Identification of an immune gene signature for predicting the prognosis of patients with uterine corpus endometrial carcinoma

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Abstract

Background: Uterine corpus endometrial carcinoma (UCEC) is a frequent gynecological malignancy with a poor prognosis especially when at an advanced stage. In the present study, we explored the potential of an immune-related gene signature to predict overall survival in UCEC patients.

Methods: We analyzed expression data of 616 UCEC patients from The Cancer Genome Atlas database and the International Cancer Genome Consortium as well as immune genes from the ImmPort database and identified the signature. We constructed a transcription factor regulatory network based on Cistrome databases and performed functional enrichment and pathway analyses for the differentially expressed immune genes. Moreover, the prognostic value of 410 immune genes was determined using Cox regression analysis then constructed a prognostic model. Finally, we performed immune infiltration analysis using TIMER-generating immune cell content.

Results: Results indicated that the immune cell microenvironment as well as the PI3K-Akt, and MARK signaling pathways were involved in UCEC development. The established prognostic model revealed a ten-gene prognosis signature , comprising PDIA3, LTA, PSMC4, TNF, SBDS, HDGF, HTR3E, NR3C1, PGR, and CBLC . This can be used as an independent tool to predict the prognosis of UCEC owing to the observed risk-score. In addition, levels of B cells and neutrophils were significantly correlated with the patient's risk score, and the expression of ten genes is associated with immune cell infiltrates.

Conclusions: In summary, we present a 10-gene signature with the potential to predict the prognosis of UCEC. This is expected to guide future development of individualized treatment approaches.

Background

Uterine corpus endometrial carcinoma (UCEC) is one of the most common malignant tumors in women. According to the current global cancer statistics, endometrial cancer has an incidence of about 4.4%[1], with related morbidity and mortality shown to increase each year despite the recent advances in treatment. This is mainly attributed to the lack of biomarkers for early diagnosis and prognosis prediction for this condition [2]. Previous studies have shown that the Grade, Stage, and TNM staging of UCEC are closely related to disease prognosis. However, some patients may manifest different clinical outcomes within the same stage group, implying that the clinical prognostic information generated by traditional clinical-pathological staging is insufficient. Therefore, identification of highly accurate, reliable and sensitive markers that, is imperative to improving prognosis of UCEC patients.

Recent studies have demonstrated the important role played by tumor microenvironment (TME)-stromal cells in tumor proliferation, invasion, and metastasis. In fact, these cells are closely related to prognosis of the disease [3, 4]. In addition, host immune responses, with multiple immune cell infiltrations, are one of the main participants in TME[5, 6]. Previous studies have also hypothesized that UCEC may be associated with long-term inflammatory stimuli, suggesting that the endometrium and menstrual cycles are essentially a chronic inflammatory process involving immune cells [7, 8]. Studies have also described

the effect of immune cell-derived cytokines on survival outcomes of UCEC patients [9-11]. However, the role of immune-related genes on systematic prediction of overall survival and response to immunotherapy in UCEC remains unknown.

Current and emerging knowledge of tumor molecular biology has led to development of numerous clinical therapeutic approaches for cancer treatment. In addition, attempts have been made to efficiently and accurately assess effects of the therapies, mainly through preclinical models that simulate characteristics of different types of cancers. For example, Muhammad et al. [12] demonstrated the anti-proliferative activity of bitter melon extract (BME) in breast cancer cells using homozygous and xenograft mouse models. In addition, advances in molecular techniques applied to different available preclinical models has greatly increased our understanding of endometrial cancer biology [13]. Currently, sequencing of the human genome and DNA microarray development have allowed identification of candidate genes of prognostic or therapeutic value. For example, the Cancer Genome Atlas (TCGA) database provides comprehensive data on the molecular basis of various types of cancer [14].

The current study sought to identify prognostic immunomarkers and construct a model for predicting UCEC. Specifically, we analyzed RNA-seq data from the TCGA database, as well as immune-related genes downloaded from the Immunology Database and Analysis Portal (ImmPort) databases. Subsequently, we assessed whether these immunity genes were associated with survival outcomes and clinical traits in a subgroup of UCEC patients. Finally, we explored the relationship between risk scores in UCEC patients with immune cell infiltration, using abundance of six immune infiltrates from the Tumor Immune Estimation Resource (TIMER) database.

Methods

Data access and analysis of differential gene expression

We downloaded expression data from the TCGA and ICGC databases. Specifically, RNA-seq and clinical data for 575 UCEC patients were downloaded from the TCGA database (https://portal.gdc.cancer.gov) and used as the training set, whereas a separate set from 41 UCEC patients were retrieved from the ICGC database, hosted on the University of California Santa Cruz (UCSC) Genome Browser (https://xena.ucsc.edu) and used as the testing set. The 2 sets of data were used to construct the signatur**.**

Differential expression gene analysis of the training set was performed using the "limma" package, at a corrected $P < 0.05$ and $|\log FC| \geq 1$. The resulting data was used to generate heatmaps and volcano plots using R version 3.5. 3[15]. Sequence data for immune-related genes and tumor-associated transcription factors were retrieved from the IMMPORT (http://www.immport.org/) and the Cistrome (http://cistrome.org/) websites [14, 16], then used for identification of differentially expressed immune genes and TFs, respectively. The resulting datasets were then used to generate heatmaps and volcano plots, as earlier described. Gene Ontology (GO), functional enrichment analyses and Kyoto Encyclopedia of Genes and Genome (KEGG) pathway analyses were then carried out for differential immune genes

using "clusterProfiler, org.Hs.eg.db, plot, ggplot2" packages implemented in R. These analyses were performed at P<0.05 and q<0.05 as cut-offs.

Construction of a regulatory network

Differentially expressed immunity genes were combined with survival time, then prognosis-related immune genes evaluated by cox univariate analysis. A forest map was drawn with significance filtering standard P< 0.01. Thereafter, correlation was performed with differential TFs at | cor |> 0.4 and P< 0.001 as the filtering criteria, and the resulting data was imported into Cytoscape version 3.7.1 for visualization of the regulatory network.

Construction and validation of the immune prognostic model for UCEC

Datasets from TCGA were used as the training whereas those from ICGA were taken as the testing set. The prognostic prediction model was constructed using the multivariate Cox regression model. Next, the most significant genes with regard to prognosis were determined by prognosis-related immune genes. ROC curves for assessing the sensitivity and specificity of the prognostic model were generated using the “survivalROC” package implemented in R.

Risk scores for each patient were calculated as follows:

$$Risk\ score = \sum_{i=1}^{n} exp_i * coef_i$$

Where “n” is the number of prognostic genes, “exp_i” is the expression value of the gene i, and “$coef_i$” is the gene i coefficient in multivariate Cox regression analysis.

The median risk value was then used to divide the patients into high and low-risk groups. The “survminer” and “survival” packages were then used to evaluate differences in survival between the two groups. A risk curve subsequently drawn using the “pheatmap” R package.

Finally, combined with clinical data for independent prognostic analysis, including single-factor and multi-factor independent prognostic analysis then used to assess the prognostic value of immune-gene signature and clinical parameters. This was also used to ascertain whether the predictive power of immunity-gene signature was independent of other clinical parameters.

Relationship among clinical parameters

To assess the association between immunity genes in the prognostic model and clinical parameters, patients were divided into two subgroups using univariate Cox regression analysis. The first group comprised patients who were ≤55 whereas the second one had those who were >55 years old. Clinically relevant immune genes across patients in the 2 groups were screened and mapped (P-value<0.05) using the “beeswarm” package.

Immunohistochemistry

The Human Protein Atlas (https://www.proteinatlas.org) contains information on tissue and cellular distribution for all 24,000 human proteins. The database applies immunohistochemistry using specific antibodies to analyze differentially expressed proteins in normal and tumor tissues. In the present study, we screened this database to analyze profiles of protein expression in ten genes across normal uterine and endometrial carcinoma tissues.

Correlation between immune cell content and immune gene expression

Data on abundance of six immune infiltrates, including B, CD4+ T, CD8+ T, and Dendritic cells as well as Neutrophils, and Macrophages, were retrieved from the TIMER official website (https://cistrome.shinyaoos.io/timer/), then used to analyze the relationship between risk scores of UCEC patients and the aforementioned immune cells. Furthermore, a correlation between the abundance of immune cells and gene expression [17].

Statistical analyses

Survival analysis was performed using the Kaplan-Meier curve, with statistical differences determined using the log-rank test. The area under curve (AUC) of the ROC was used to analyze prediction accuracy of the prognostic model, whereas effects of clinical traits on OS were assessed by univariate Cox and multivariate Cox regression analyses. The hazard ratio (HR) and 95% confidence interval (CI) were generated using the Cox proportional hazards model. In addition, Univariate Cox regression analysis was used to evaluate the correlation of immune cells and gene expression. Data with $p< 0.05$ were considered statistically significant.

Results

Identification of DEGs in UCEC

Finally, we identified 6,268 DEGs, 410 candidate prognostic immune genes and 100 differential TFs (**Fig. 1 and 2**). Enrichment analysis of differentially expressed immunity genes showed that Biological processes (BP), mainly chemotaxis migration of anti-inflammatory cells, including leukocyte and neutrophils, were primarily enriched (**Fig. 3A**). Enriched Cellular components (CC) were mainly extracellular matrix whereas the main Molecular function (MF) comprised growth factor and cytokine activity. These results indicated that most differentially expressed immunity genes were associated with UCEC development, progression, and prognosis through immune cells. The enriched top 30 KEGG pathways are as shown in **Fig. 3B**. Here, several signaling pathways involved in UCEC development, including PI3K-Akt, MAPK, Ras, and JAK-STAT, were identified.

Association between immunity genes and survival rates

Univariate Cox regression analysis of differentially expressed immunity genes revealed a significant correlation between 21 of these genes and survival rates (P < 0.01). The genes with a significant association were *PDIA3, LTA, PSMC4, IL6, TNF, KCNH2, SYTL1, BACH2, PCSK1, BIRC5, SBDS, ANGPTL7, GPI, HDGF, ADCYAP1R1, HTR3E, NPR1, NR3C1, PGR, THRB, and CBLC.* Among them, *LTA, ADCYAP1R1, PGR, SYTL1,* and *PDIA3* were low-risk, while the remaining 16 are high-risk genes. Detailed information of all 21 genes is shown in **Fig. 4A**.

To assess the relationship between the 21 prognosis-related immunity genes and TFs, a univariate Cox regression analysis was performed at | cor |>0.4 and P<0.001, then a TF regulatory network constructed (**Fig. 4B**). According to the regulatory network diagram, that comprised low- (*PGR, SYTL1,* and *LTA*), and high-risk (*BIRC5, HDGF, HTR3E, THRB, NR3C1, BACH2)* genes, as well as TFs (*AR, BATF, CBX2, CENPA, E2F1, E2F3, ETS1, EZH2, FOXK1, FOXP3, GREB1, H2AFX, LMNB1, LYL1, NCAPG, NR3C1, RFX2, SNAI2, SOX17, SPDEF, SPIB, STAT5A,* and *WWTR1*) revealed a positive relationship between immunity genes and TFs. Notably, *BIRC5* was associated with several transcription factors, including *CBX2, CENPA, E2F1, EZH2, FOXM1, H2AFX, LMNB1,* and *NCAPG.*

The prognostic prediction model

To establish a model for predicting prognosis of UCEC patients, we employed Cox regression analysis and identified a ten-gene signature. The genes in the signature included *PDIA3, LTA, PSMC4, TNF, SBDS, HDGF, HTR3E, NR3C1, PGR,* and *CBLC* (**Table 1**). The prognostic model was then used to calculate a risk score for each patient, and the median value subsequently used to divide all patients into either a high- or low-risk groups. Prediction power of the ten-gene signature for patients in training and testing sets are outlined in **Fig. 5**, while distribution of risk scores, gene expression levels and patient survival status are shown in **Fig. 5A**. In the training set, lower overall survival rates were recorded for patients in the high-risk compared to those in the low-risk group (P-value = 5.502e−09). In addition, the 5-year OS rates of 63.1 and 89.9%, were recorded for patients in the high- and low-risk groups, respectively, whereas 9-year OS rates were 34.6 and 78.7% for patients in the high- and low-risk groups, respectively. In the testing set, a shorter overall survival rate was recorded for patients in the high- than those in the low-risk group (P-value = 2.022e−02) **(Fig. 5B**). AUC for the training and testing sets were 0.756 and 0.797, respectively (**Fig. 5C**), indicating good accuracy of the prognostic prediction-values across the ten-gene signature. To determine whether the prognostic model risk score was an independent prognostic factor for patient survival, we employed univariate and multivariate Cox regression analyses. Results revealed P-values that were less than 0.05, across both analyses, indicating that the risk score derived from this prognostic model can be independent of other clinical traits, hence an independent prognostic factor. In addition, univariate Cox regression analysis showed that age (P=0.002, hazard ratio=1.035) and grade (P<0.001, hazard ratio=2.595) were significantly associated with prognosis. In fact, prognosis of patients was worse with increase in age and grade. (**Fig. 6**)

Clinical parameters, immunohistochemical examination, and immune cells

The correlation between immune genes involved in the prognostic model and clinical traits was assessed using Univariate Cox regression analysis. In addition, patients were divided into two groups, based on clinical traits: Group 1 comprised patients aged <55 and Age ≥ 55) and Group 2 (G1 & G2 and G3). Results revealed a significant correlation between *HDGF, PGR, PSMC4, TNF, NR3C1, HTR3E,* and *CBLC* with age, whereas expression of *HDGF, PSMC4, TNF, NR3C1, HTR3E,* and *CBLC* increased with age. On the other hand, *HDGF, PGR, PSMC4, TNF, NR3C1, PDIA3,* and *SBDS* were significantly associated with grade. Moreover, an increase in grade resulted in upregulation of *HDGF, PSMC4, TNF, NR3C1,* and *SBDS* (**Fig. 7**). Immunohistochemical analysis based on The Human Protein Atlas database revealed significant upregulation of PSMC4, NR3C1, SBDS, and CBLC in endometrial cancer tissues, relative to normal bladder tissues. On the other hand, immunohistochemical analysis of PGR and PDIA3 expression showed significant downregulation of these factors in endometrial cancer relative to normal tissues (**Fig. 8**).

Finally, a correlation analysis between risk scores in UCEC patients with abundance of six immune infiltrations revealed a significant positive association between B cells (p=3.408e−10, cor=0.265) and Neutrophils (p=0.011, Cor = 0.109) with the patient's risk score (**Fig. 9**). To explain the relationship, we analyzed infiltration abundance, and found a positive relationship between B cells and expression of LTA (Cor=0.594, P=5.55e-29) (**Fig. 10B**), TNF (Cor=0.117, P=4.60e-02) (**Fig. 10D**), and NR3C1 (Cor=0.301, P=1.85e-07) (**Fig. 10H**). Moreover, the infiltration abundance of neutrophils was positively correlated with expression of LTA (Cor=0.339, P=2.65e-09) (**Fig. 10B**), PSMC4 (Cor=0.209, P=3.23e-04) (**Fig. 10C**), TNF (Cor=0.408, P=3.56e-13) (**Fig. 10D**), SBDS (Cor=0.418, P=7.89e-14) (**Fig. 10E**), HDGF (Cor=0.309, P=6.50e-08) (**Fig. 10F**), and NR3C1 (Cor=0.48 P=2.70e-18) (**Fig. 10H**).

Discussion

Numerous reports have described the relationship between differentially expressed genes and various aspects of tumors, including tumorigenesis and prognosis [18-20]. However, a vast majority of genes implicated in predicting tumor prognosis are limited by certain factors, such as insufficient sample sizes. In the present study, we employed a large sample size comprising TCGA genome-wide expression data to develop a ten-gene prognostic signature for UCEC patients. This prognostic model is expected to guide the identification of potential biomarkers that can monitor the prognosis, and response to immunotherapy in UCEC patients.

Our results revealed an association between differentially expressed immune genes with immune cell responses to extracellular matrix and tumor microenvironment in UCEC, in line with previous studies [21]. In addition, KEGG enrichment analysis showed that UCEC may be associated with several well-known cancer-related pathways, including the PI3K-Akt, MAPK, Ras, and JAK-STAT signaling pathways. Previous studies have demonstrated activation of the PI3K-AKT signaling pathway in UCEC patients, as well as its role in promoting tumor development [22]. Numerous studies have revealed multiple factors that activate MAPK, Ras, and JAK-STAT signaling pathways, thereby mediating proliferation, infiltration and other biological behaviors to promote the occurrence and progression of UCEC [23-25]. Our TF-related regulatory network showed that BIRC5 was positively regulated by multiple TFs, and BIRC5 was a high-

risk gene. This was consistent with previous studies demonstrating that up-regulation of BIRC5 leads to the development and progression of many malignant tumors in humans [26]. Similarly, BIRC5 was reportedly overexpressed in more than 90% of UCEC [27], while another study demonstrated frequent overexpression of BIRC5 in recurrent UCEC relative to primary tumors [28].

In the present study, we developed a ten-gene prognostic signature, comprising *PDIA3, LTA, PSMC4, TNF, SBDS, HDGF, HTR3E, NR3C1, PGR,* and *CBLC,* for prediction of overall survival rates in UCEC patients. Our results indicated that the signature effectively predicted OS of UCEC patients, with a statistically significant correlation between the training and testing sets. These findings suggest potential of the signature as a powerful prognostic tool for the entire cohort of UCEC patients. In addition to hepatoma-derived growth factor (HDGF) and Protein disulfide-isomerase A3 (PDIA3), the remaining 8 genes have not been well validated in gynecologic oncology, especially in UCEC. HDGF is a heparin-binding growth factor that has been implicated in differentiation, growth, and division of various tissues. Previous studies have demonstrated its involvement in the occurrence and development of malignant tumors, promoting proliferation and differentiation of tumor cells, as well as enhancing the metastatic ability of tumor cells through EMT [29, 30]. In addition, HDGF was reported to be an independent risk factor for prognosis of various malignancies, including liver, gastric, cholangiocarcinoma and non-small cell lung cancers [31]. However, in endometrial cancer, HDGF has been implicated in multiple abnormalities. For example, a higher FIGO stage mediated HDGF upregulation, a potential adverse factor for progression and prognosis of UCEC [32].

On the other hand, PDIA3, also known as ERp57/GRP58, has been implicated in in malignant transformation of cells through STAT3 and Wnt signaling pathways. In addition, this factor has been closely associated with the occurrence and development of various tumors[33]. Interestingly, PDIA3 has been reported to enhance the ability of cervical and ovarian cancer cells to proliferate and invade, indicating its potential as a sensitive marker for reflecting tumor prognosis during gynecologic oncology [34,35]. In the present study, these two immune genes were key DEGs (P-value <0.0001), suggesting their possible role in the development and progression of UCEC. Notably, the overall survival rate of patients in the high-risk group was lower than those in the low-risk group, whereas the AUC values showed that a combination of the ten immune genes had prognostic value in UCEC patients. Moreover, our prognostic model was an independent prognostic factor, owing to the fact that the predicted survival rates were not related to other clinical traits

In the present study, our results revealed that age and grade were associated with OS of UCEC patients, and were high-risk factors. This was consistent with previous studies showing that age, stage, and body weight are clinical prognostic factors for UCEC [36]. On the other hand, age and grade were also associated with prognosis of endometrial cancer, and were further a high-risk factor for the disease, in line with previous studies that have described age, stage, and body weight as clinical prognostic factors for endometrial cancer. Further correlation analysis revealed a significant positive correlation between HDGF and PSMC4 with age and grade. This may be attributed to the fact that the up-regulation of these genes could promote tumor development [37,38]. In terms of survival prediction, the current staging

system is far from accurate at the individual level. Moreover, age is not a survival indicator for cancer because older people are less likely to receive adjuvant therapy [39]. Therefore, risk scores present a more reliable tool for prognosis of UCEC patients compared to age and stage.

Currently, numerous studies have hypothesized the involvement of immune cells and related inflammatory factors in the UCEC interstitial, which is an important component of the tumor inflammatory microenvironment and generates a marked influence on the biological behavior of UCEC[40]. Consequently, we analyzed the relationship between UCEC risk-score and immune cells using immune cell infiltration abundance data from TIMER. Results indicated a significant positive correlation between B cells and neutrophils with the patient's risk-scores. Furthermore, we found a close relationship between prognostic model genes and immune cells. Among them, neutrophils were positively correlated with the expression of several genes, including *LTA, PSMC4, TNF, SBDS, HDGF,* and *NR3C1*. This phenomenon may be attributed to the secretion of HDGF, which was shown to promote neutrophil infiltration and induce inflammatory signals [41]. To add strength to this, Wikberg et al. [42] demonstrated that neutrophils of the innate immune system play a major role in acute inflammation as well as in anti-tumor immune responses. Despite the close association between neutrophil infiltration with other immune cell infiltration, studies have shown that neutrophil infiltration may have additional prognostic values in various cancers. For example, neutrophils persist in tissues, during chronic inflammation, causing cancer progression. Previous studies have also shown that elevated numbers of neutrophils in many human cancers or a higher Neutrophil/Lymphocyte Ratio (NLR) are associated with poor prognosis possibly because neutrophils secrete matrix metalloproteinase-9 to stimulate angiogenic activity of cancer cells [43, 44]. Different proportions of infiltrating B cells were included in solid human tumors. Although the search for immune-related factors associated with a cancer diagnosis, prognosis, and survival has been largely limited to T cell responses, recent reports have suggested that B cells may also play critical roles in prognosis of cancer patients. For example, results by Schimdit et al. [45, 46] demonstrated that the B cell marker was the strongest prognostic factor in breast cancer and other human tumors, given the immunoglobulin kappa chain (IGKC) secreted by plasma cells. On the other hand, Nielsen et al. [47] found that increase in CD20+ B cells resulted in higher survival rates of patients with advanced ovarian cancer. Therefore, an increase in the risk-score is likely to elevate levels of these two immune cells and influence immune escape or suppression.

Despite the important clinical value of these findings in UCEC, there were several limitations in our study. Firstly, age and grade were the only clinical traits in the TCGA database of UCEC, although related such as stage and TMN may strengthen value of the identified genes. Secondly, most of the ten-gene prognosis signature and immune cells have rarely been reported in UCEC patients. Therefore, more prospective studies are needed to validate the intrinsic relevance of these genes in the prognosis of UCEC patients.

Conclusions

In summary, TCGA, ImmPort, and Cistrome databases allowed screening of 410 candidate prognostic immune genes, and subsequent construction of a TF regulatory network. These differential immune

genes play a role in UCEC development through immune cells. Moreover, tumors were closely associated with the PI3K-Akt, MAPK, Ras, and JAK-STAT signaling pathways. A prognostic model, comprising a ten-gene prognostic signature that included *PDIA3, LTA, PSMC4, TNF, SBDS, HDGF, HTR3E, NR3C1, PGR,* and *CBLC*, was successfully established. The resulting risk-score can be used as an independent prognostic factor for UCEC patients. Finally, a significant association was found between B cells and neutrophils based on risk-scores of patients. Future prospective studies with larger sample size are advocated to validate our findings.

Declarations

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Author's contributions

Cankun Zhou and Yuhua Zheng performed data analysis work and aided in writing the manuscript. Cankun Zhou and Chaomei Li designed the study, assisted in writing the manuscript. Fangli Yan edited the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

All data generated or analyzed during this study and included in this published article.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Southern Medical University Affiliated Maternal & Child Health Hospital of Foshan

Competing interests

The authors declare that they have no competing interest.

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Table

Table 1 The prognostic model of prognosis-related 10 immune genes

ID	Coef	HR (95% CI)	P-value
PDIA3	-0.00226	0.997739 (0.994865-1.000621)	0.124063
LTA	-1.0635	0.345246 (0.170688-0.698318)	0.003086
PSMC4	0.003143	1.003148 (1.000176-1.00613)	0.037905
TNF	0.02748	1.027862 (1.00758-1.048551)	0.006879
SBDS	0.017278	1.017428 (1.00557-1.029426)	0.00387
HDGF	0.002992	1.002996 (0.999056-1.006952)	0.136281
HTR3E	0.548394	1.730472 (1.42863-2.096087)	2.05E-08
NR3C1	0.153559	1.165977 (0.996923-1.363698)	0.054681
PGR	-0.02721	0.973152 (0.945302-1.001823)	0.066204
CBLC	0.01077	1.010828 (1.004709-1.016984)	0.000508

Abbreviation: Coef, coefficient. HR, hazard ratio. CI, confidence interval.

Figures

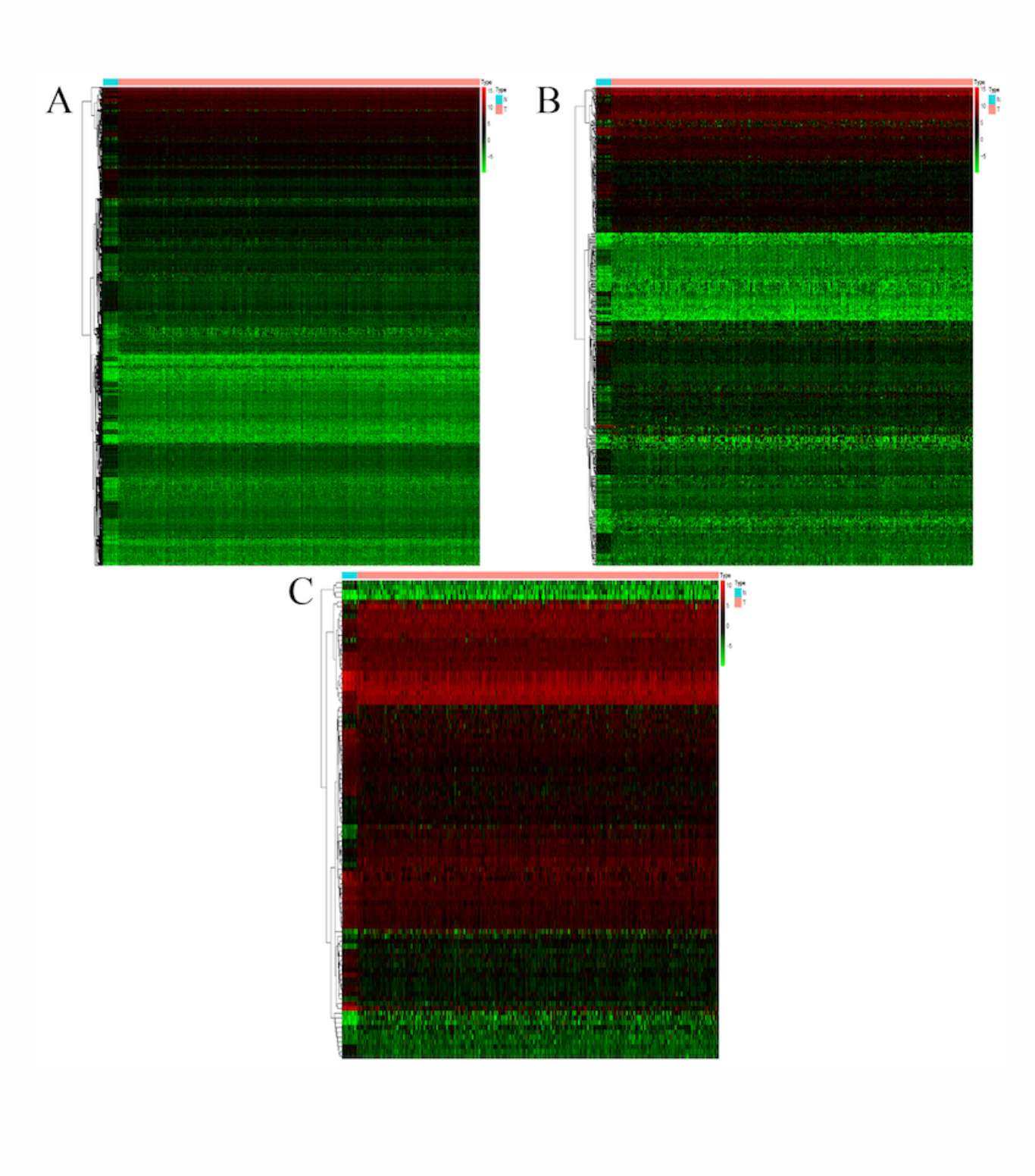

Figure 1

Hierarchical clustering heatmap of DEGs. The genes with higher expression in the heatmap are shown in red, lower expression in green, and genes with the same expression level in black. Tiffany blue represents the adjacent tissue, and the pink represents the cancer tissue. (A) DEGs of RNA-seq gene expression. (B) DEGs of immune genes. (C) DEGs of TFs. Abbreviation: DEGs, differentially expressed genes. TFs, transcription factors.

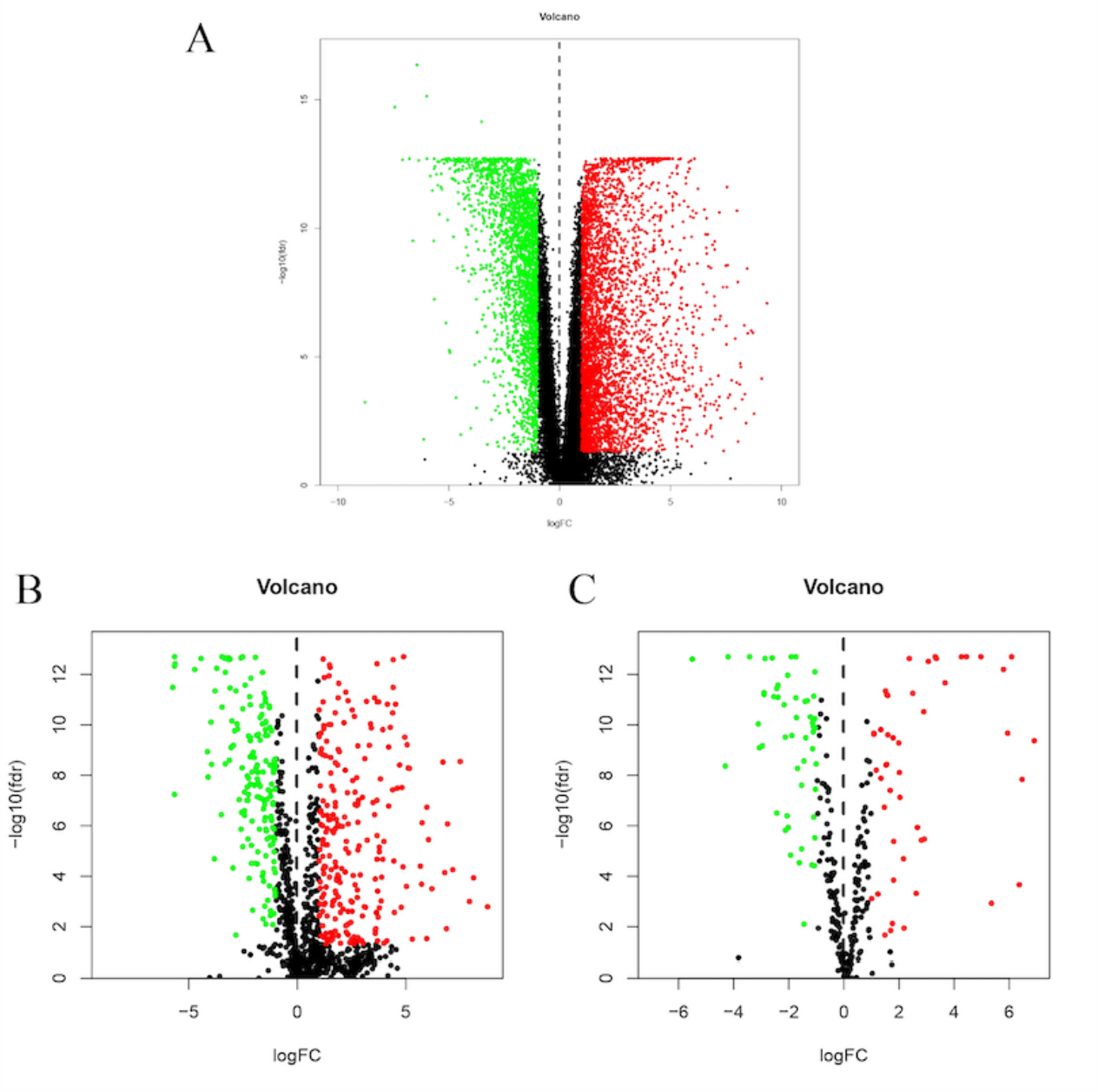

Figure 2

Volcano plot of DEGs. The red dots represent the up-regulated genes screened on the basis of corrected P < 0.05 and logFC≥1. The green dots represent the down-regulated genes screened on the basis of corrected P < 0.05 and logFC≤-1. The black dots represent genes with no significant differences. (A) DEGs of RNA-seq gene expression. (B) DEGs of immune genes. (C) DEGs of TFs. Abbreviation: DEGs, differentially expressed genes. TFs, transcription factors. FC, fold change.

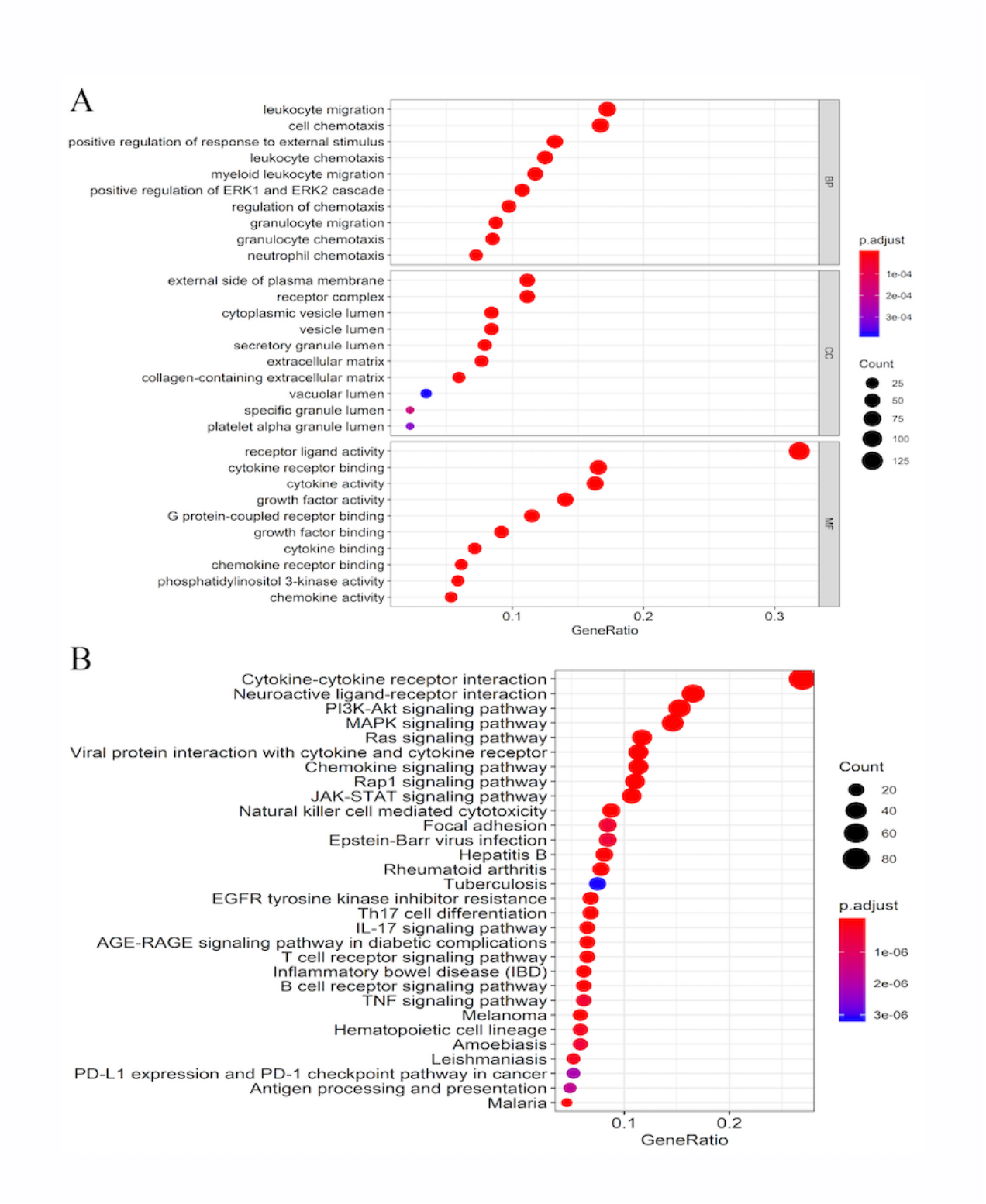

Figure 3

Functional enrichment analysis of DEGs of immune genes. (A) Biological process, Cellular composition, and molecular function of GO enrichment analysis. (B) Enrichment analysis of KEGG pathway. The results were as follows: BP (Biological process) mainly included the chemotaxis migration of anti-inflammatory cells, including leukocyte and neutrophil, CC (Cellular composition) mainly included extracellular matrix, and MF (Molecular function) mainly included growth factor and cytokine activity. The

enriched KEGG pathway mainly included PI3K-Akt, MAPK, Ras, and JAK-STAT signaling pathways. Abbreviation: DEG, differentially expressed gene. GO, Gene Ontology. KEGG, Kyoto Encyclopedia of Genes and Genomes.

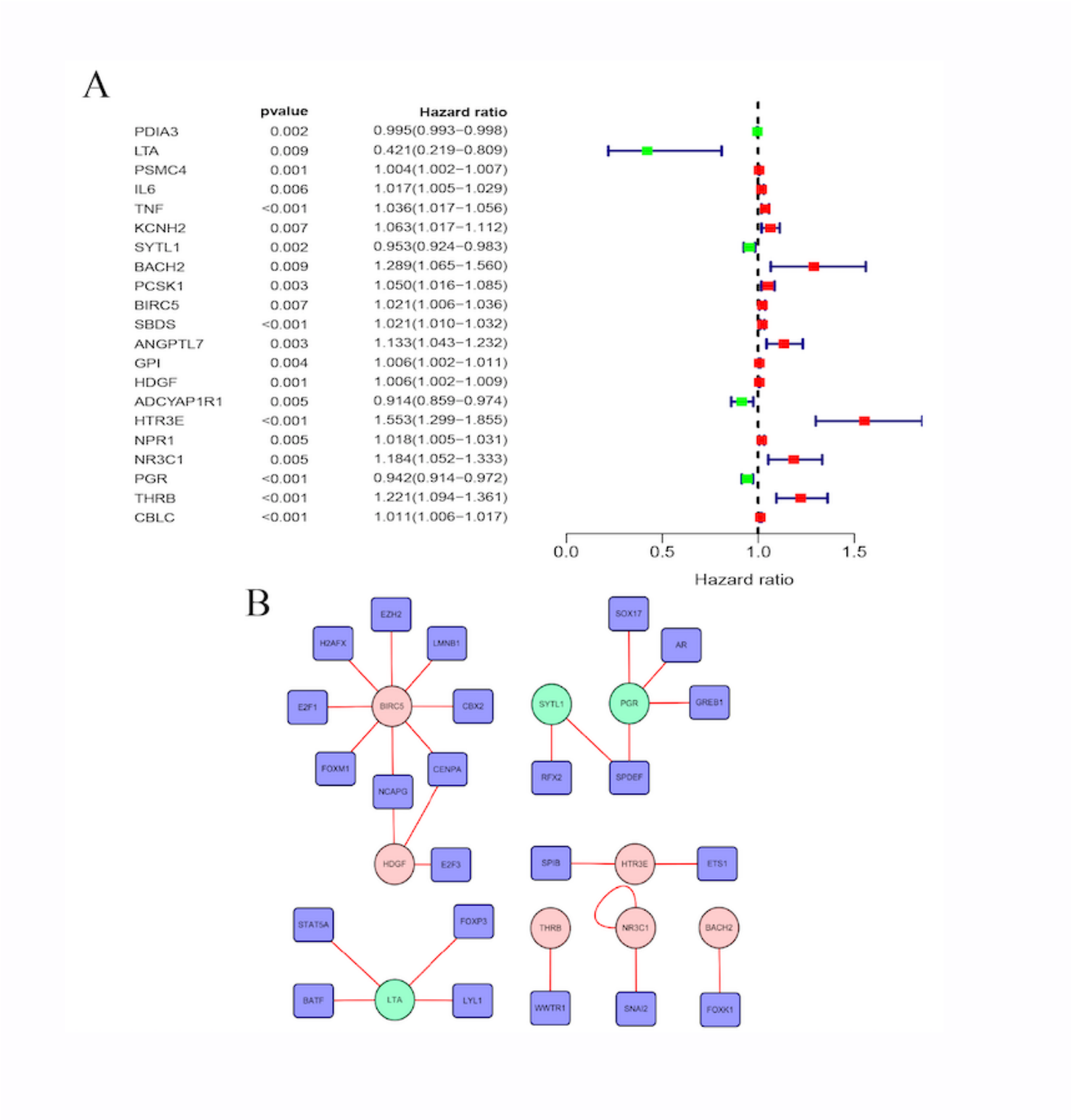

Figure 4

Correlation analysis of prognosis-related immune genes and TFs. (A) Forest of prognosis-related immune genes on the basis of P-value□0.001. Red means high risk, green means low risk. The higher the Hazard

ratio, the higher the prognostic risk. (B) TFs and prognosis-related immune gene regulatory networks on the basis of | cor |0.4 and P-value0.001. Blue round rectangle represents TFs, dark pink and light green ovals represent high-risk and low-risk prognosis-related immune genes, respectively, and red lines represent positive regulation. Abbreviation: TFs, transcription factors.

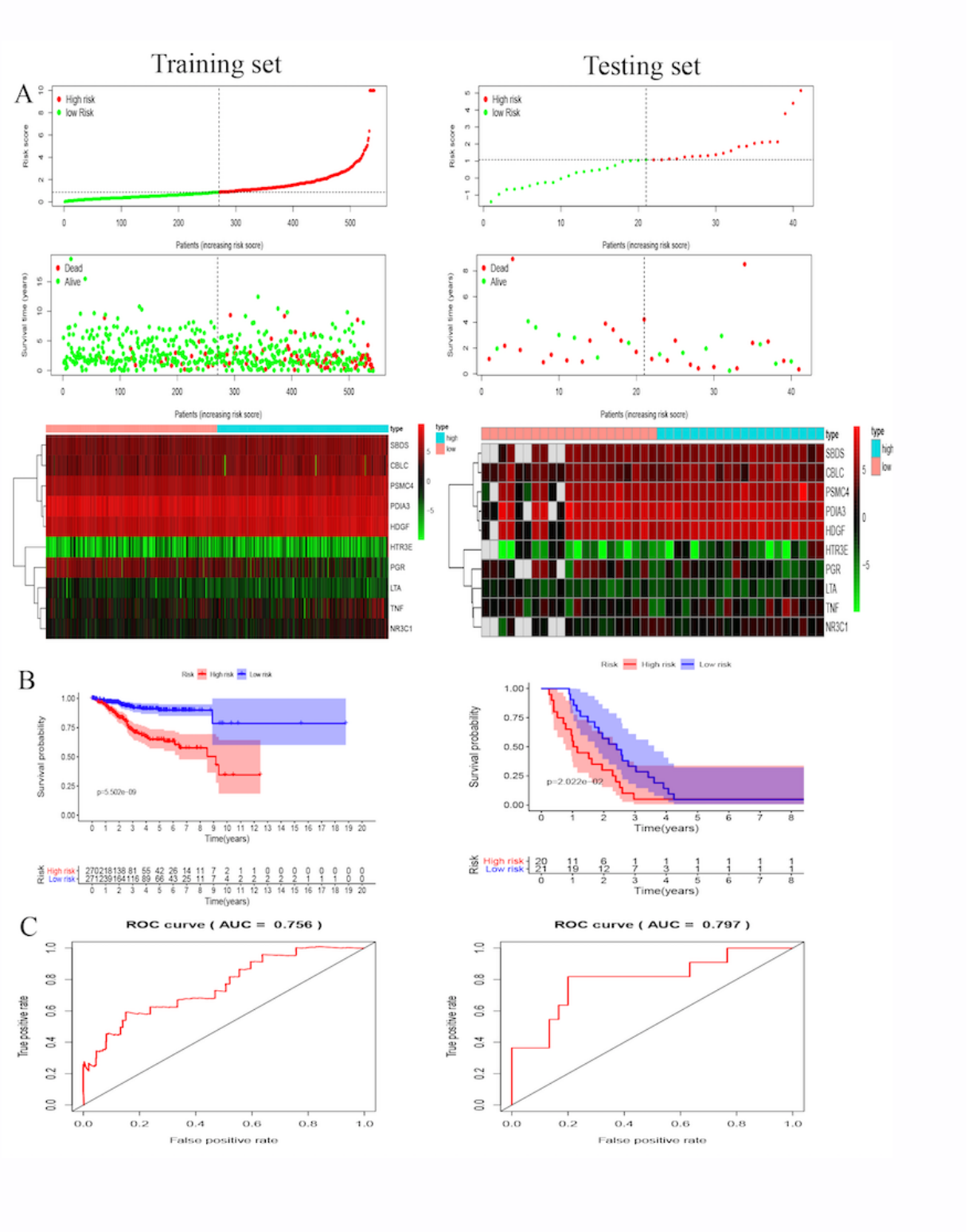

Figure 5

Correlation between the ten-gene prognosis signature and the OS of patients in two datasets, including training set (TCGA database) and testing set (ICGC database). (A) The distribution of risk scores, gene expression levels and patient survival status. The black dotted line represents the median cut point and divides patients into low-risk and high-risk groups. (B) Kaplan–Meier curves of OS of high- and low-risk groups. (C) ROC curve for judging the accuracy of the prognostic model. Abbreviation: OS, overall survival. UCEC, Uterine corpus endometrial carcinoma.

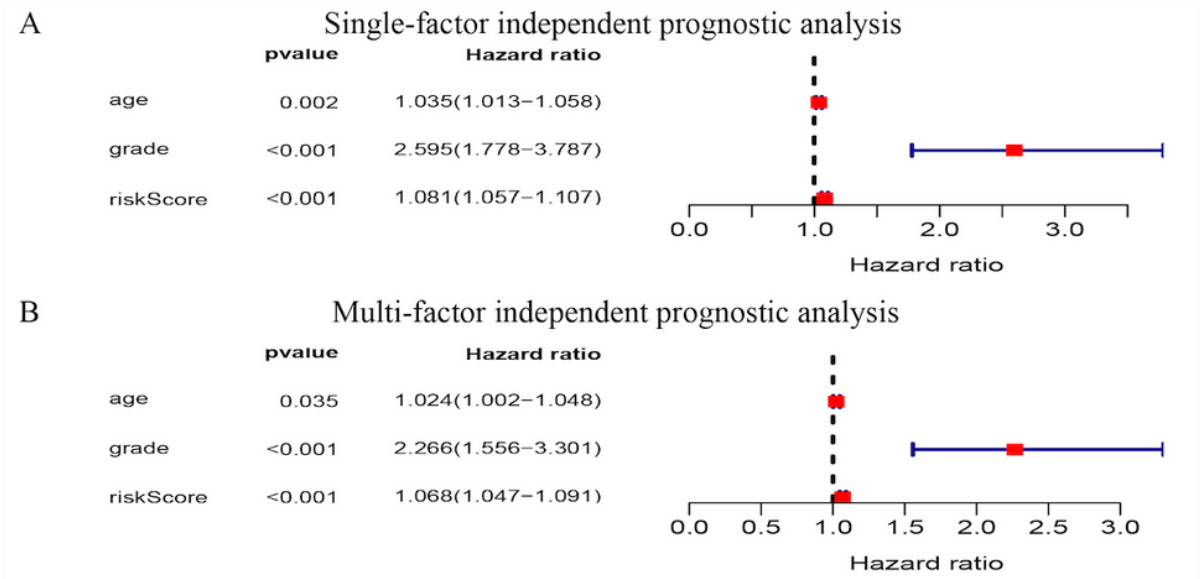

Figure 6

Univariate and multivariate Cox regression analysis of the ten-gene prognosis signature of UCEC patients in TCGA Cohort. (A) Univariate Cox regression analysis. (B) Multivariate Cox regression analysis. Abbreviation: UCEC, Uterine corpus endometrial carcinoma

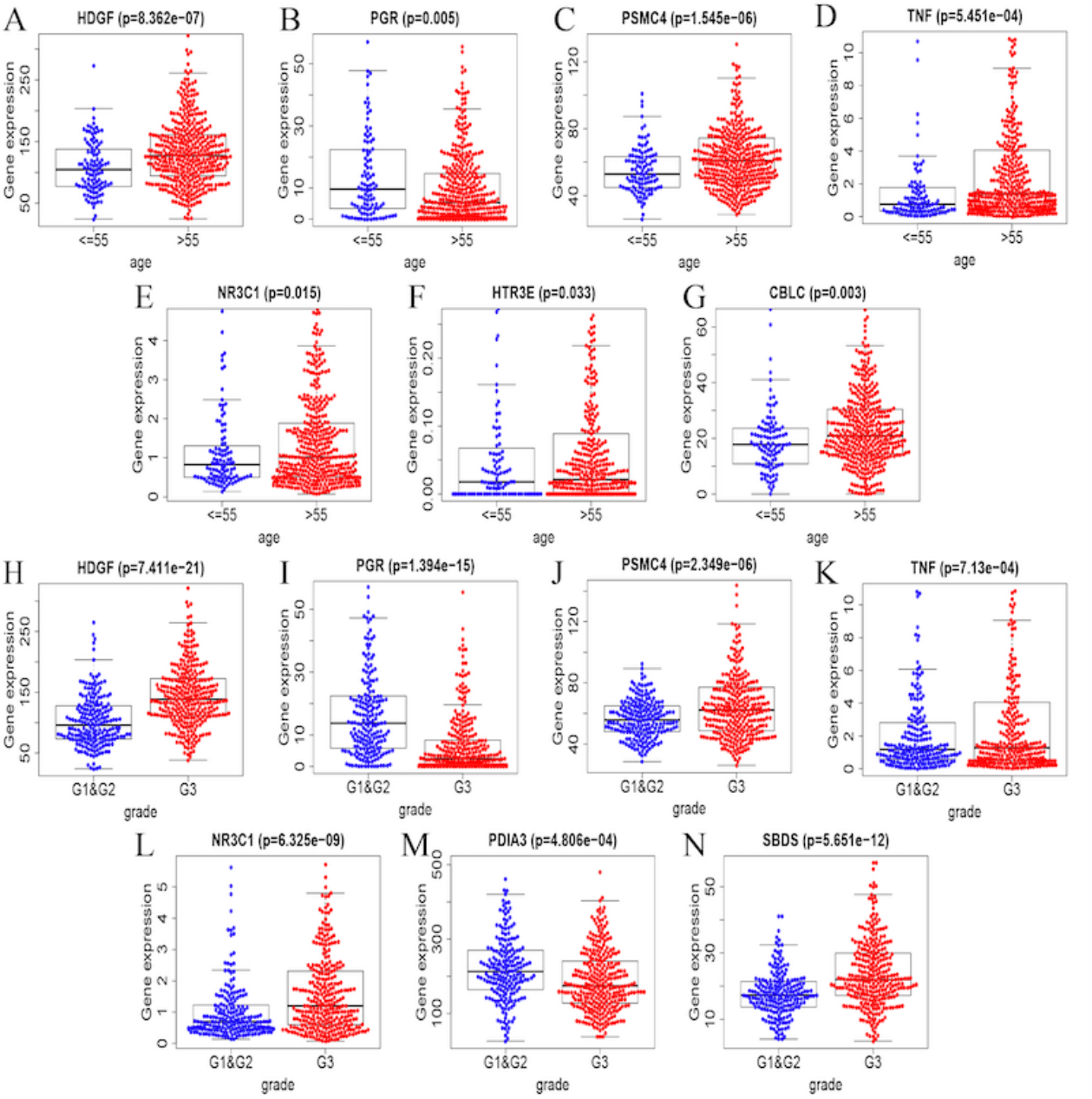

Figure 7

Gene expression levels of (A) HDGF, (B) PGR, (C) PSMC4, (D) TNF, (E) NR3C1, (F) HTR3E, and (G) CBLC between different age of UCEC. Gene expression levels of (H) HDGF, (I) PGR, (J) PSMC4, (K) TNF, (L) NR3C1, (M) PDIA3, and (N) SBDS between different clinical grade of UCEC. Abbreviation: UCEC, Uterine corpus endometrial carcinoma

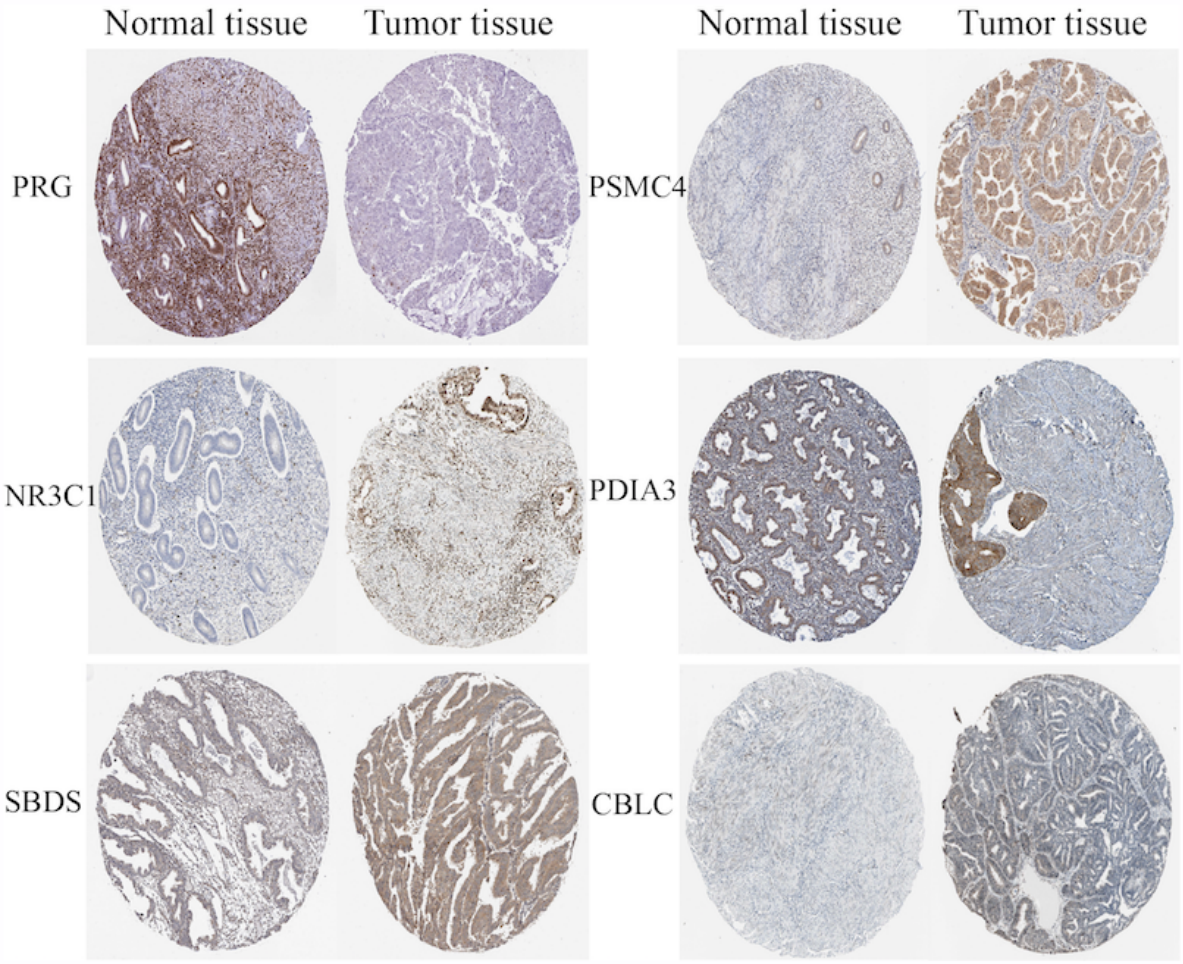

Figure 8

The level of ten genes in endometrial cancer patients in protein level (The Human Protein Atlas). Immunohistochemical examination for expression of PSMC4, NR3C1, SBDS, and CBLC were significant up-regulated in endometrial cancer tissue compared with normal tissues, while PGR and PDIA3 expression were significantly down-regulated in endometrial cancer tissues compared to normal tissues.

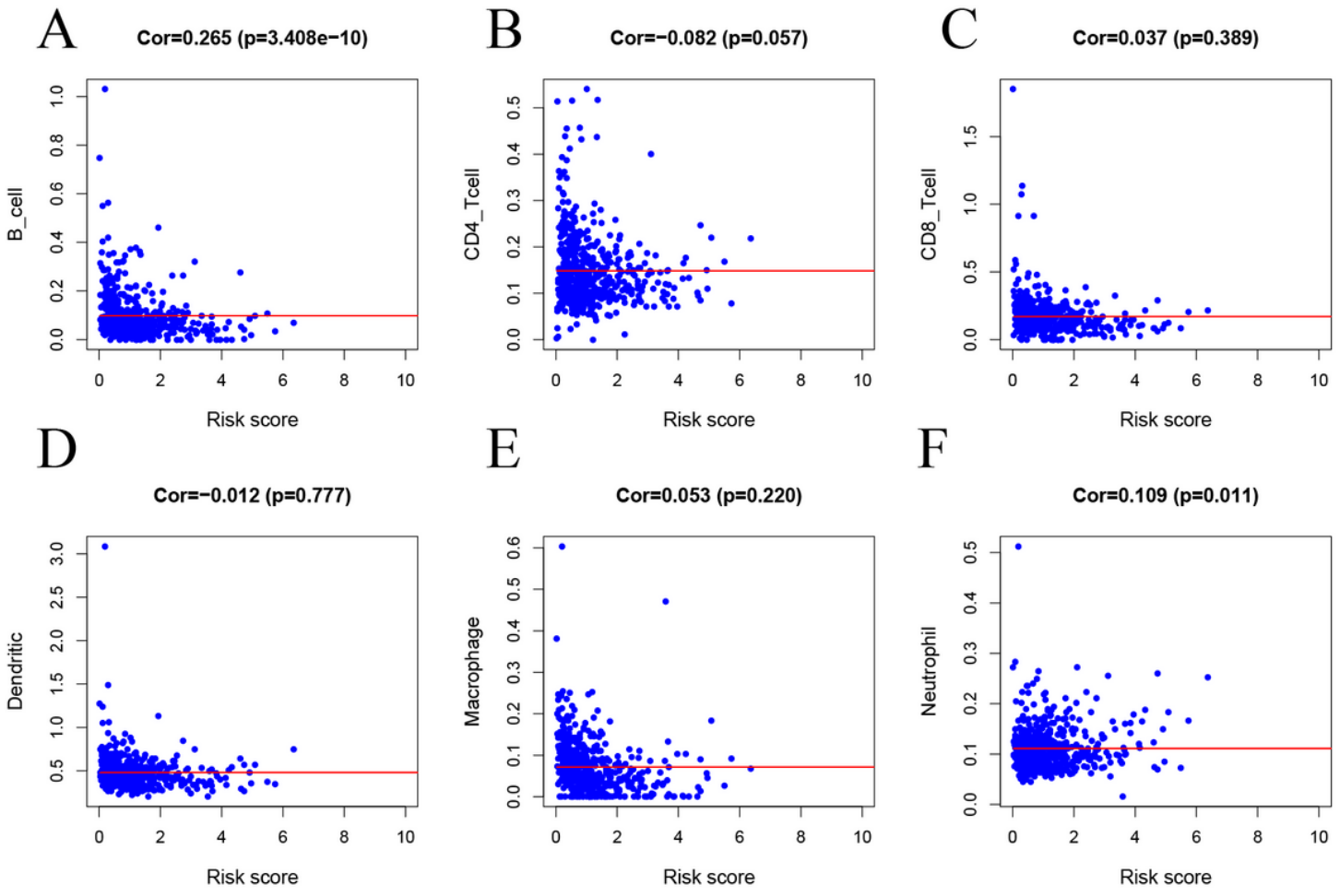

Figure 9

Association between the risk score of the ten-gene prognosis signature and the abundance of 6 immune infiltrates, where (A) B cells and (F) Neutrophils was significantly correlated with the patient's risk score, and were positively correlated.

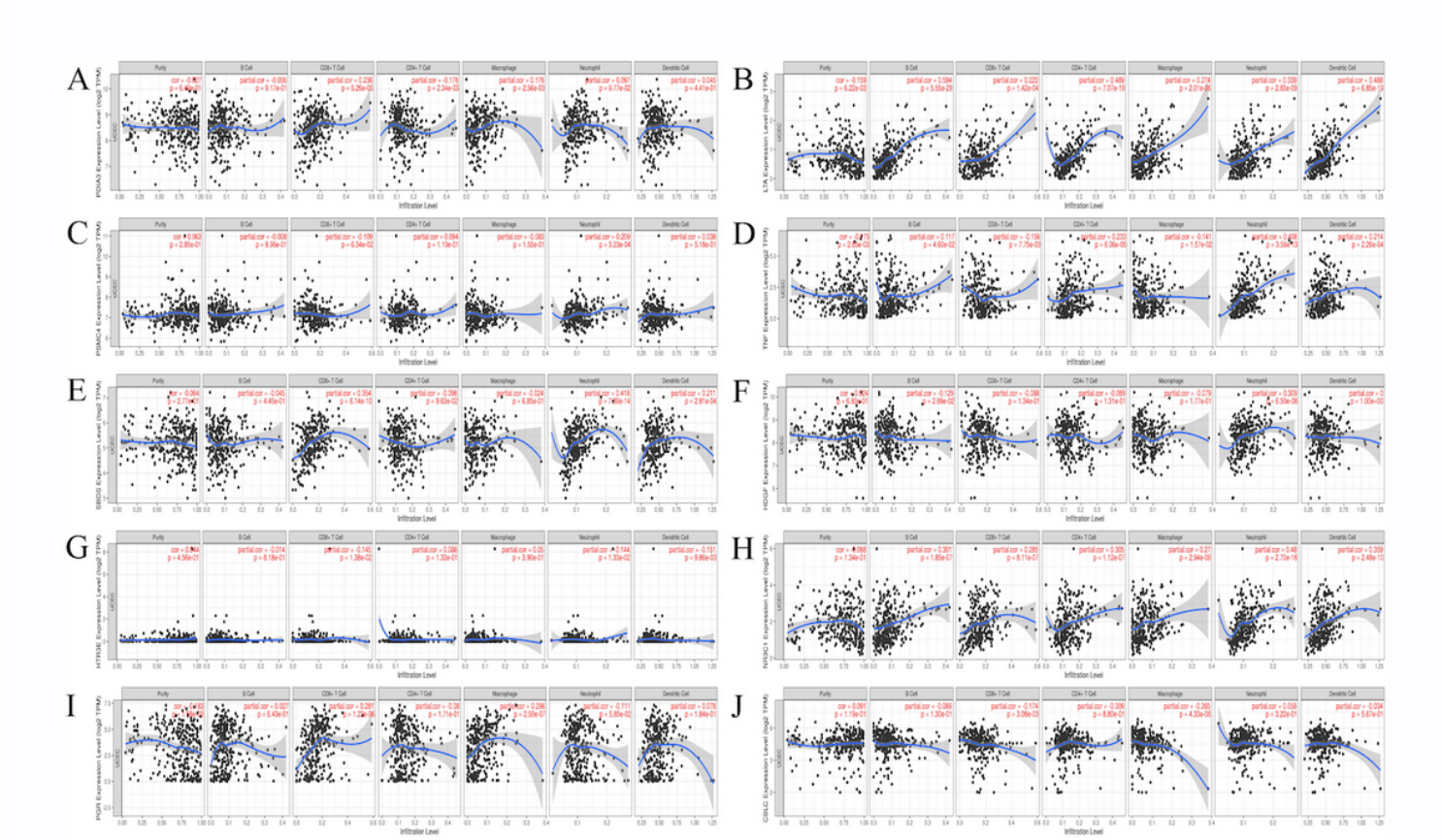

Figure 10

The correlation between (A)PDIA3, (B)LTA, (C)PSMC4, (D)TNF, (E) SBDS, (F)HDGF, (G)HTR3E, (H)NR3C1, (I)PGR, (J)CBLC and the immune infiltration level in UCEC. Abbreviation: UCEC, Uterine corpus endometrial carcinoma