstract

ATF3 is an essential transcription activator in regulating cancer-related genetic expression. To identify the role of *ATF3* in ovarian, we investigated the correlation between *ATF3* expression and the clinicopathological properties using multiple database. The cBioPortal and GEPIA database displayed the clinical information of ovarian patients harboring or without harboring *ATF3* mutation. Furthermore, we assessed the relationship between survival and *ATF3* expression level using Kaplan-Meier plotter, which reveals that the ovarian patients with higher expression of *ATF3* suffered the worse overall survival and progression-free survival. The differentially expressed genes were analyzed using Gene Ontology, protein-protein interaction network and gene set enrichment analysis to identify the hub gene and critical pathways, significantly affecting the tumorigenesis of ovarian tumor. Finally, we assessed the correlation between *ATF3* and immune cell infiltration using Tumor Immunoassay Resource (TIMER) database. The results demonstrated that higher expression is positive correlation with macrophage infiltration, expression for M1 and M2 type macrophages. Our study suggests that *ATF3* can regulate the cell cycle and heme-related oxidative phosphorylation process, and it may be a critical factor to regulate the macrophage cell to be infiltrated into ovarian cancer. ATF3 can be as a biomarker for diagnosis and therapy of ovarian.

Introduction

Ovarian cancer is the fourth commonest cancer to lead the death of females and more than 220000 women were diagnosed as the epithelial ovarian cancer for every year.[1–2] The main reason to lead the patient death is owing to the late diagnosis and serious metastasis within the abdomen.[3] By performing the genomic analysis of high-grade serous ovarian cancer including 92 patients, the critical reason for chemotherapy resistance is owing to the genetic inactivation of *RB1*, *NF1*, *RAD51B* and *PTEN* tumor suppressors.[4] Moreover, the most frequent amplification and mutation genes are *BRCA1* and *BRCA2*.[5] These oncogenes drives the serious drug resistance and lead the poor survival of patients.[6–7] Among these genetic factors, immunity-related reason can significantly lead the ovarian metastasis, for example tumor-associated macrophage[8], inflammatory cytokines[9], microRNA[10]. Consequently, to explore the correlation between tumorigenesis-driven genes and immune response will be helpful for patient to perform the precise therapeutic interventions.

Activating transcription factor 3, as called *ATF3*, is attributed to the ATF/CREB family[11] and anticipates amount of biological processes to regulate the cancer progression.[12] ATF3 protein can bind with the ATF/CRE cis-regulatory element moiety (5'-TGACGTCA-3') or AP-1 site (5'-TGA(C/G)TCA-3'), which is performed by the basic region-leucine zipper domain (zZip).[13] To response the external inflammation, ATF3 protein can negatively regulate the downstream gene expression levels to adaptive the stress environment[14–15], for example up-regulating MMP13 for inflammatory joint disease[16], regulating the intracellular Ca2+/ROS dependent IL-1β activation of streptococcus pneumoniae injection,[17] negatively regulating the cellular antiviral signaling against the virus infection[18]. As a result, the ATF3 expression

levels plays a critical role in inflammation response process and the correlation with immune-response may provide more information for early diagnosing and therapeutic approaches.

Immune microenvironment is of great impact on the tumor growth and drug resistance.[19–20] Tumor environment comprises multiple types of immune cells, including T cell, B cell, macrophage etc. The host immune reaction can recruit these immune cell to invade the tumor tissues, which forms the complex immune microenvironment and lead the serious drug resistance or metastasis[21–22]. After the immune cell infiltration, the genetic and epigenetic alterations will lead the tumor genetic expression level, which leads the intertumoral heterogeneity.[23] To target the tumor-related immune cells have significantly improve the clinical outcomes[24]. To explore the genetic expression level with the immune cell infiltration, the correlation can provide more potential therapeutic and diagnosis biomarkers.

Herein, we performed one comprehensive study to assess and explore the roles of *ATF3* in ovarian tumor tumorigenesis and immune cell infiltration. The correlation among *ATF3* expression level, overall survival and progression-free survival of ovarian patients were conducted by Kaplan-Meier analysis. By identifying the differentially expressed genes (DEGs), the gene ontology analysis and protein-protein interaction network were performed based on the up- and down-regulation, respectively. Then, the gene set enrichment analysis of DEGs was performed to identify how the overexpression of *ATF3* gene dysfunctions the cellular signaling pathways. Finally, the correlation between *ATF3* expression and immune cell infiltration was performed utilizing the TIMER and GEPIA database. Our result gave a novel glance at the role of ATF3 in ovarian tumor and highlight the potential clinical applications in early diagnosis.

Method

Database and clinical information for ovarian patients

In our study, we utilized the public datasets. The patient information and related RNA-seq data were obtained from TCGA database (https://portal.gdc.cancer.gov/) and cBioPortal website (http://www.cbioportal.org/). To explore the patient information in cBioPortal website, the key word is set as "*ATF3*" and "ovarian". By performing the analysis, the mutating sites and expression level was obtained from the cBioPortal website.

Protein-protein interaction (PPI) network and Gene Ontology analysis

The PPI analysis was performed in the STRING website (https://string-db.org/). Here, the minimum required interaction score was set as 0.9 and number of kmean is 5. The further PPI analysis was performed using Cytoscape3.6.0 and all the parameters were set as default. All the genetic functions are confirmed by NCBI database. The GO analysis was performed in the go:profiler website

(https://biit.cs.ut.ee/gprofiler/gost), and all the parameters are set as default except marked the ordered query.

Gene set enrichment analysis (GSEA)

All the DEGs with foldchange and p-value was saved as .rnk files. Then, the GSEAPreranked analysis was performed using GSEA v4.1.0 software (https://www.gsea-msigdb.org/gsea/msigdb/search.jsp). Number of permutations was set as 10000 and collapse to gene symbols was set as no collapse. The threshold value is set as NOM p-value < 0.05 & FDR q-value < 0.05 & |NES|>1.0. All the gene sets were obtained from the GSEA Molecular Signatures Database (MSigDB) (https://www.gsea-msigdb.org/gsea/msigdb/index.jsp).

Kaplan-Meier Analysis

The overall survival and progression-free survival were analyzed by using Kaplan-Meier plotter (http://kmplot.com/analysis/index.php?p=service). The database of ovarian cancer is TCGA database, which includes 565 patients. When performing the survival analysis, the cutoff value was set based on the auto-optimization. Other parameters are set as default.

Immune cell infiltration analysis

To explore the correlation between ATF3 gene expression and immune cell infiltration, TIMER 2.0 (http://timer.cistrome.org/) was employed to analyze the genetic correlation.

Profile of ATF3 **expression by GEPIA**

The ATF3 differential expression between TCGA tumor and normal tissue was performed using GEPIA 2.0 (http://gepia2.cancer-pku.cn/#index). The normal tissue database is GTEx data sets. The method for correlation coefficient is by spearman analysis. The differentially expressed genes were obtained from GEPIA2.0 website with |log2(FoldChange)|>1.0 & p-value < 0.01

Statistical analysis

The clinical information of ovarian patients includes the tumor grade, stage, *TP53* status. The clinical endpoints were overall survival and progression-free survival. All the results generated from the relative website with the HR and p-value data. P value < 0.05 is the cutoff value to identify whether significance. The GO analysis and GSEA results were presented by using Graphpad_prism 5.0.

Results

Clinical information summary of ATF3 **in ovarian patients**

The ovarian patient information was obtained from the TCGA and cBioprtal database. As shown in Fig. 1, the database collected 585 patients suffering ovarian tumor. In TCGA ovarian database, the diagnosis-year range of ovarian cancer is 35–85 and the median is about 57.5 year old. This result is consistent

that diagnosis stage of ovarian patients is always too late and cause the poor prognosis.[25] *ATF3*, as a activation transcription factor and a member of ATF/CREB transcription factor family, can bind to transcription promoters (E2, E3 and E4) with common sequence "CGTCA"[26]. The overexpression of ATF3 gene may activate the transcription process of many critical genes to promote the tumorigenesis.

Here, we carefully analyzed the mRNA expression of *ATF3* in ovarian tumor. As shown in Fig. 1A, all the patients considering the genetic condition of *ATF3* gene can be classified into two sub-groups, alteration and no alteration. By analyzing the *ATF3* status in these ovarian patients, we found that the mutation ratio of *ATF* gene is ~ 2.4%, including missense mutation (0.17%, 1 case), amplification (10 cases, 1.71%), deep deletion (0.51%, 3 cases). And, the most frequency of ATF3 protein occurred at the NO. 145 site (mutated from methionine to Threonine), while this mutation site is not attributed to the leucine-zipper binding region (Fig. 1B). By analysis the overall survival dependence on the alteration (n = 14) and no alteration (n = 571), no significance was observed (longrank test *p*-value = 0.872).

To analysis the expression level of ATF3 based on the genetic condition, we found that the genetic condition did not significantly affect the expression of *ATF3* (Fig. 2A). However, the expression level of *ATF3* in ovarian tumor tissue is significantly lower than the normal tissue (Fig. 2B). To further analysis the expression level of ATF3 in different stages of ovarian tumor, we found that the expression level of ATF3 in early stage of patients is significantly higher than normal tissue (Fig. 2C). Dependence on the progression of ovarian tumor, the expression level of ATF3 is increasing (Fig. 2D-E). These results implied that ATF3 gene plays a positive role in promoting the tumor proliferation.

Effect of ATF3 expression level on the OS and PFS

In order to explore the effect of *ATF3* expression level on the overall survival and progression-free survival, we performed the Kaplan-Meier analysis utilizing Kaplan-Meier Plotter. As shown in Fig. 3, the ovarian patients with lower expression of ATF3 suffered the better survival than higher expression groups. The median survival time of low and high expression of *ATF* gene are 48.07 months and 44.3 months, respectively. Meanwhile, progression-free survival (PFS) of low expression of ATF3 genes also was better than high expression group (p value = 0.012). The median survival of PFS is 22.57 month and 17.9 months, respectively. Compared with the decreasing of median survival of OS (8.5%), *ATF3* expression level deeply affected the median survival of PFS (26.7%). By performing the K-M analysis based on the various types of clinicopathological factors (Table 1), we found that patients can benefit from the lower expression of *ATF3* in ovarian tumor, expect in Stage 4, grad 1 or 1 + 2 and Endometrioid. These results reveal that the ovarian patients can benefit from the low expression of *ATF3*.

Table 1
Kaplan-Meier plotter to determine the effect of different clinicopathological factors on the expression of *ATF3* in ovarian cancer

Clinicopathological characteristics	Overall survival (n = 557)			Progression-free survival (n = 522)		
	N	Hazard ratio	p-value	N	Hazard ratio	p-value
Stage						
2 + 3	454	1.52(1.13–2.05)	0.0053	425	1.49(1.13–1.96)	0.004
2 + 3 + 4	539	1.4(1.08–1.81)	0.011	506	1.42(1.1–1.84)	0.0068
3	427	1.64(1.21–2.21)	0.0012	400	1.51(1.14–2.01)	0.0039
3 + 4	512	1.48(1.16–1.89)	0.0017	481	1.44(1.1–1.87)	0.0067
4	85	1.58(0.89–2.81)	0.11	81	1.56(0.8–3.04)	0.19
Grade						
1 + 2	75	1.62(0.79–3.34)	0.18	71	1.47(0.81–2.65)	0.2
2	69	1.6(0.77–3.32)	0.2	65	1.47(0.8–2.72)	0.21
2 + 3	538	1.45(1.11–1.88)	0.0057	505	1.36(1.08–1.72)	0.0096
3	469	1.47(1.13–1.91)	0.0037	440	1.45(1.09–1.93)	0.011
TP53						
Mutated	382	1.67(1.2–2.32)	0.002	359	1.65(1.24–2.19)	0.00046
Wild-type	75	2.02(1.01–4.06)	0.043	65	0.67(0.32–1.37)	0.27
Histology						
Endometrioid	51	3.08(0.89–10.66)	0.062	N.A.	N.A.	N.A.
Serious	522	1.35(1.06–1.69)	0.012	557	1.41(1.09–1.83)	0.0079

Go Functional Analysis Of Degs In Ovarian Tumor

In order to explore the mechanism of *ATF3* in promoting the tumorigenesis, we identified the DEGs using GEPIA2.0 with |log2(FoldChange)|>1.0 & p-value < 0.01. Here, there are 3195 genes identified as the DEGs and these genes were employed to perform the GO analysis based on the up- and down-regulation, respectively. As shown in Fig. 4A, the GO analysis of down-regulated genes showed that cell skeleton-related annotations were enriched, for example actin binding, actin cytoskeleton organization, actin filament-based process, extracellular. Cytoskeleton is highly related with the cell invasion[27] and metastasis[28] of cancer cells. These enriched annotations of down-regulated genes indicated that *ATF3* may participate into regulating the transcription of these genes and further affecting the cell cytoskeletons. Meanwhile, the matrix-related annotations were also enriched, i.e. extracellular structure organization, extracellular matrix organization, extracellular matrix structure constituent, extracelluar region and collagen-containing extracellular matrix. The lower expression of matrix-related genes can rebuild the microenvironment of tumor and further affected the tumor proliferation.

For the up-regulated DEGs, we found that heme-related mitotic cell cycle process-related annotations were enriched. Heme is the critical cofactor in forming the electron transport chain complexes and participates into the oxidative phosphorylation process[29]. Higher expression of heme-related annotations indicated that the ovarian tumor tissue strengthened the oxygen consumption process. The enhancement of oxidase activity, i.e. cytochrome-c oxidase activity and heme-copper terminal oxidase activity, were consistent with the up-regulation of heme-related functions. The enhancement of oxygen consumption of heme-related oxidation process also can affect the mitotic process, which are cell division, mitotic cell cycle process and mitotic nuclear division.

Protein = protein Interaction Network Analysis

In order to identify the hub genes, PPI analysis of DEGs was performed and these network was further clustered by kmean method. As shown in Fig. 5A, we can find several hub genes, i.e. *C3AR1, FCFR1G, FRR1, IGFBP3, TMP2, SOCS1, SOCS3, CCNB1, UBE2C and CDK1. C3AR1, FCFR1G, IGFBP3, SOCS1/3* were the inflammation-related genes, which participated the RTK signaling and regulated the mitotic process[30]. The network of these clustered groups were regenerated in Fig. 5B-E, respectively. Then, we ranked the nodes of PPI network based on the connecting degree and the top 10 genes were selected to further analysis of genetic functions. As shown in Table 2, we found that the most of these genes are related with the mitotic processes. The PPI results indicated that the overexpression level of ATF3 may affect the progression process through regulating the mitotic process.

Table 2
Biological functions of top 10 hub genes.

Gene	Biological functions	Gene	Biological functions
C3AR1	an anaphylatoxin released during activation of the complement system.	CCNB1	a regulatory protein involved in mitosis
ITGAM	leukocyte-specific integrin referred to as macrophage receptor 1 ('Mac-1'), or inactivated-C3b (iC3b) receptor 3 ('CR3').	CXCL12	plays a role in many diverse cellular functions, including embryogenesis, immune surveillance, inflammation response, tissue homeostasis, and tumor growth and metastasis.
IGF1	Involved in mediating growth and development.	POMC	preproprotein that undergoes extensive, tissue-specific, post-translational processing via cleavage by subtilisin-like enzymes known as prohormone convertases
CDK1	essential for G1/S and G2/M phase transitions of eukaryotic cell cycle	THBS1	an adhesive glycoprotein that mediates cell-to-cell and cell-to-matrix interactions
UBE2C	required for the destruction of mitotic cyclins and for cell cycle progression, and may be involved in cancer progression	IGF2	involved in development and growth

Gene Set Enrichment Analysis (Gsea)

In order to explore the impact of overexpression of *ATF3*, we performed the GSEA (Fig. 6) to identify the highly relative pathways using Hallmark, Reactome, KEGG and Wikipathway gene sets. As shown in Fig. 6A, the GSEA analysis using Hallmark gene set showed that the spermatogenesis, glycolysis, cholesterol homeostasis, mitotic spindle, MTORC1 signaling, MYC targets V1, G2M checkpoint and E2F targets are up-regulated, implying that the ATF3 gene can promote the mitotic process of ovarian cells. And, the inflammatory response, apoptosis, epithelial mesenchymal transition and xenobitic metabolism showed that the overexpressed *ATF3* may inhibit the inflammation-related pathways. Moreover, other types of GSEA (Fig. 6B-D and Fig. 7–8) based on Reactome, KEGG and Wikipathway gene set also confirmed these results and also were consistent with GO functional analysis.

Correlation between ATF3 expression level and immune cell infiltration

The most lethal reason of cancers is the metastasis to organism and it leads the multiple organism dysfunctions[31]. Moreover, the immune cells always anticipate the metastasis process of cancer tumors[32–33]. Consequently, we carefully investigated the correlation between immune cell infiltration and *ATF3* expression levels by using TIMER database. As shown in Fig. 9, most of immune cell inflitration (B cell, macrophage, T cell CD8+, neutrophil, NK, T cell CD4+) are highly associated with the ATF3

expression, while higher expression of *ATF3* will decrease the immune cell infiltration of macrophage (R=-0.152, p value = 1.65e-02) and T cell CD8+ (R=-0.16, p value = 1.15e-02). Then, we explored the impact of immune cell infiltration on the patient' survival period by Kapplan-Meier analysis. As shown in Fig. 10, we can find that the immune cells did not affect the survival period. These results indicated that immune cell infiltration may affect the *ATF3* expression. Moreover, we also explored the correlation between *ATF3* and immune cell biomarker expression by using TIMER database. As shown in Table 3, we only observed that M1 macrophage biomarker (*PTGS2*), M2 macrophage (*CD163, VSIG4, MS4A4A*) TAM biomarker (*CCL2*, *IL10*), Th1 (*STAT1*), Th2 (*STAT6*, *STAT5A*), Tfh (*BCL6*), Th17 (*STAT3*), Treg (*STAT5B*, *TGFB1*), T cell exhaustion (*CLTA4*) is significantly associated with ATF expression. And, the correlation between ATF3 expression and related immune cells (M1 & M2 macrophage, TAM and monocyte) is showed in Fig. 10. By analysis these results, we found that only M2 macrophage markers (*PTGS2* and *IRF5*) is highly correlated with *ATF3* expression, which is consistent with GEPIA 2 results (Table 4). M2 macrophage, activated by immune factors (e.g. IL4 and IL10), can improve the tumor metastasis and invasion by secreting some factors[34]. By considering the clinicopathology of higher *ATF3* expression ovarian cancer, our results demonstrated that *ATF3* may play a critical role in improving the ovarian invasion, which will lead serious metastasis.

Table 3
Correlation analysis between ATF3 and relative immune cell biomarkers in ovarian by TIMER.

Immune cells	General markers	Ovarian	
		None	
		Cor	p-value
CD8 + T cell	CD8A	-0.001	0.987
	CD8B	-0.078	0.222
T cell (general)	CD3D	-0.001	0.993
	CD3E	0.002	0.973
	CD2	-0.024	0.701
B cell	CD19	0.015	0.81
	CD79A	-0.048	0.452
Monocyte	CD86	0.074	0.245
	CD115	0.091	0.154
TAM	CCL2	0.195	0.00201
	CD68	0.046	0.467
	IL10	0.312	5.21e-07
M1 Macrophage	NOS2	0.047	0.459
	IRF5	0.077	0.223
	PTGS2	0.375	9.41e-10
M2 Macrophage	CD163	0.133	3.6e-02
	VSIG4	0.114	7.2e-02
	MS4A4A	0.114	7.3e-02
Neutrophils	CEACAM8	0.14	0.0269
	ITGAM	0.095	0.136
	CCR7	0.085	0.179
Natural killer cell	KIR2DL1	0.103	0.0723
	KIR2DL3	0.107	0.0625

Note: correlation coefficiency and p-value of purity in immune cell infiltration is -0.001 and 0.993, respectively.

	KIR2DL4	0.055	0.343
	KIR3DL1	0.025	0.698
	KIR3DL2	0.002	0.74
	KIR3DL3	0.099	0.0615
	KIR2DS4	0.065	0.259
Dendritic cell	HLA-DPB1	0.015	0.797
	HLA-DQB1	0	1
	HLA-DRA	-0.001	0.981
	HLA-DPA1	0.019	0.738
	CD1C	-0.057	0.325
	NRP1	0.081	0.161
	ITGAX	0.062	0.281
Th1	TBX21	-0.018	0.755
	STAT4	0.029	0.614
	STAT1	0.126	0.0279
	IFNG	0.006	0.913
	TNF	0.127	0.0265
Th2	GATA3	-0.039	0.504
	STAT6	0.236	3.44E-05
	STAT5A	0.157	6.12E-03
	IL13	0.104	0.0697
Tfh	BCL6	0.125	0.0292
	IL21	-0.047	0.419
Th17	STAT3	0.116	0.0444
	IL17A	-0.068	0.239
Treg	FOXP3	0.102	0.107
	CCR8	0.058	0.361

Note: correlation coefficiency and p-value of purity in immune cell infiltration is -0.001 and 0.993, respectively.

	STAT5B	0.166	8.52E-03
	TGFB1	0.132	3.69E-02
T cell exhaustion	PDCD1	0.055	0.388
	CTLA4	0.13	0.0406
	LAG3	0.008	0.903
	HAVCR2	0.071	0.261
	GZMB	-0.007	0.91
Note: correlation coefficiency and p-value of purity in immune cell infiltration is -0.001 and 0.993, respectively.			

Table 4
Correlation analysis between *ATF3* expression levels and immune cell biomarkers (monocyte, TAM and macrophages) by using GEPIA 2

Immune cells	Biomarkers	Ovarian			
		Tumor tissue		Normal tissue	
		R	p-value	R	p-value
Monocyte	CD86	0.099	0.04	0.52	0.069
	CSF1R	0.11	0.029	-0.1	0.35
TAM	CCL2	0.19	6.6E-05	0.43	3.1e-05
	CD68	0.037	0.44	-0.031	0.77
	IL10	0.36	2.9E-14	0.039	0.72
M1 Macrophage	NOS2	-0.0051	0.92	-0.11	0.2
	IRF5	0.089	0.065	-0.1	0.35
	PTGS2	0.16	0.0012	0.66	4.4E-12
M2 Macrophage	CD163	0.03	0.54	-0.061	0.57
	VSIG4	0.051	0.29	-0.072	0.5
	MS4A4A	0.1	0.038	-0.063	0.56

Discussion

Activating transcription factor 3, as called *ATF3*, is attributed to the ATF/CREB family and anticipates amount of biological processes to regulate the cancer progression[26]. The critical roles of *ATF3* for these cancers is owing to the rapid response for external cell stress, for example DNA damage and oxidative

stress. Except the binding to p53 protein to resist the oncogenic challenge, *ATF3* also can anticipate the immune response via activating the NF-κB signaling pathway. By response for these cellular processes, *ATF3* is an important factor to regulating the tumor metastasis.

In our work, we firstly found that the *ATF3* expression level is highly associated with the patient's overall survival and progression-free survival. By performing the analysis between Clinicopathological characteristics and *ATF3* expression levels, the *ATF3* can be as the reliable predictor for ovarian invasion to near tissues (Table 1). By using the GO functional analysis, PPI analysis and GSEA analysis, we found that the higher expression of ATF3 can promote the regulating process of transcription factors, such as MYC targets V1, G2M checkpoint and E2F targets pathways. Moreover, the higher of *ATF3* also enhanced the oxidative phosphorylation process, by regulating the heme-related processes.

The invasion of tumor is always driven by the immune cells. We explored the *ATF3* expression levels and immune cell infiltration. The high correlation between *ATF3* expression level and different types of immune cell infiltration demonstrated that higher *ATF3* expression can drive the infiltration of relative immune cells (B cell, neutrophil NK and T cell CD4+) and decrease the infiltration of T cell 8 + and macrophage.

Although the immune cell infiltration did not significantly affect the survival of ovarian patients, the expression levels of immune cell biomarkers (including *PTGS2, CD163, VSIG4, MS4A4A, CCL2, IL10, STAT1, STAT6, STAT5A, BCL6, STAT3, STAT5B, TGFB1, CLTA4*) is positive correlation with ATF3 expression levels (Table 3–4 and Fig. 9–10). These markers are always regulated by the inflammation factors or proinflammatory cytokines and *ATF3* can response for the external cellular factors. As a result, these results demonstrated that *ATF3* can be as the ovarian biomarker for predicting the metastasis to near tissues.

In summary, we found that the overexpression of *ATF3* genes in ovarian tumor can up-regulated the heme-related processes and further enhance the cell mitotic processes. Furthermore, the higher activation of mitosis process leads the worse of progression of ovarian patients. Owing to the correlation among *ATF3* enhancement of cell cycle and immune cell infiltration, *ATF3* may be an important predictor and potential target for ovarian therapy.

Declarations

Acknowledge

None.

Conflict of interest

All the authors declared no competing interests.

References

1. Jayson GC, Kohn EC, Kitchener HC, Ledermann JA. Ovarian cancer. The Lancet. 2014;384(9951):1376–88.
2. Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, Gaudet MM, Jemal A, Siegel RL. Ovarian cancer statistics, 2018. *CA: A Cancer Journal for Clinicians* 2018, *68* (4), 284–296.
3. Coffman LG, Burgos-Ojeda D, Wu R, Cho K, Bai S, Buckanovich RJ, New models of hematogenous ovarian cancer metastasis demonstrate preferential spread to the ovary and a requirement for the ovary for abdominal dissemination. *Translational Research* 2016, *175*, 92–102. e2.
4. Patch A-M, Christie EL, Etemadmoghadam D, Garsed DW, George J, Fereday S, Nones K, Cowin P, Alsop K, Bailey PJ. Whole–genome characterization of chemoresistant ovarian cancer. Nature. 2015;521(7553):489–94.
5. Network CGAR. Integrated genomic analyses of ovarian carcinoma. *Nature* **2011**, *474* (7353), 609.
6. Madariaga A, Lheureux S, Oza AM. Tailoring ovarian cancer treatment: implications of BRCA1/2 mutations. Cancers. 2019;11(3):416.
7. Naora H, Montell DJ. Ovarian cancer metastasis: integrating insights from disparate model organisms. *Nature Reviews Cancer* 2005, *5* (5), 355–66.
8. Yin M, Li X, Tan S, Zhou HJ, Ji W, Bellone S, Xu X, Zhang H, Santin AD, Lou G. Tumor-associated macrophages drive spheroid formation during early transcoelomic metastasis of ovarian cancer. *The Journal of Clinical Investigation* 2016, *126* (11), 4157–4173.
9. Browning L, Patel MR, Horvath EB, Tawara K, Jorcyk CL. IL-6 and ovarian cancer: Inflammatory cytokines in promotion of metastasis. Cancer Management Research. 2018;10:6685.
10. Zhang R, Shi H, Ren F, Feng W, Cao Y, Li G, Liu Z, Ji P, Zhang M. MicroRNA-338-3p suppresses ovarian cancer cells growth and metastasis: implication of Wnt/catenin beta and MEK/ERK signaling pathways. Journal of Experimental Clinical Cancer Research. 2019;38(1):1–13.
11. Duncan RM, Reyes L, Moats K, Robinson RM, Murphy SA, Kaur B, Stessman HA, Dolloff NG. ATF3 Coordinates Antitumor Synergy between Epigenetic Drugs and Protein Disulfide Isomerase Inhibitors. *Cancer research* 2020, *80* (16), 3279–3291.
12. Chang YS, Jalgaonkar SP, Middleton JD, Hai T. Stress-inducible gene Atf3 in the noncancer host cells contributes to chemotherapy-exacerbated breast cancer metastasis. *Proceedings of the National Academy of Sciences* **2017**, *114* (34), E7159-E7168.
13. Yan C, Boyd DD. ATF3 regulates the stability of p53: a link to cancer. Cell Cycle. 2006;5(9):926–9.
14. Lim R, Barker G, Liong S, Nguyen-Ngo C, Tong S, Kaitu'u-Lino T u.; Lappas M, ATF3 is a negative regulator of inflammation in human fetal membranes. *Placenta* 2016, *47*, 63–72.
15. Zhang Z-B, Ruan C-C, Chen D-R, Zhang K, Yan C, Gao P-J. Activating transcription factor 3 SUMOylation is involved in angiotensin II-induced endothelial cell inflammation and dysfunction. J Mol Cell Cardiol. 2016;92:149–57.

16. Chan CM, Macdonald CD, Litherland GJ, Wilkinson DJ, Skelton A, Europe-Finner GN, Rowan AD. Cytokine-induced MMP13 expression in human chondrocytes is dependent on activating transcription factor 3 (ATF3) regulation. *Journal of Biological Chemistry* 2017, *292* (5), 1625–1636.
17. Lee S, Kim G-L, Kim NY, Kim S-J, Ghosh P, Rhee D-K, ATF3 stimulates IL-17A by regulating intracellular Ca2+/ROS-dependent IL-1β activation during Streptococcus pneumoniae infection. *Frontiers in immunology* 2018, *9*, 1954.
18. Sood V, Sharma KB, Gupta V, Saha D, Dhapola P, Sharma M, Sen U, Kitajima S, Chowdhury S, Kalia M. ATF3 negatively regulates cellular antiviral signaling and autophagy in the absence of type I interferons. *Scientific Reports* 2017, *7* (1), 1–17.
19. Zhang Y, Xu J, Zhang N, Chen M, Wang H, Zhu D. Targeting the tumour immune microenvironment for cancer therapy in human gastrointestinal malignancies. Cancer Lett. 2019;458:123–35.
20. Almeida FV, Douglass SM, Fane ME, Weeraratna AT. Bad company: Microenvironmentally mediated resistance to targeted therapy in melanoma. Pigment Cell Melanoma Research. 2019;32(2):237–47.
21. Li J, Byrne KT, Yan F, Yamazoe T, Chen Z, Baslan T, Richman LP, Lin JH, Sun YH, Rech AJ. Tumor cell-intrinsic factors underlie heterogeneity of immune cell infiltration and response to immunotherapy. Immunity. 2018;49(1):178–93. e7.
22. Yang S, Liu T, Cheng Y, Bai Y, Liang G. Immune cell infiltration as a biomarker for the diagnosis and prognosis of digestive system cancer. Cancer Sci. 2019;110(12):3639.
23. Burrell RA, McGranahan N, Bartek J, Swanton C. The causes and consequences of genetic heterogeneity in cancer evolution. Nature. 2013;501(7467):338–45.
24. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJ, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbé C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A, Urba WJ. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711–23.
25. Brüggmann D, Pulch K, Klingelhöfer D, Pearce CL, Groneberg DA. Ovarian cancer: density equalizing mapping of the global research architecture. *International journal of health geographics* 2017, *16* (1), 3.
26. Lee KA, Hai TY, SivaRaman L, Thimmappaya B, Hurst HC, Jones NC, Green MR. A cellular protein, activating transcription factor, activates transcription of multiple E1A-inducible adenovirus early promoters. *Proceedings of the National Academy of Sciences of the United States of America* 1987, *84* (23), 8355–9.
27. Yilmaz M, Christofori G. EMT, the cytoskeleton, and cancer cell invasion. *Cancer metastasis reviews* 2009, *28* (1–2), 15–33.
28. Fife CM, McCarroll JA, Kavallaris M. Movers and shakers: cell cytoskeleton in cancer metastasis. *British journal of pharmacology* 2014, *171* (24), 5507–23.
29. Fiorito V, Chiabrando D, Petrillo S, Bertino F, Tolosano E. The Multifaceted Role of Heme in Cancer. Frontiers in oncology. 2019;9:1540.

30. Strainic MG, Pohlmann E, Valley CC, Sammeta A, Hussain W, Lidke DS, Medof ME. RTK signaling requires C3ar1/C5ar1 and IL-6R joint signaling to repress dominant PTEN, SOCS1/3 and PHLPP restraint. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 2020;34(2):2105–25.
31. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* **2011**, *144* (5), 646–674.
32. Kaur A, Ecker BL, Douglass SM, Kugel CH 3rd; Webster MR, Almeida FV, Somasundaram R, Hayden J, Ban E, Ahmadzadeh H, Franco-Barraza J, Shah N, Mellis IA, Keeney F, Kossenkov A, Tang HY, Yin X, Liu Q, Xu X, Fane M, Brafford P, Herlyn M, Speicher DW, Wargo JA, Tetzlaff MT, Haydu LE, Raj A, Shenoy V, Cukierman E, Weeraratna AT. Remodeling of the Collagen Matrix in Aging Skin Promotes Melanoma Metastasis and Affects Immune Cell Motility. Cancer discovery. 2019;9(1):64–81.
33. Vilgelm AE, Richmond A. Chemokines Modulate Immune Surveillance in Tumorigenesis, Metastasis, and Response to Immunotherapy. Front Immunol. 2019;10:333.
34. Lan J, Sun L, Xu F, Liu L, Hu F, Song D, Hou Z, Wu W, Luo X, Wang J, Yuan X, Hu J, Wang G. M2 Macrophage-Derived Exosomes Promote Cell Migration and Invasion in Colon Cancer. Can Res. 2019;79(1):146–58.

Figures

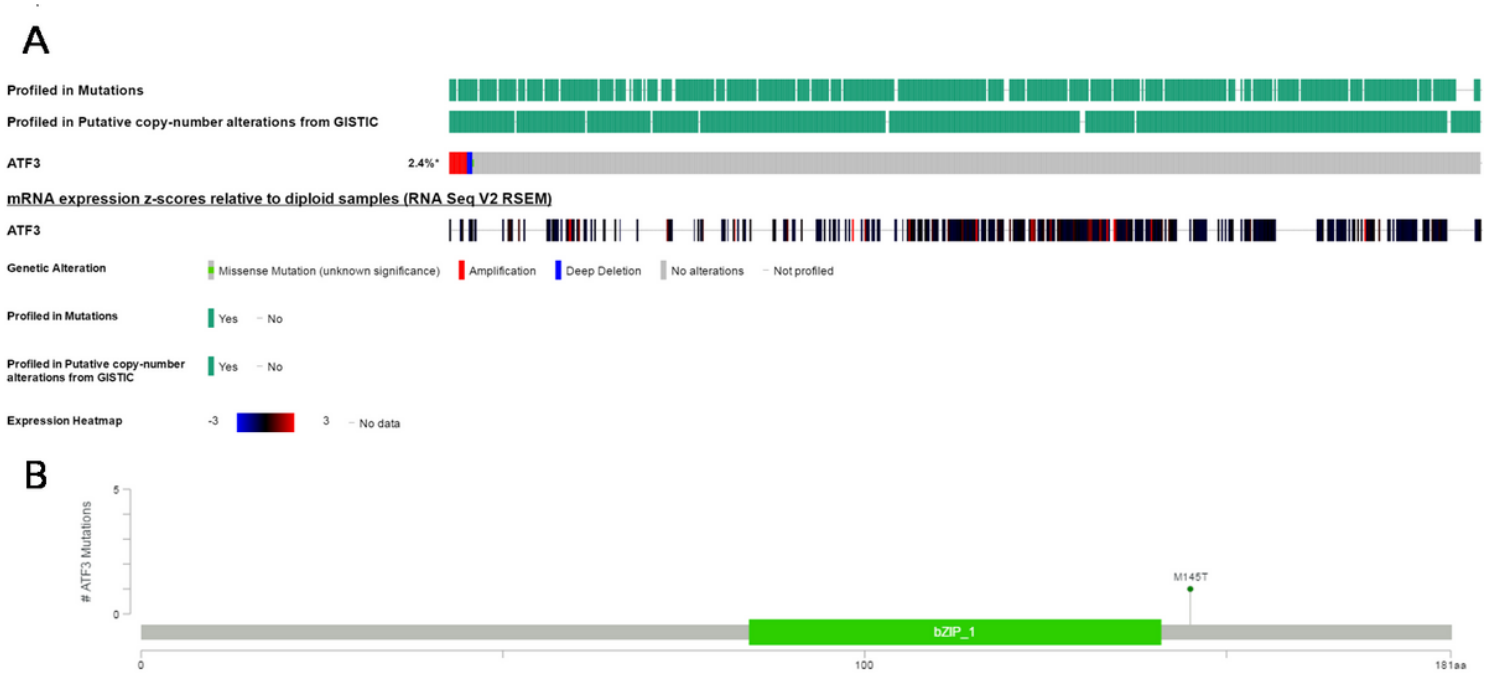

Figure 1

A. Clinical information and mRNA expression status of AFT3 in Ovarian cancer. B. Common mutation sites of ATF3 protein in ovarian cancer. The most frequency site of ATF3 is M145T.

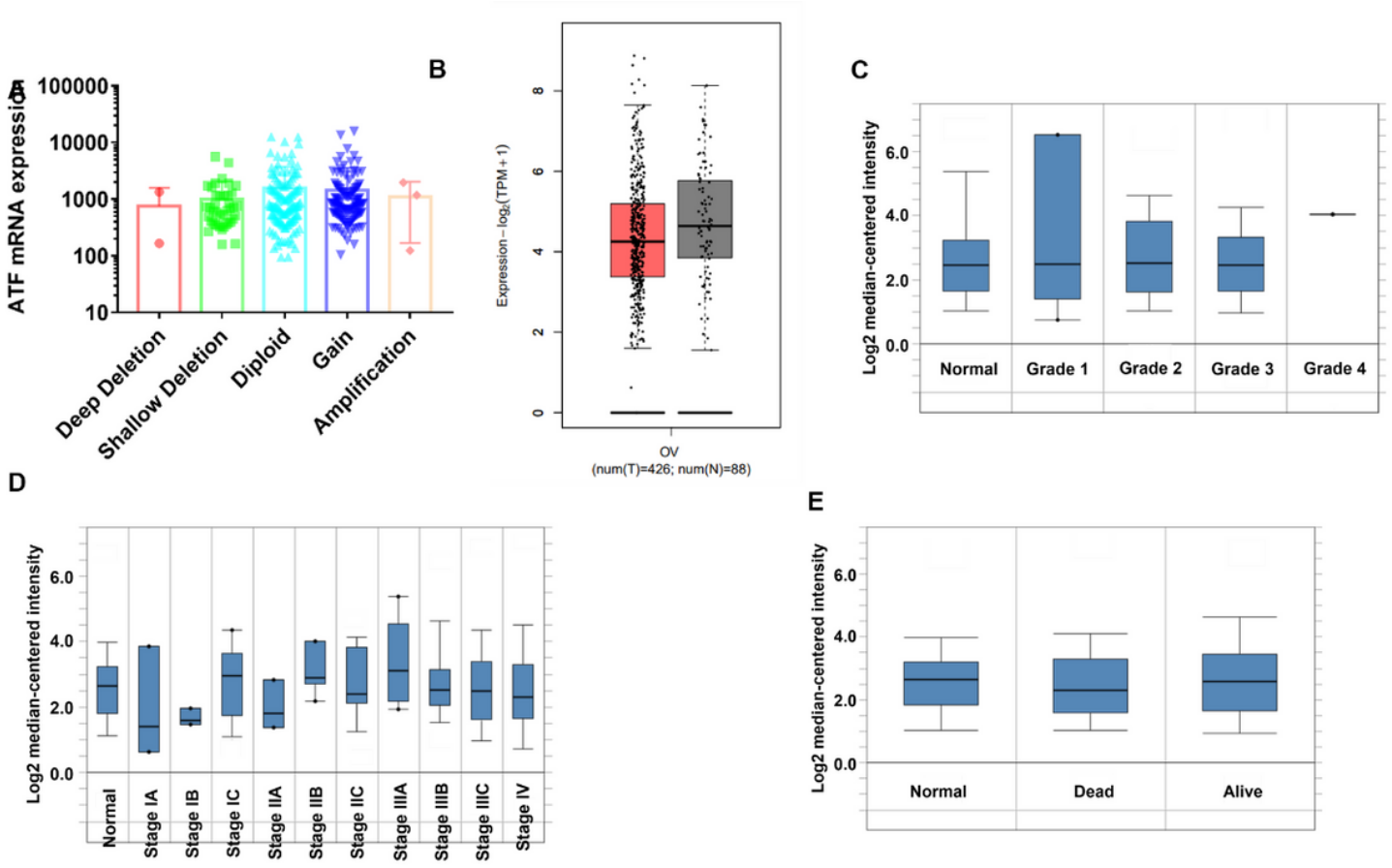

Figure 2

A. Expression level of ATF3 gene based on the different conditions: deep deletion, shallown deletion, diploid gain and amplification, respectively. B. The expression level of ATF3 compared with normal tissue. C. The expression level of ATF3 gene based on the different disease stage.

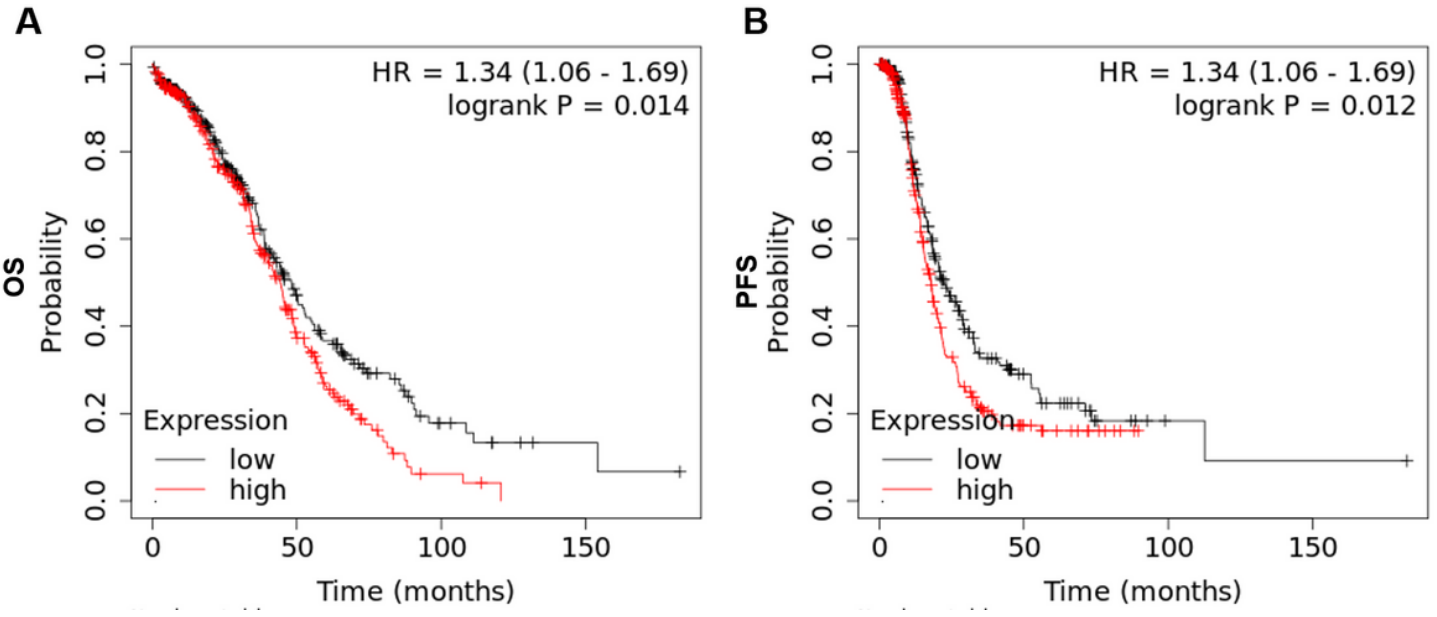

Figure 3

A. Overall survival curve of ovarian cancer patients depends on the ATF expression level. The p-value of ranking curve is 0.014. The median survival times are 48.07 month (low expression) and 44.3 months (low expression), respectively. B. Progression-free survival curve of ovarian cancer patients depends on the ATF expression level. The p-value of ranking curve is 0.012. The median survival times are 22.57 month (low expression) and 17.9 months (low expression), respectively.

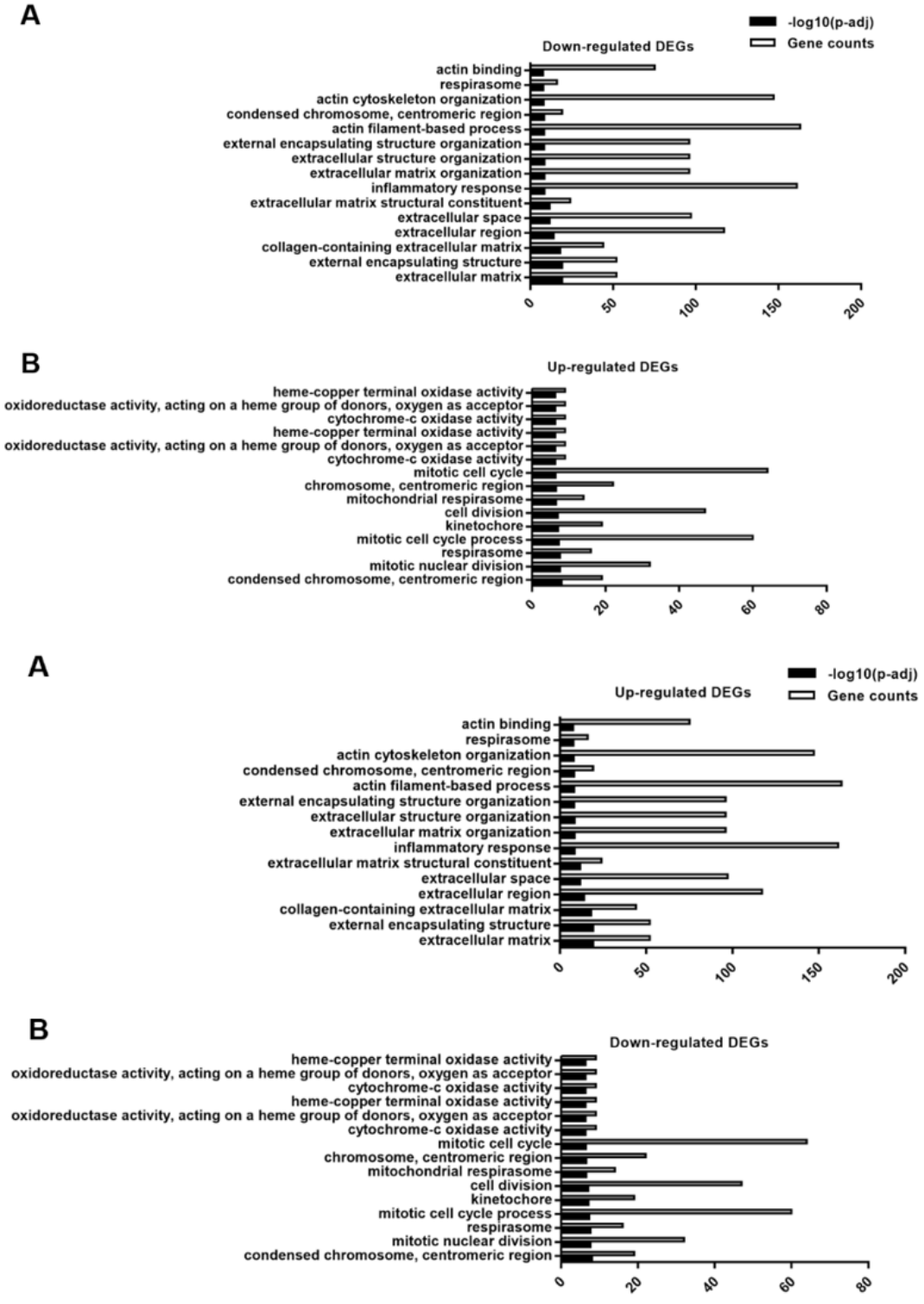

Figure 4

Functional analysis of up- and down-regulated differentially expressed genes with log2|FoldChange|>1.5 & p-value <0.01.

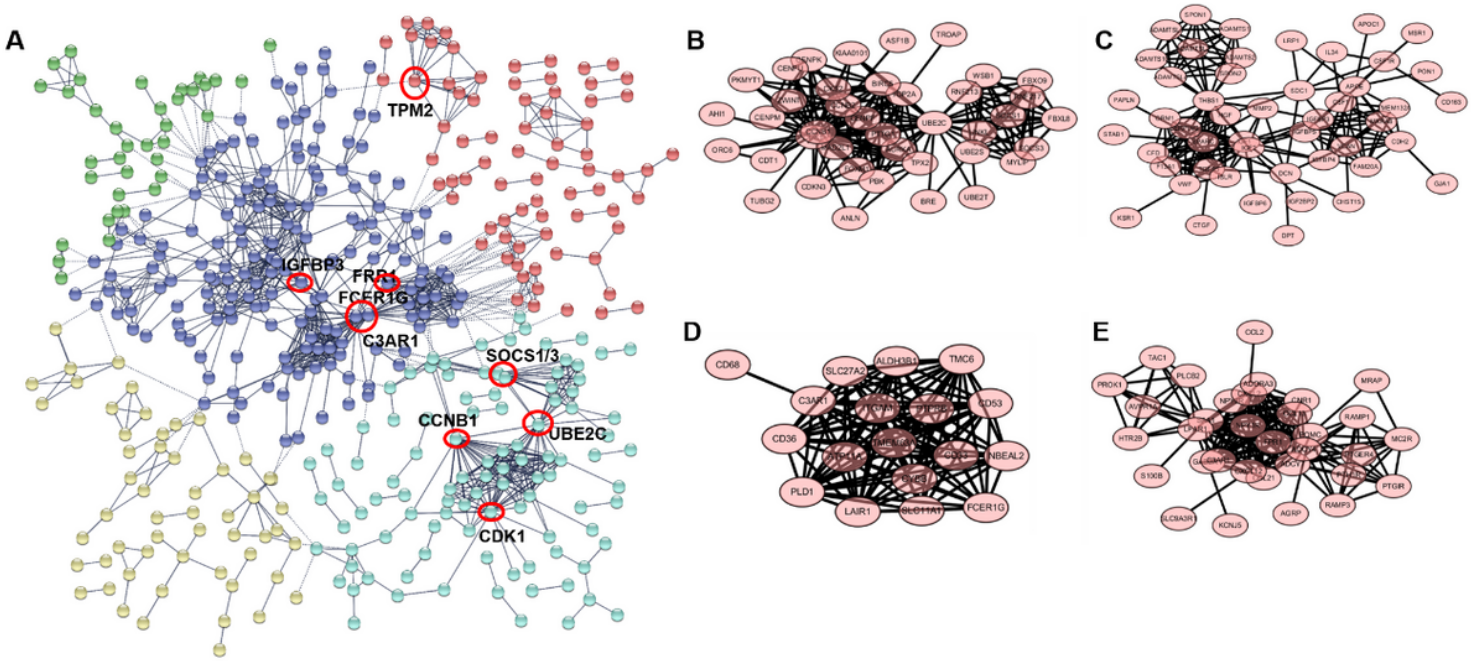

Figure 5

Protein-protein interaction network of differentially expressed genes. A. Total PPI network. B-E. Sub-PPI network of four differentially expressed genes cluster.

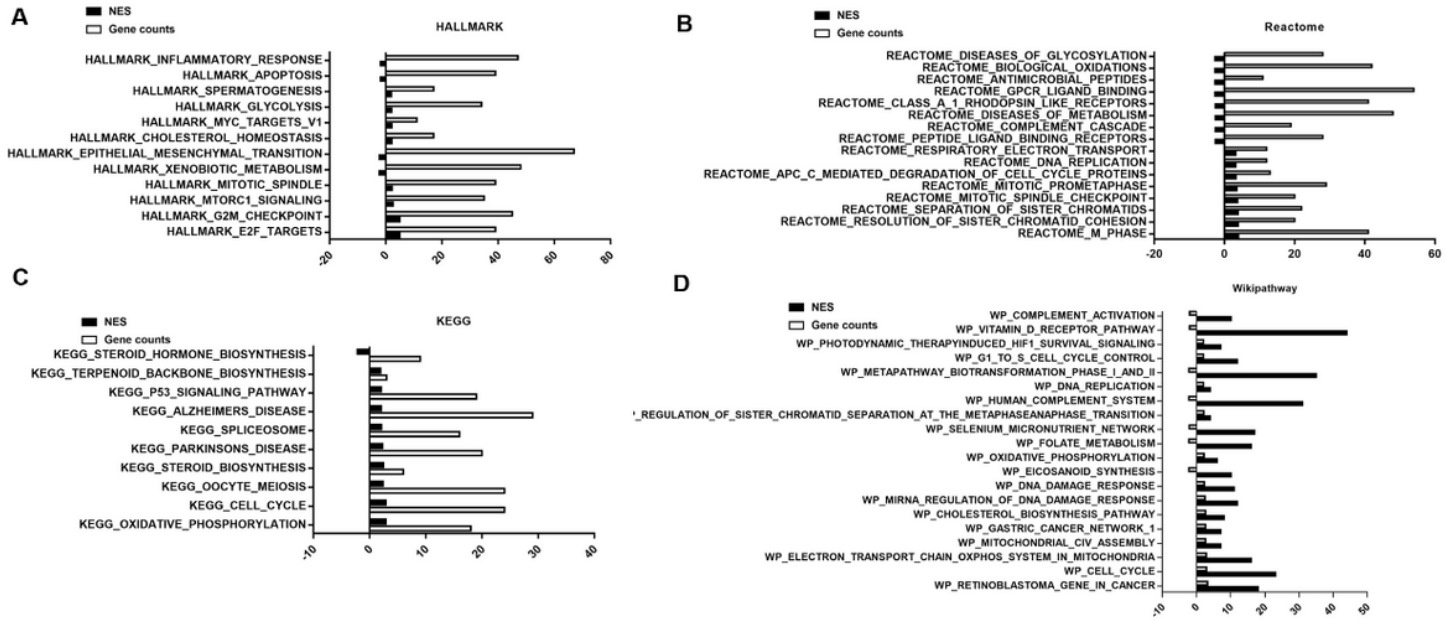

Figure 6

GSEA analysis of differentially expressed genes based on the Hallmark (A), Reactome (B), KEGG (C) and Wikipathway (D) gene sets.

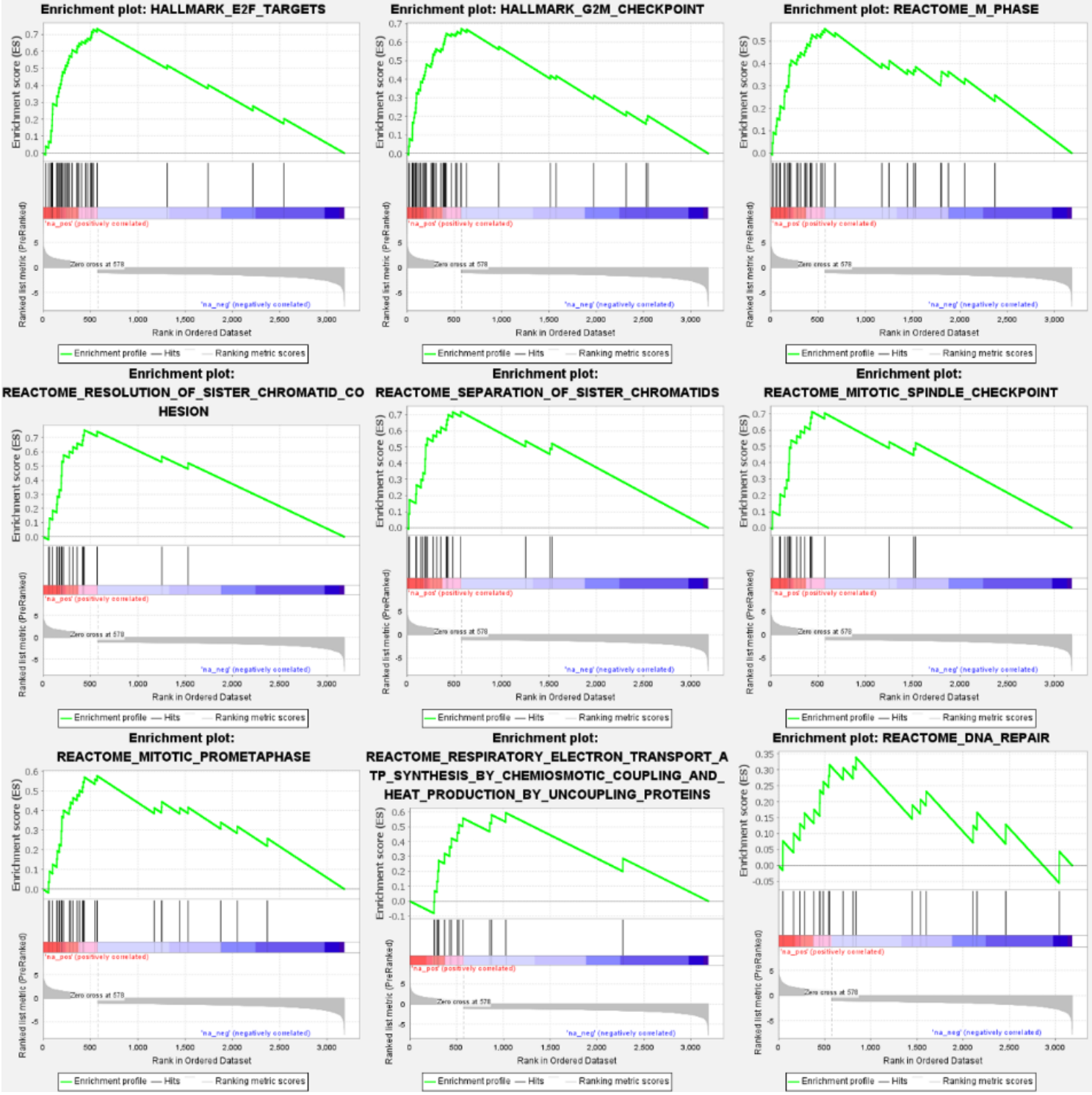

Figure 7

GSEA profile of top 9 enriched and up-regulated pathways (NES > 1.0).

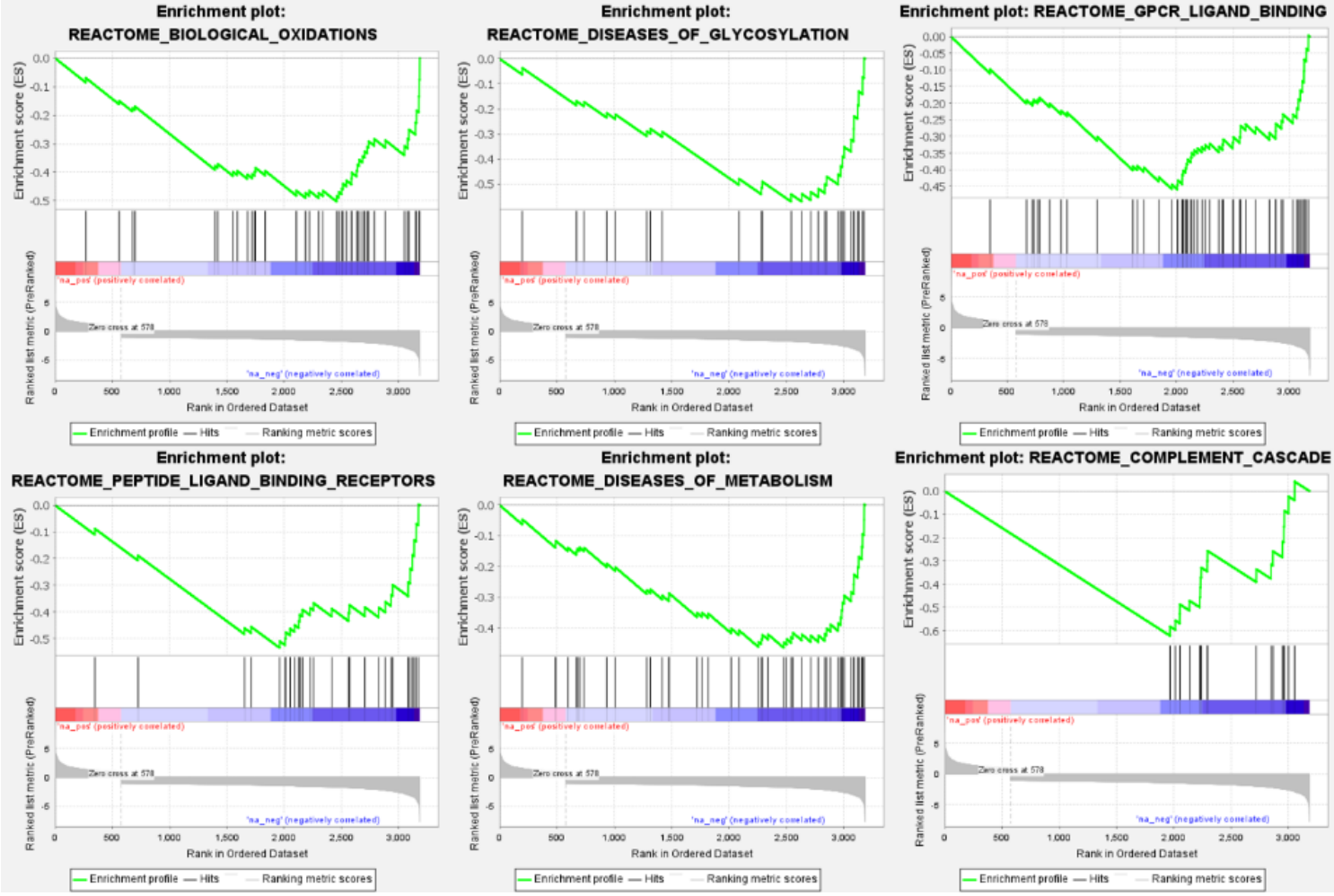

Figure 8

GSEA profile of top 6 enriched and down-regulated pathways (NES < 1.0).

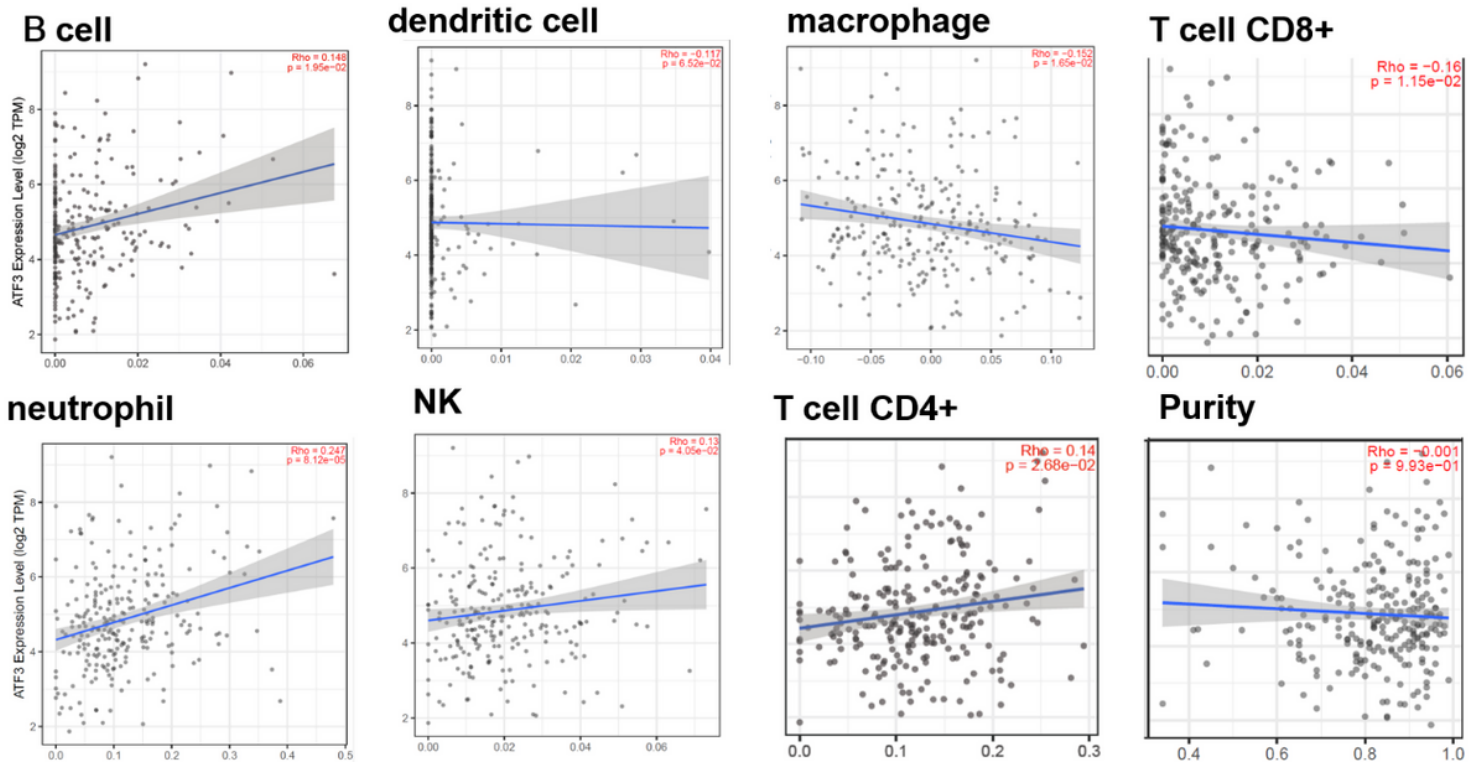

Figure 9

Higher ATF3 expression promotes the immune cell infiltration levels in ovarian tumor.

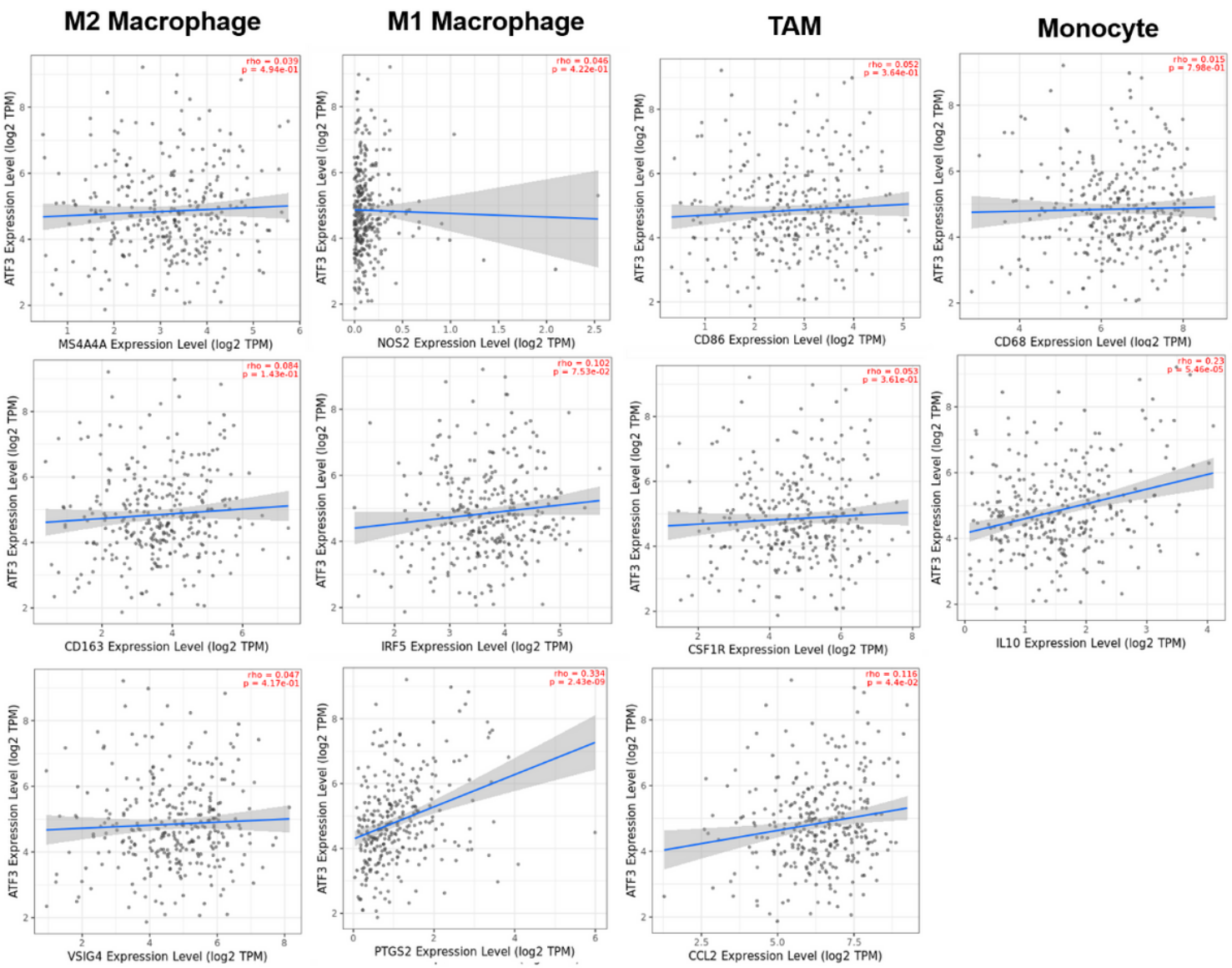

Figure 10

Correlation analysis between ATF3 expression and immunological marker sets in ovarian cancer.