Prognostic Value of Interferon-γ-Related Signature Involved in Tumor Immune Infiltration in Bladder Cancer

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Research Article

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Abstract

Background

The molecular biological characteristics and unique anatomical structure of bladder cancer (BC), become cancer immune reaction mechanism and a predictor of good model and help to improve the level of cancer immunotherapy. Interferon-γ (IFN-γ) plays a key role in activating cellular immunity and stimulating anti-tumor immune responses. However, the role of IFN-γ in BC is unclear. We aimed to clarify the biological occurrence and development of BC and identify reliable biomarkers.

Method

We downloaded data on patients with BC from The Cancer Genome Atlas (TCGA) database and constructed a prognostic model. We analyzed the relationships between clinicopathological features and the IFN-γ signature by univariate and multivariate Cox regression analyses and evaluated the prognostic and predictive values of the IFN-γ signature by survival analysis and nomogram construction. We also conducted Gene Ontology (GO) and Kyoto Encyclopedia of and Genes and Genomes (KEGG) pathway enrichment analyses to explore the potential biological pathways related to the IFN-γ signature in BC. Immune infiltration was evaluated using CIBERSORT algorithms and the correlation between the signature and antineoplastic drug sensitivity was analyzed using the CellMiner platform.

Results

Five genes (*RIPK2*, *RBCK1*, *PTPN6*, *ITGB7*, *LATS2*) were selected to construct IFN-γ-related signatures. The pathological features were identified as an independent risk factor. Patients with BC were divided into high-risk and low-risk groups according to their signatures. Patients with higher risk scores had shorter overall survival and a worse prognosis. GO and KEGG enrichment and CIBERSORT analysis showed significant relationships between the signature and survival, independent risk factors, immune infiltration, and antineoplastic drug sensitivity.

Conclusion

We obtained a risk profile for a regression model consisting of IFN-γ signature genes to predict the prognosis of patients with BC. This model may be used to improve the prognostic accuracy of the immune microenvironment in BC.

Introduction

Bladder cancer (BC) is the fifth most common cancer and the most prevalent urinary tract cancer worldwide, with an estimated 81,400 new cases and 17,980 deaths in the United States in 2020[1]. A

national registry of advanced cancer in Denmark (N = 31,771) reported that BC was associated with higher risks of pain and constipation and a lower quality of life than other cancers[2]. BC can present as non-muscle-invasive bladder cancer (NMIBC), muscle-invasive bladder cancer (MIBC), or as a metastatic form of the disease, of which MIBC (≥ T2) usually progresses to metastasis and has a poor prognosis, with a 5-year survival rate of < 50% [3].

BC is one the most common mutated cancers in humans, second only to lung and skin cancers in terms of mutation rates[4, 5]. Sequencing and gene expression studies have revealed numerous DNA, RNA, and protein biomarkers for BC[6]. In addition, immune checkpoint inhibitors (ICIs) have demonstrated prognostic and therapeutic potential [7, 8] and have thus been approved for the treatment of metastatic BC, leading to renewed interest in the immune components of the tumor microenvironment (TME). Further information on the mechanisms underlying BC biogenesis and development is thus required, and reliable response biomarkers are needed to allow the selection of patients likely to benefit from such treatments.

Interferon-γ (IFN-γ) is the sole member of the type II IFN family discovered nearly 60 years ago. It is encoded by the *IFNG* gene and consists of two polypeptide chains joined in an antiparallel fashion [9]. Wheelock first described IFN- γ as a phytohemagglutinin-induced viral inhibitor produced by leukocyte stimulation[10]. In addition, a recent review reported that IFN-γ was involved in tumor progression and regression[11], with a key role in activating cellular immunity and subsequent anti-tumor immune responses. However, IFN-γ can also lead to immune escape by inhibiting the T cell immune response and can induce programmed death-ligand 1 (PD-L1) and indolamine-2,3-dioxygenase expression and regulate tumor immune-resistance mechanisms. The role of IFN-γ signaling in regulating the immune state and anti-tumor immunity is thus controversial.

Patients with NMIBC have high rates of recurrence and progression[12]. Recent studies found that immunotherapy could help to avoid surgery in patients with high grade NMIBC, and pembrolizumab has been used in patients who have failed second-line therapy or who are intolerant to first-line platinum-based chemotherapy[13]. PD-L1 was induced by typical IFN-γ signaling in clear cell renal cell carcinoma-like cell lines, and a high level of PD-L1 mRNA in tumor tissues was positively correlated with IFN-γ signature and was associated with a beneficial prognosis in renal cell cancer[14]. Changes in Toll-like receptor 4 expression were associated with changes in the expression of key cytokines (transforming growth factor-β, tumor necrosis factor-α, and IFN-γ), which affect tumor progression and metastasis [15]. These results suggested that IFN-related genes may be used to guide the use of ICIs in patients with BC.

We conducted this study to validate the hypothesis that IFN-γ promotes tumor immune infiltration in BC. Using The Cancer Genome Atlas (TCGA) database as a training set, we evaluated the mRNA expression data, clinical information, signaling pathways, and immune infiltration in patients with BC. We also constructed an optimized IFN-γ estimation model, and hypothesized that this could be used as a predictive marker for immunotherapy and as a prognostic biomarker for BC patients. We also verified the drug sensitivity of the model.

Materials And Methods

Patient Data Extraction

Transcriptome profiles and clinical information, including sex, age, clinicopathological characteristics, stage, and survival data for patients with BC were obtained with the HTSeq-FPKM format from TCGA database via the GDC portal (https://portal.gdc.cancer.gov/). Data collected from TCGA database were used as the training set for model construction. Exclusion criteria were patients with incomplete data. Drug sensitivity data were identified by NCI-60 and downloaded from the CellMiner dataset (https://discover.nci.nih.gov/cellminer/).

Construction of IFN-γ Signature Prediction Models

We first constructed a signature prediction estimation model. IFN-related genes were identified from relevant research and prognostic genes were further identified by univariate Cox analysis. Significant genes with a cut-off point of $P < 0.05$ were selected and a stepwise Cox regression model was established. On the basis of the results, we calculated the risk score using the following formula:

$$\mathrm{RiskScore} = \sum_{i=1}^{n} Coef(i) * x(i)$$

Coef(i) and x(i) represent the estimated regression values. Patients were then divided into high and low risk groups according to the median risk score. A Kaplan-Meier curve was plotted using the R package “survival” to compare survival differences between the two groups. A receiver operating characteristic (ROC) curve was drawn using the R package “survivalROC” to assess the predictive effect of the signature on overall survival (OS).

Independent Risk Factor of the IFN-γ Signature

Univariate and multivariate Cox regression analyses were used to determine if the IFN-γ-related signature was a risk factor independent of other clinicopathological information (sex, age, grade, and stage) in TCGA database. Patients were divided into subgroups according to age (> 65 and ≤ 65 years), sex (male and female), grade (G1/2 and G3/4), stage (I/II and III/IV), and risk (high and low risk) according to TCGA database. OS analysis was performed for each subgroup using the R package “survival”.

Nomogram of the IFN-γ-related Signature

We also constructed a nomogram of the most influential prognostic and clinical characteristics of IFN-γ-response genes, such as age and pathological TNM stage, which could be used to calculate the risk of recurrence in an individual patient using the “rms” R package. The nomogram was established based on the results of multivariate Cox proportional hazards analysis, to predict survival recurrence at 1, 3, and 5 years.

Functional Enrichment and Signaling Pathway Analysis

We evaluated immune infiltration in the model by dividing the BC patients in TCGA into high- and low-risk groups according to their IFN-γ-related signature, and applied Gene Ontology (GO) enrichment analysis to identify the related biological processes. The main signaling pathways regulated by the signature were established by KEGG analysis.

Immune Cell Infiltration

We explored cell infiltration among the immune subtypes of BC patients in TCGA database using the immune R package normalized via the “limma” package. CIBERSORT algorithms were used to evaluate immune infiltration. The correlations between target gene expression and immune cell infiltration levels were assessed using Spearman’s test. Differences in infiltration between the high and low risk groups were calculated by Wilcoxon’s rank-sum test and the results were presented using the “vioplot” package.

Antineoplastic Drug Sensitivity of the Model

We downloaded the gene expression file RNA-seq and NCI-60 drug sensitivity file via CellMiner (https://discover.nci.nih.gov/cellminer/), and selected drugs with USA Food and Drug Administration approval to evaluate the relationship between the IFN-γ-related signature and the therapeutic effects of antineoplastic drugs in BC patients.

Statistical Analysis

Statistical analyses were performed using R software 4.0.2. All statistical analyses were two-sided, and a value of $p < 0.05$ was considered significant. The associations between gene expression and clinicopathological data were evaluated using Wilcoxon’s rank sum test and visualized using ggplot2 R package.

Results

Construction of the IFN-γ Response Gene Signature

We selected 24 IFN-γ response genes. The patient characteristics from TCGA are shown in Table 1. We screened out nine IFN-γ response genes by univariate Cox regression analysis (Table 2). We selected genes with $p < 0.05$ and established a stepwise Cox regression model to optimize the signatures. Five genes were subsequently selected to construct IFN-γ-related signatures by stepwise Cox regression: *RIPK2*, *RBCK1*, *PTPN6*, *ITGB7*, and *LATS2*. Among these five genes, *LATS2* was a high-risk factor and *RBCK1*, *PTPN6*, *ITGB7*, and *RIPK2* were low risk factors. The risk score was formulated by the expression levels of the four genes and the Cox coefficient: risk score = -0.1972 × *RIPK2–0*.2682 × *RBCK1–2*.2664 × *PTPN6–0*.6172 × *ITGB7*+ 0.3084 × *LATS2.* BC patients were divided into high risk and low risk groups based on the median risk score. The distribution characteristics and related risk scores of the five genes are shown in Figs. 1 and 2. Kaplan-Meier analysis was applied to evaluate the predictive value of OS in BC patients, and the results showed that OS was better in the low-risk group (Fig. 3A). The ROC curves for

5-year OS showing the prognostic accuracy for the IFN-γ response gene-related signature is shown in Fig. 3B (area under the curve [AUC] = 0.702).

Independence of IFN-γ-related Signature as an Independent Risk Factor

The clinical features of all BC patients were analyzed, including T, N, and M stage, grade, sex, and age (Fig. 4). The relationships between the five IFN-γ response signatures and the pathological features of BC were analyzed by univariate and multivariate Cox analyses according to TCGA database to confirm the independence of IFN-γ signature as a risk factor for BC (Fig. 5A, B). The pathological features age, lymph node (N), and risk score were significantly different in the high-risk group (age < 0.001, hazard ratio [HR] = 1.971; N < 0.001, HR = 2.007; risk sore < 0.001, HR = 1.818). Based on the above data, the risk score was valid and the IFN-γ-related signatures were identified as an independent risk factor.

Construction of Nomogram Predicting the Prognosis of BC patients

To further optimize the prediction model and prove the good predictive effect of the IFN-γ signature, we established a nomogram for the prognosis of BC using the four independent factors (age, grade, stage, risk) that were most significantly related to BC. Univariate and multivariate Cox regression analyses were used to prove that the IFN-γ-related signature was an independent risk factor and had a significant prognostic role in BC patients. The nomogram combining age, sex, stage, and risk score predicted the 1-, 3-, and 5-year survival outcomes for BC patients, and indicated the scores for each risk factor (Fig. 6).

Biological Pathways Related to the IFN-γ-related Signature

We performed edgeR filtration (false discovery rate < 0.05, $|\log_2$fold change > 1|) to further study the potential functions of the IFN-γ-related features by GO and KEGG pathway enrichment analyses. In the Biological Process (BP) category, T cell activation, regulation of T cell activation, skin development, extracellular matrix organization, extracellular structure organization, regulation of T cell activation, antigen processing and presentation of exogenous peptide antigen via MHC class II, antigen processing and presentation of peptide antigen via MHC class II, and antigen processing and presentation of peptide or polysaccharide antigen via MHC class II were all related to the IFN-γ signature. In the Cellular Component (CC) category, the IFN-γ-related signature was highly enriched in collagen-containing extracellular matrix, external side of plasma membrane, and MHC class II protein complex, and the extracellular matrix structural constituent was relatively enriched in the Molecular Function (MF) category (Fig. 7A). KEGG pathway enrichment analysis showed that the IFN-γ-related signature was highly enriched in pathways related to cell adhesion molecules, Epstein-Barr virus infection, rheumatoid arthritis, hematopoietic cell lineage, human T-cell leukemia virus 1 infection, cytokine-cytokine receptor interaction, Th1 and Th2 cell differentiation, and Th17 cell differentiation (Fig. 7B).

Immune Infiltration of the IFN-γ-related Signature

The above results demonstrated that the IFN-γ-related signature was associated with immunity. We then used CIBERSORT to derive and further verify the relationship between the IFN-γ response signatures and immune-infiltration status in all BC patients in the high and low risk groups. According to Wilcoxon's rank-sum test, M0 and M2 macrophages and resting mast cells were positively associated with the risk score ($p < 0.001$), while CD8 T cells, CD4 memory resting T cells, CD4 memory activated T cells, follicular helper T cells, and resting natural killer (NK) cells were negatively correlated with the risk score (Fig. 8).

Antineoplastic Drug Sensitivity of the IFN-γ Signature

Our results suggested that the IFN-γ signature was associated with immune infiltration. We therefore further analyzed the correlation between the signature and antineoplastic drug sensitivity. Sensitivities to alectinib, denileukin diftitox (Ontak), LDK-378, isotretinoin, fluphenazine, estramustine, and irofulven were significantly correlated with the IFN-related *ITGB7* gene signature (Spearman's $\rho = 0.662$, 0.612, 0.579, 0.538, 0.531, 0.510, -0.505, respectively, $p < 0.001$). *LATS2* was also significantly correlated with irofulven sensitivity ($\rho = 0.504$, $p < 0.001$), and hydroxyurea sensitivity was significantly correlated with *PTPN6* ($\rho = 0.499$, $p < 0.001$) (Fig. 9).

Discussion

BC is one of the most common and aggressive malignant diseases. Due to the unique urinary-storage function of the bladder, intravesical instillation was used to treat NMIBC in a BC patient in 1976, thus establishing bacillus Calmette-Guérin (BCG) instillation as the gold standard adjunctive therapy for NMIBC[16], and opening a new chapter in the immunotherapy of BC. BCG instillation and anti-programmed cell death protein 1 (PD-1)/PD-L1 immune-checkpoint blocking have been used successfully to treat early and late BC via different immunotherapeutic approaches [17], thus providing a good model for studying the mechanism of tumor immune response and improving the efficiency of immunotherapy.

The development of high-throughput sequencing and biomolecular technology has facilitated breakthroughs in immunotherapy, making it a promising therapeutic approach for cancers. However, only 25% of advanced/metastatic BCs respond to anti-PD-1/PD-L1 ICIs [18], indicating the need to develop new immunotherapy approaches and predict new biomarkers to fully explore the curative potential of immunotherapy in patients with BC.

IFN-γ stimulates the immune editing of tumor cells and modulates the tumor immune-resistance mechanism, thus promoting tumor progression. Immune activation of IFN-γ in tumor cells can promote lymphocyte migration and inhibit angiogenesis, mainly due to the influence of tumor cells, monocytes, endothelial cells, and fibroblasts, to induce the expression of MHC and secrete CXCL9, CXCL10, and CXCL11 [19–21]. In order to design better therapeutic targets, differentiate immunotherapy populations, and balance the antitumor and immune-escape abilities of BC, we therefore established an IFN-γ signature containing five genes (*RIPK2*, *RBCK1*, *PTPN6*, *ITGB7*, *LATS2*) to assess the prognosis of BC patients.

We explored the efficacy of this signature by combining the five genes and examining the survival and ROC curves, which showed that the IFN-γ signature had good prognostic performance (AUC = 0.702). We then established a nomogram using four independent factors (age, grade, stage, risk) that were most significantly related to the prognosis of BC, which confirmed the good predictive effect of the IFN-γ signature.

The IFN-γ signature-related genes play an important role in immunobiological pathways. For example, T cells are the key cells in cellular immunity [22], with important roles in immune tolerance and immune homeostasis. High infiltration by Treg cells has been associated with poor survival in various types of cancer [23]. M2 macrophages are closely related to the growth and survival of various tumor cells [24, 25], and exhausted T cells in the TME are major targets of immunotherapies in BC [26]. The current results showed that M0 and M2 macrophages and resting mast cells were positively associated with the risk score, suggesting that M0 and M2 macrophages were significantly up-regulated in the high-risk group, while CD8 T cells, CD4 memory resting T cells, CD4 memory activated T cells, follicular helper T cells, and resting NK cells were negatively correlated with risk scores. In addition, GO enrichment analysis showed that, in the BP category, T cell activation, regulation of T cell activation, regulation of T cell activation and presentation of exogenous peptide antigen via MHC class II, antigen processing and presentation of peptide antigen via MHC class II, and antigen processing and presentation of peptide or polysaccharide antigen via MHC class II were related to the IFN-γ-related signature, while the signature was highly enriched in MHC class II protein complex in the CC category. These results were consistent with the analysis of the IFN signature, and further confirmed the effectiveness of the signature and its risk profile for predicting tumor-infiltrating immune cells and guiding the selection of clinical immunotherapies.

Integrin β7 (*ITGB7*) is associated with immune cell infiltration. It is expressed on the surface of leukocytes and plays an important role in the homing of immune cells to intestinal-related lymphoid tissues and facilitating the retention of lymphocytes in the gut epithelium. The role of *ITGB7* expression in promoting tumor progression has also been reported in different types of tumors, such as colorectal cancer, fibrosarcoma, multiple myeloma, pancreatic cancer, and cervical cancer [26–30]. A study of patients with colorectal cancer found a significant reduction in the number of β7 + cells in the tumor tissue compared with the adjacent normal tissue. β7 expression was decreased in tumor-derived CD8 + T cells compared with normal tissue-derived CD8 + T cells. In addition, analysis of bulk RNA expression data from a public platform showed that high *ITGB7* expression was associated with longer patient survival, higher cytotoxic immune cell infiltration, lower somatic copy number alterations, decreased mutation frequencies of *APC* and *TP53*, and a better immunotherapy response[31]. *ITGB7* deficiency reduced the infiltration of activated CD8 + T cells, effector memory CD8 + T cells, IFNγ + CD8 + T cells, IFNγ + NK cells, and CD103 + dendritic cells, and thus accelerated the development and progression of colorectal cancer in $Apc^{min/+}$ spontaneous and MC38 orthotopic models[32]. *ITGB7* downregulation also inhibited focal adhesion kinase and Src phosphorylation in a cell co-culture model of multiple myeloma [28]. In pancreatic cancer, *ITGB7* transcription was shown to be regulated in a reactive oxygen species-related nuclear factor erythroid 2-related factor 2-dependent manner, and *ITGB7* was inhibited by N-acetyl-L-cysteine in

pancreatic cancer cells, thus accelerating the progression of pancreatic cancer[29]. All the above evidence suggests that *ITGB7* may inhibit cancer pathogenesis via maintaining antitumor immunity.

Protein tyrosine phosphatase nonreceptor type 6 (*PTPN6*) is a nonreceptor protein tyrosine phosphatase, which mainly acts as a tumor suppressor through phosphorylation of carcinogenic kinases[32]. *PTPN6* was shown to be associated with the prognosis and progression of gastric cancer and hepatocellular carcinoma[33, 34], and can be used as a prognostic factor in peripheral T cell lymphomas[35]. Recent studies suggested that *PTPN6* was overexpressed in BC tissues and was significantly correlated with grade, T stage, N stage, and low *PTPN6* expression was significantly associated with poorer OS in BC patients. Based on analysis of TCGA database, *PTPN6* may be a new prognostic biomarker of BC [36]. *LATS2* encodes a serine/threonine protein kinase and has been reported to be a member of the *LATS* tumor-suppressor gene family involved in the hippocampus signaling pathway[37]. The above results confirmed that the genes included in our signature were significantly correlated with tumor development and prognosis. In addition, the current drug sensitivity results confirmed the correlation between these genes and antineoplastic drug sensitivity.

In this study, we determined the prognostic value of an IFN-γ signature in BC based on TCGA database. We also discussed the relationship between this IFN-γ signature and immune cell infiltration in the BC microenvironment. However, the study had several limitations. First, the sample size of TCGA database was limited. Second, this was a retrospective study and lacked experimental verification of the findings. Further clinical trials are therefore needed to confirm our observations, and to clarify the mechanism responsible for the prognostic value of the IFN-γ-related signature in BC.

Conclusion

In conclusion, this study investigated the risk characteristics of an IFN-γ signature. We identified a promising model for prognostic risk assessment in BC patients and improved the prognostic accuracy of the immune BC microenvironment. The model could serve as a powerful tool for guiding immunotherapy in BC patients.

Declarations

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Author contributions

Y.L. and M.W. contributed equally to the literature research, drafting, interpretation, and writing of the manuscript. W.Z contributed to the supervision and writing of the manuscript. J.T and X.X. contributed to the literature research of the manuscript. All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Competing Interests

The authors declare no competing financial interests.

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Tables

Table 1. Bladder cancer patient characteristics for TCGA

Characteristics	Variable	Total	Percentages (%)
Age	≤ 65	161	39.36
	65	248	60.34
Gender	Male	303	74.08
	Female	106	25.92
Grade	High	385	94.13
	Low	21	5.14
	Unknown	3	0.73
Stage	StageI	2	0.49
	StageII	130	31.78
	StageIII	139	33.99
	StageIV	136	33.25
	Unknown	2	0.49
T	T0	1	0.24
	T1	3	0.73
	T2	120	29.34
	T3	194	47.43
	T4	59	14.43
	TX	1	0.24
	Unknown	31	7.58
N	N0	237	57.95
	N1	47	11.49
	N2	76	18.58
	N3	8	1.96
	NX	36	8.80
	Unknown	5	1.22
M	M0	194	47.43
	M1	11	2.69
	MX	202	49.39

	Unknown	2	0.49
Survival rate	Survival	251	61.37
	Dead	158	38.63

Table 2. 24 IFN-γ Response Genes ssociated with patients’OS.

gene	HR	z	pvalue
CD69	0.90576	-1.2449	0.213169
CD74	0.903791	-2.24966	0.02447
CD86	0.92398	-0.96258	0.335756
CDKN1A	0.993033	-0.09185	0.926818
CIITA	0.755691	-2.82052	0.004795
CSF2RB	1.054883	0.754494	0.450553
IL10RA	0.919944	-1.00846	0.313235
IRF4	0.738926	-1.9931	0.046251
IRF8	0.95445	-0.59747	0.550196
ITGB7	0.46529	-3.96765	7.26E-05
LATS2	1.307091	2.16621	0.030295
LCP2	0.899599	-1.13229	0.257513
MT2A	1.035004	0.87063	0.383956
NMI	0.834622	-1.82505	0.067993
NOD1	0.903036	-0.54127	0.588323
OAS3	0.875975	-1.81542	0.069459
PFKP	1.143493	1.557691	0.119306
PNP	1.25711	1.954446	0.050648
PTPN6	0.56505	-4.74742	2.06E-06
RBCK1	0.618663	-3.207	0.001341
RIPK2	0.758894	-2.16129	0.030673
SELP	0.992782	-0.08349	0.933466
SOD2	0.936558	-0.87892	0.379443
TRAFD1	0.617443	-3.28903	0.001005

HR,hazard ratio; Z, Z test.

Figures

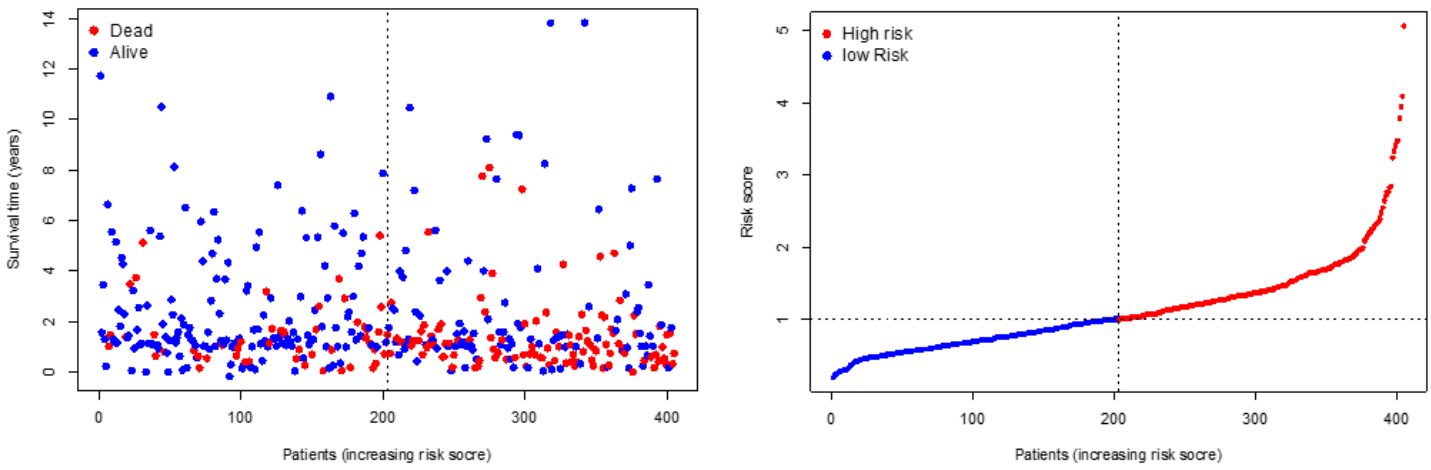

Figure 1

IFN-γ related signatures were constructed from TCGA database. (A, B) Contribution of risk score and survival status.

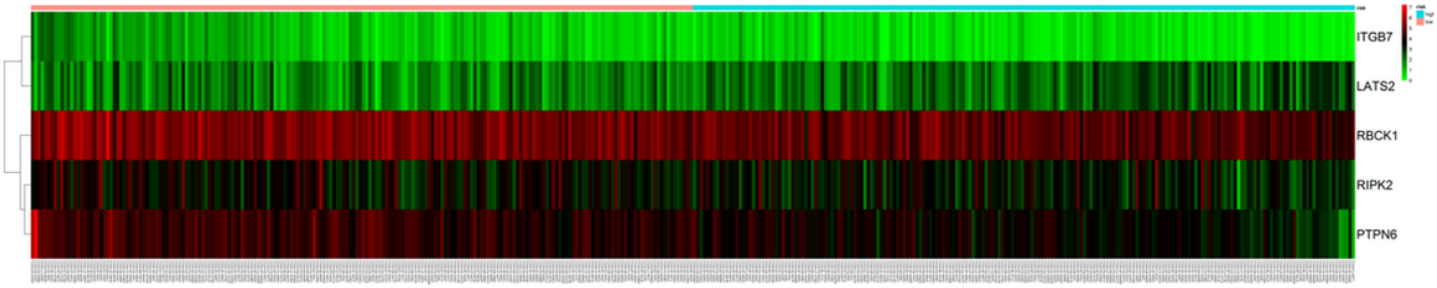

Figure 2

The relationship between survival status and gene expression of the IFN-γ signature. PTPN6 and RIPK2 were highly expressed in high-risk group. ITGB7、LATS2 and RBCK1 were highly expressed in low-risk group.

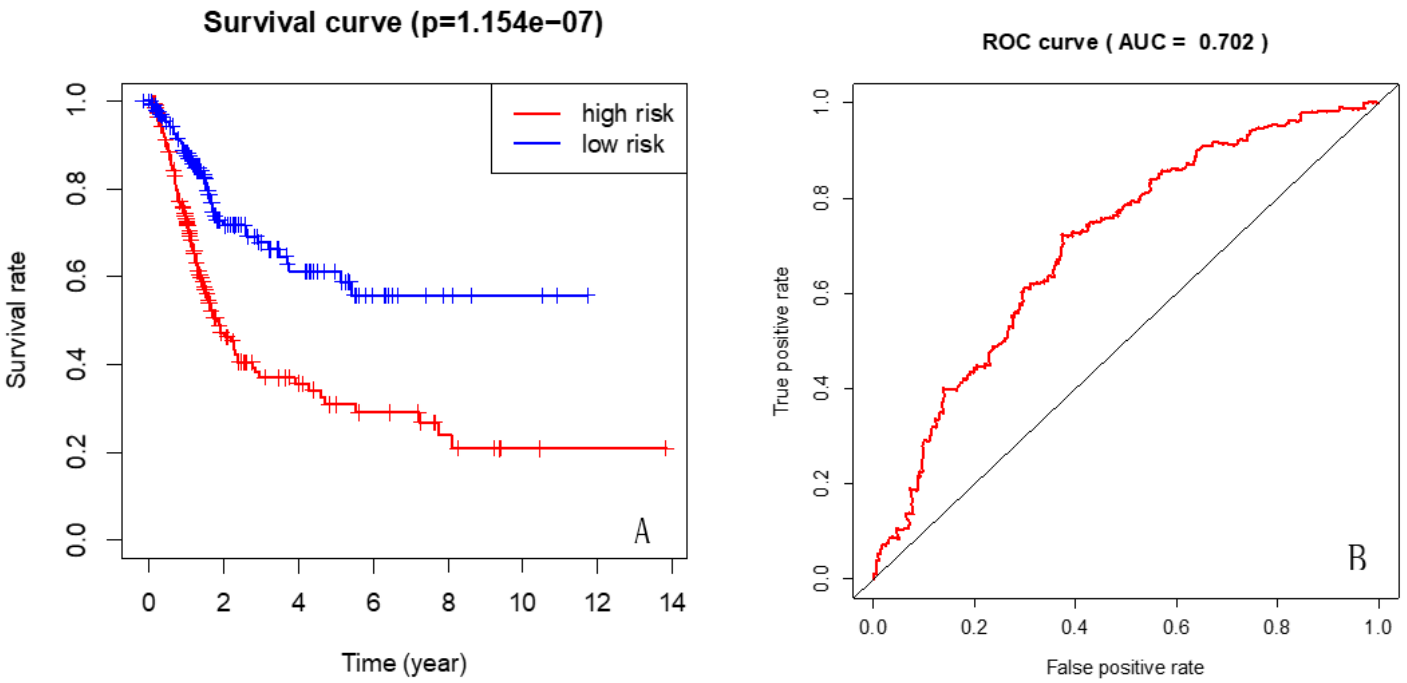

Figure 3

Construction of the IFN-γ related signature by the TCGA database. (A) Kaplan-Meier survival curve of total patients OS for BC patients were divided into high and low risk groups based on IFN-γ related signatures. (B) ROC curve showing the values of the signature for 5-year OS amonAg BC patients

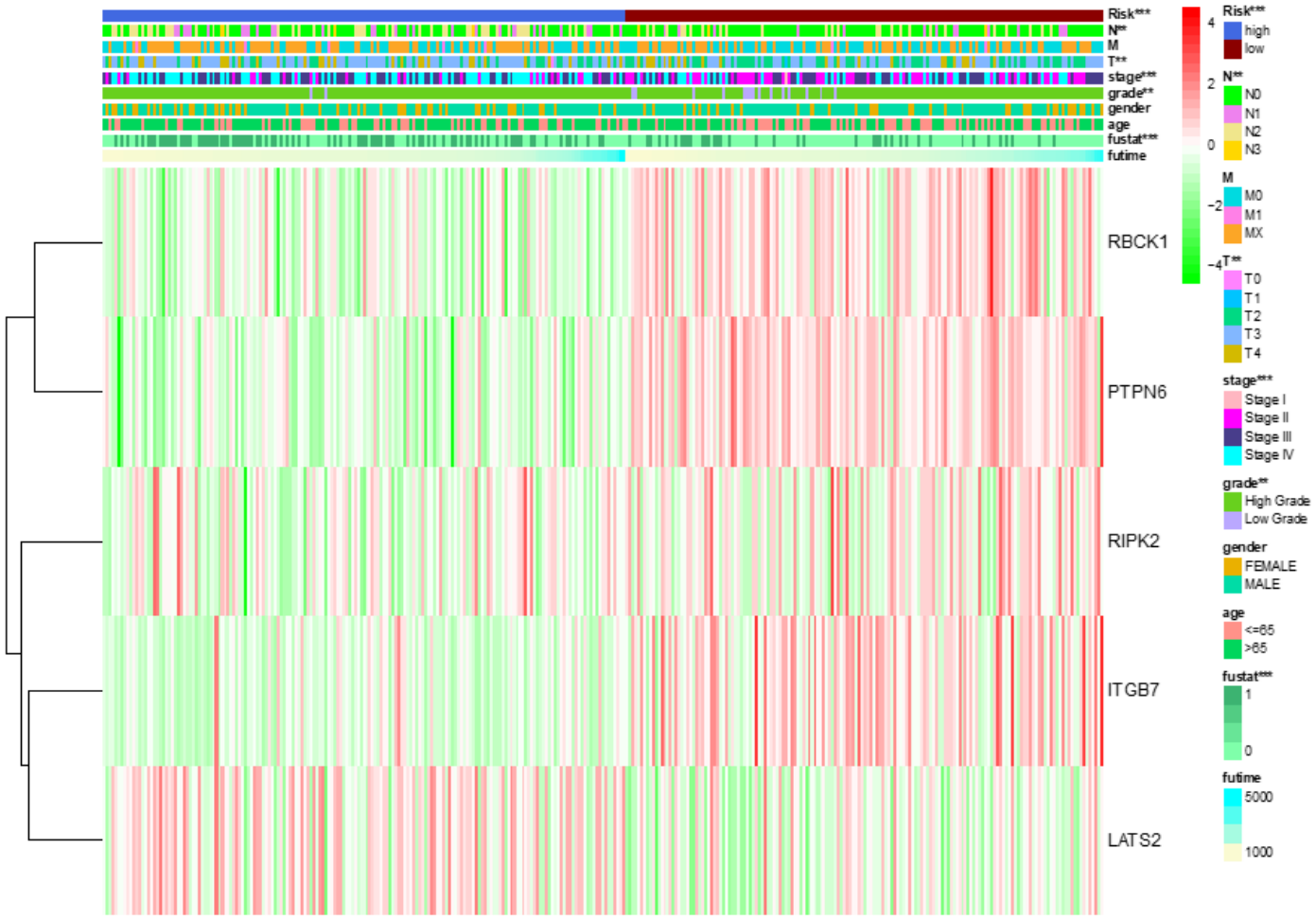

Figure 4

All 5 survival-related genes were significantly higher expressed ($p < 0.001$). The clinicopathological features, which included N, M,T, stage, grade, gender, age, fustat and futime, and the expressions of survival-related genes distributed in the heatmap of 2 defined clusters of BC patients. *$p<0.05$, **$p < 0.01$, ***$p < 0.001$

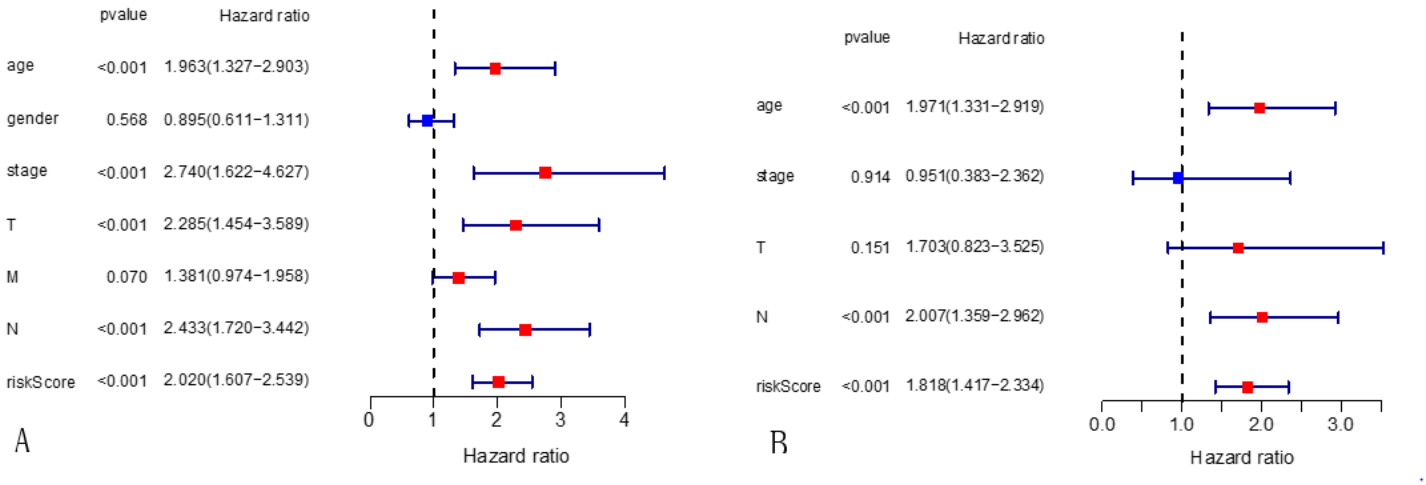

Figure 5

The signature identified as an independent risk factor in BC patients. (A) Univariate regression analysis was used to calculate risk ratio (HR), risk score, and 95% confidence intervals for all clinical characteristics. (B) Multivariate regression analysis calculated risk ratio (HR), risk score, and 95% confidence intervals for all clinical characteristics.

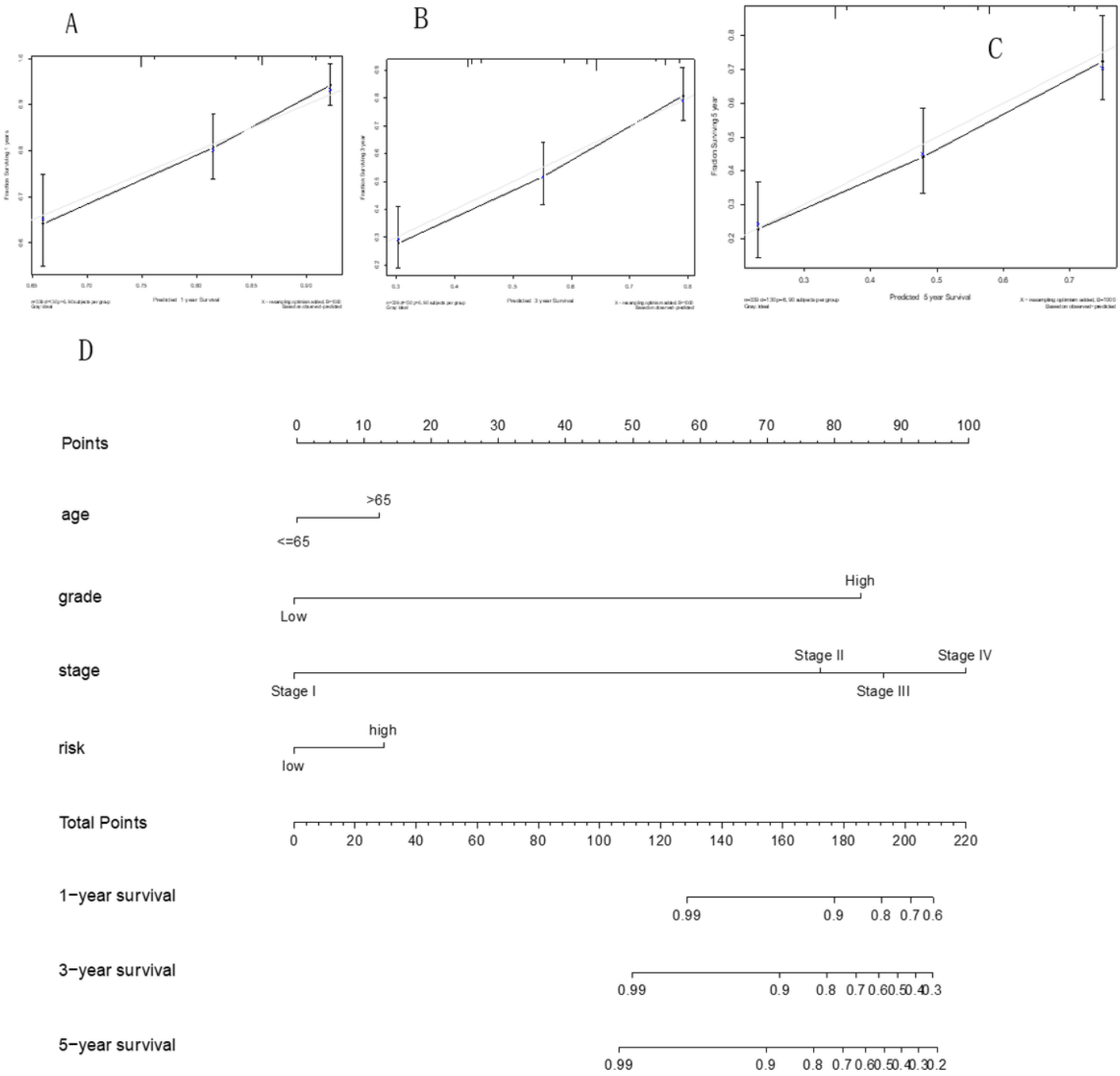

Figure 6

Construction of the nomogram predicting the prognosis of BC. (A) The contrast figure of between fraction surviving 1 years and predicted 1 year survivial with IFN-γ related signatures. (B) The contrast figure of between fraction surviving 3 years and predicted 3 year survivial with IFN-γ related signatures. (C) The contrast figure of between fraction surviving 5 years and predicted 5year survivial with IFN-γ related signatures.(D) The Nomogram shows the clinicopathological features and IFN-γ signatures response of the patients.

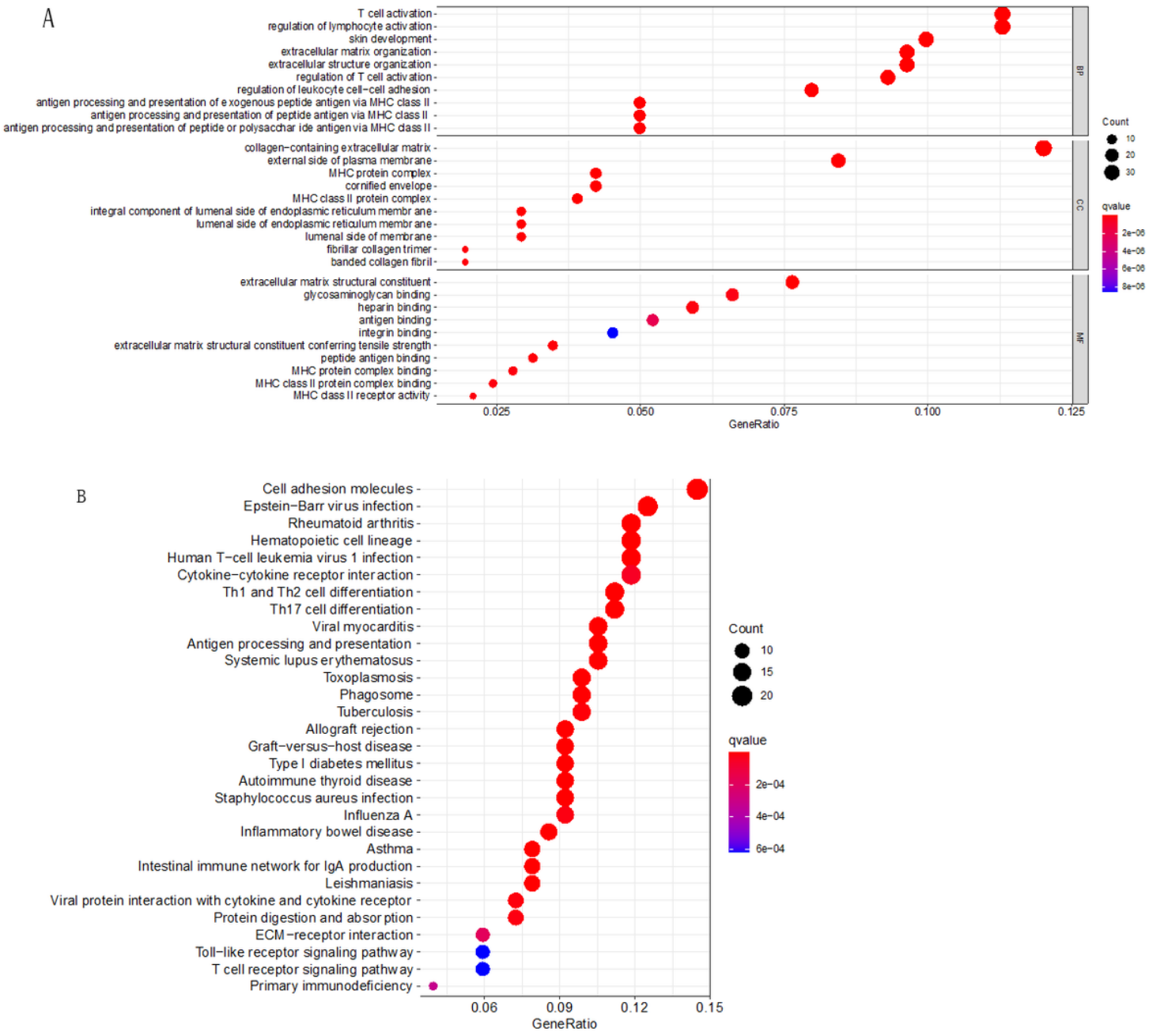

Figure 7

Explore of the Biological Pathway About the IFN-γ-Related Signature (A) GO enrichment analysis of the top 30 corresponding genes. (B) KEGG pathways was enriched in the top 30 corresponding genes.

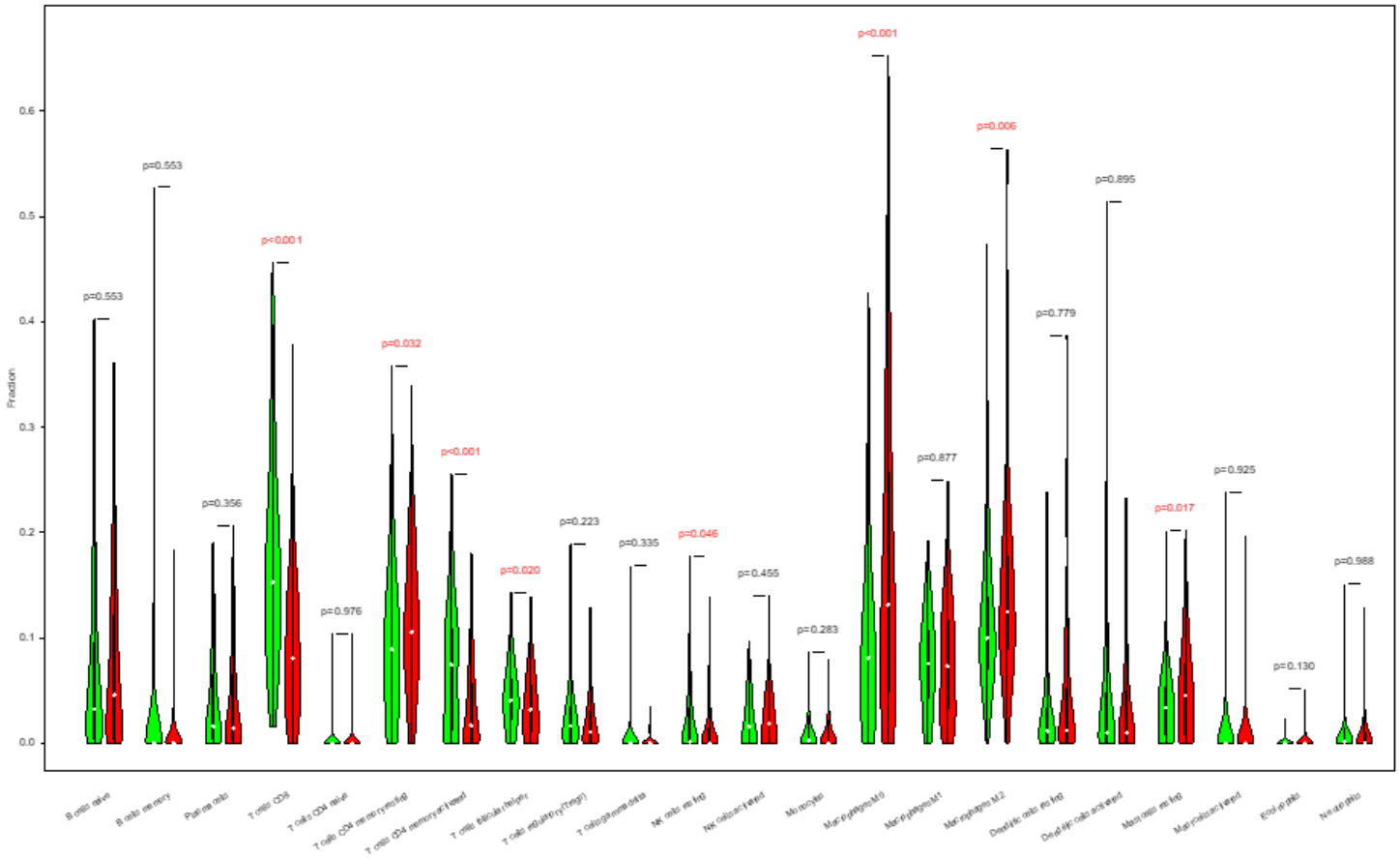

Figure 8

Differences in the abundance of immune cell infiltrates between high-risk and low-risk patients. Red represents high-risk patients. Green is low-risk patients.

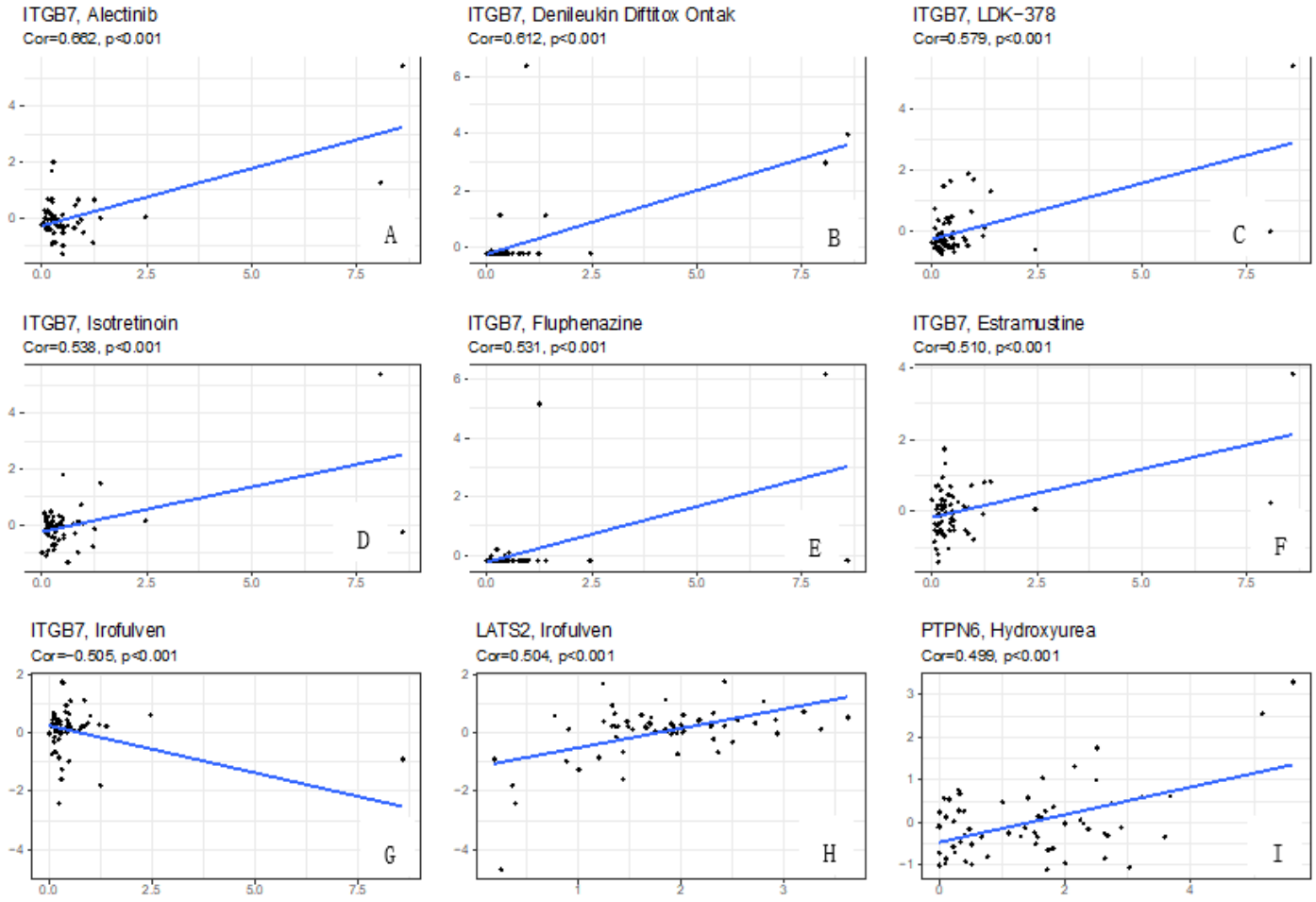

Figure 9

Antineoplastic Drug sensitivity of IFN-γ Signature