Comprehensive Analysis of a 14 Immune-related Gene Pairs Signature to Predict the Prognosis of Gastric Cancer and Immune Features

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Research

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Abstract

Background: As a new method for predicting tumor prognosis, the predictive effect of immune-related gene pairs (IRGPs) has been confirmed in several cancers, but there is no comprehensive analysis of the clinical significance of IRGPs in gastric cancer (GC).

Methods: The clinical and gene expression profile data GC patients were obtained from the GEO database. Based on the ImmPort database, differently expressed immune-related genes (DEIRGs) events were determined by a comparison of GC samples and adjacent normal samples. Cox proportional regression was used to construct an IRGPs signature, and its availability was validated with three external validation sets. In addition, we explored the association between clinical data and immune features and established a nomogram to predict outcomes of GC patients.

Result: A total of 88 DEIRGs were found in GC from the training set, which formed 3828 IRGPs. 14 overall survival (OS)-related IRGPs were used to construct the prognostic signature. As a result, the patients in the high-risk group have a poorer OS compared with the low-risk group. In addition, the fraction of CD8+ T cells, plasma cells, T cells CD4 memory activated, and macrophages M1 were higher in the high-risk group. The expression of two immune checkpoints, CD276 and VTCN1, was significantly higher in the high-risk group. Based on the independent prognostic factors, a nomogram was established and showed excellent performance.

Conclusion: The 14 OS-related IRGPs signature was associated with the OS, immune cells, and immune checkpoints of GC patients, which can provide the basis of related immunotherapy.

Background

From the worldwide epidemiological survey, the incidence and mortality of gastric cancer (GC) rank fifth and third among all cancers, respectively [1, 2]. In recent years, a series of treatments of GC has been continuously optimized, including microsurgery, immunotherapy, and molecular targeted therapy, and the prognosis of patients has been effectively improved [3-5]. However, the five-year survival rate is still lower than 25%, and one of the reasons is that most patients are already in the advanced stage or metastatic status when first diagnosed [6, 7]. Additionally, since patients with the same stage sometimes have a different prognosis, it is essential to understand the mechanism of GC and find relative prognostic predictors to estimate disease prognosis and guide the therapy. It was reported in previous studies that several clinicopathologic factors, genes, microsatellite instability, and noninvasive markers can be used as prognostic factors, but most of them lack clinical practice or have limitations [8-10]. Therefore, effective predictors that can be used for accurate prediction and prognosis improvement of GC patients are highly needed.

Recent studies have shown that immune-related genes (IRGs) are involved in the regulation of the immune system, which plays an important role in the generation and progression of cancers [11, 12]. Increasing pieces of evidence have reported important characteristics of IRGs in the complex regulatory network of cancers, which can be used as biomarkers to predict the prognosis of cancer patients [13]. For instance, Qiu et al. [14] and Yang et al. [15] have found that IRGs were related to the prognosis of GC patients and established an effective prediction model to predict their prognosis. However, with the upgraded data of gene sequencing methods, new sequencing and traditional sequencing require appropriate standardization of gene expression levels, which is a challenge for IRGs-based models currently. Luckily, researchers have proposed new methods to eliminate data processing limitations, such as standardization and scaling based on the relative ranking of gene expression levels, which has produced reliable results in various studies [16-18].

Therefore, in this study, we integrated one of the new methods—immune-related gene pairs (IRGPs) as predictors to comprehensively study their effects on prognosis, immunity, and immune checkpoints of GC patients.

Methods

Data acquisition and processing

Retrospective research and comprehensive analysis of the relationship between IRGPs and the prognosis of GC patients were performed based on public data sets. The clinical data of GC patients and the corresponding gene expression profile data were obtained from the Gene Expression Omnibus (GEO) database (<http://www.ncbi.nlm.nih.gov/geo>). In total, six GC cohorts were found, including GSE13911, GSE118916, GSE84437, GSE13861, GSE26901, and GSE66229. Specifically, GSE13911 and GSE118916 were used to identify differentially expressed immune-related genes (DEIRGs) between GC and normal tissues. GSE84437 set was used to construct a prognostic signature and the remaining three sets were used as validation sets to verify the effectiveness of the IRGPs signature. For training and validation sets, patients without complete survival data will not be included in this study.

Identification of tumor-associated IRGs and enrichment analysis

There are gene expression profiles of both GC tissues and adjacent normal gastric tissues in GSE13911 (T:38, N:31) and GSE118916 (T:15, N:15). In previous studies, comparing biomarkers in different pathological conditions to determine tumor-related biomarkers has been widely used [19]. Thus, we performed the analysis by the GEO2R tool, and those genes with adjusted $P < 0.05$ and $|\text{Log}_2 \text{FC}| > 1$ were defined as differentially expressed genes (DEGs). Then, we downloaded IRGs from the ImmPort database (<https://www.immport.org/shared/>) [20] to identify DEIRGs. After that, the interaction DEIRGs between GSE13911 and GSE118916 were used for later studies.

To initially understand the mechanism and function involved in DEIRGs, these DEIRGs were then incorporated into the enrichment analysis, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways with metasplice [21].

Construction of a prognostic signature based on IRGPs

It was reported that IRGPs have a robust prognostic ability in different cancers [17, 22]. the cohort of GSE84437 ($n=433$) that contain both IRGs and clinical data was used as the training set to form various IRGPs (Supplementary file1). And each IRGP was formed by comparing the gene expression level of a specific sample or profile of IRGs. Specifically, in a pairwise comparison, if the expression of the first element is greater than the latter element, the output is 1. Otherwise, the output is 0. This method of forming IRGPs can avoid the difficulties caused by different gene expression profiles, and does not need to be standardized for personal evaluation. And those IRGPs with a constant value (more than 80% of the samples are assigned the same score on the training set) would be eliminated in the subsequent model construction because they do not provide enough discriminatory information [23].

To identify the prognostic value of IRGPs in GC patients, the univariate Cox analysis was used to identify OS-related IRGPs in the training set, and those with $p < 0.05$ were considered as candidate prognostic IRGPs. Then, based on the candidate IRGPs identified in the univariate Cox analysis, LASSO penalized Cox regression was performed to choose the most significant pairs [24]. Finally, multivariate Cox analysis was used to select the most appropriate OS-related pairs and construct an IRGPs signature. To assess the performance of the IRGPs signature for GC patients, we generated receiver operating characteristic (ROC) curves at 1-, 3-, 5-years [25]. In addition, based on the individual risk score, GC patients were divided into high- and low-risk groups by the median risk score. Then, the Kaplan-Meier (K-M) survival curve was used to compare the differences in OS between the two groups with the log-rank test. Moreover, the subgroup analysis was applied for validation of the signature in different subgroups, including age (≥ 65 , < 65), gender (female, male), N stage (N0-1, N3-4), T stage (T1-3, T4).

Validation of the expression of IRGPs in Oncomine

Oncomine is a cancer microarray database and integrated data-mining platform (<http://www.oncomine.org>) that contains more than 65 gene expression data sets comprised of 48 million gene expression measurements. By using expression data and clinical data from this database, all the IRGs of OS-related IRGs in the prognostic signature were explored to verify their differential expression in GC and normal gastric tissues.

Validation of the IRGPs signature

In order to verify the prognostic value of the IRGPs signature, three validation sets were applied, including GSE13861 (n=65), GSE26901 (n=109), and GSE66229 (n=300). According to the risk score formula in the training cohort, the risk score of each patient in the validation cohort was calculated. Based on the optimal cut off value identified in the training cohort, all patients in the validation cohort were divided into high- and low-risk groups. The K-M survival curves with the log-rank test were used to compare the differences of prognosis between these two groups.

The explosion of immune features of IRGPs in GC

CIBERSORT is a common algorithm to obtain cell composition from volume tumors or gene expression profiles [26], which was used in our study to understanding the enrichment of immune cells in different risk groups. For each sample, CIBERSORT infers the relative proportion of 22 infiltrating immune cells, including T cells, B cells, natural killer cells, macrophages, plasma cells, neutrophils, dendritic cells (DCs) and so on. Additionally, associations between IRGPs and a variety of immune checkpoints were explored. The associations between risk groups and infiltration levels of 22 kinds of immune cells and immune checkpoints were assessed using the Wilcoxon's rank-sum test or Kruskal-Wallis test. A Circos diagram was generated by R software to display the genes in IRGPs on the corresponding chromosomes and visualize their interactions.

Development of a nomogram based on the immune and clinical prognostic factors

In order to improve the clinical utilization of IRGPs signature for GC patients, we conducted the univariate Cox analysis based on risk score and clinical variables in the training set, including age, sex, T stage, and N stage. As for the N stage, it was found that the prognosis of GC patients with N0 stage was significantly better than other stage patients in previous studies, and the prognosis of N1, N2, and N3 differed less [27]. Therefore, we divided the N stage into N0 and N1-3 groups. Then, the variables with $p < 0.05$ were included in the multivariate Cox analysis to identify the independent OS-related factors. Based on independent OS-related factors, a nomogram was developed by rms package. The concordance index (C-index), the calibration curve, and decision curve analyses (DCA) were used to evaluate the performance of the nomogram.

Statistical analysis

All statistical analyses and graphics in our study were performed using R software (version 3.6.1). The student's t-test or Wilcoxon rank-sum test was used to comparing the mean standard error of the mean of continuous variables among groups. The Cox proportional hazards regression model was used for univariate and multivariate survival analysis. The gene model was conducted with multivariate Cox analysis, and the ROC curves were conducted by "survival ROC". A p-value of less than 0.05 (two sides) was considered as statistical significance.

Results

Identification of DEIRGs and relative enrichment analysis

To better find IRGs specifically related to GC, we analyzed the difference in gene expression between normal tissues and tumor tissues. Totally, there are 303 DEIRGs in the GSE13911 set, including 136 upregulated and 167 downregulated

(Supplementary file2). Meanwhile, 147 DEIRGs were confirmed in the GSE118916 set, including 91 upregulated and 56 downregulated (Supplementary file3). After the intersection of these two DEIRGs sets, a total of 88 IRGs were confirmed, which included 47 upregulated and 41 downregulated IRGs (Supplementary file4 and Supplementary Fig. 1). Interestingly, among the 88 interaction IRGs, some were also differentially expressed in colorectal cancer and ovarian cancer as previously reported [18, 28]. For example, the IRGs of MMP12, PLAU, ILIR2, and NR3C2 were associated with GC and ovarian cancer, while CCL8, MMP9, GAL, PLAU, BIRC5, and LIF were related to both GC and colorectal cancer. The comparison indicated the role of these IRGs in tumorigenesis, which should be paid more attention in later research and experiments.

The results of GO analysis were illustrated in Fig. 1A, which showed that specific GO categories were significantly related to DEIRGs, like leukocyte migration, receptor ligand activity, and positive regulation of MAPK cascade. In addition, some KEGG pathways related to GC development were enriched (Fig. 1B), including neutrophil mediated immunity, neutrophil activation, and regulation of inflammatory response. In a word, the enrichment analysis suggested DEIRGs play an immune role in GC, which helped reveal the potential modification mechanisms of protein function.

Development and validation of the prognostic IRGPs signature

The gene expression profiles of 88 DEIRGs were obtained from the set of GSE84437, which was used to establish an IRGPs signature and the IRGPs-clinicopathologic nomogram. 1546 IRGPs were retained and selected as the initial candidate IRGPs after eliminated those with a constant value (Supplementary file5). With the univariate Cox analysis, 234 OS-related IRGPs were identified (Supplementary file6), and the 20 most significant IRGPs related to the prognosis of GC are shown in Supplementary Fig. 2A, including 12 negative and 8 positive pairs. Then, we performed Lasso Cox proportional hazards regression on the training set and selected 27 IRGPs (Supplementary file7 and Supplementary Fig. 2B-2C), which were included in the multivariate Cox analysis. Finally, 14 IRGPs were selected to construct a prognostic signature (Table 1). As shown in the coefficients in the table, three IRGPs were positively correlated with patient prognosis, while eleven were negatively correlated.

Table 1 The information of IRGPs signature

	Immune processes	IRG 2	Immune processes	Coe	P value
CHGA	Cytokines	INBHA	Cytokines	0.374	0.065
CHGB	Cytokines	OSMR	Cytokine Receptors	-0.541	0.002
CXCL14	Antimicrobials	CXCL6	Antimicrobials	-0.605	0.000
CXCL9	Antimicrobials	GREM1	Cytokines	-0.401	0.030
F2RL1	Antimicrobials	TNFRSF12A	Cytokine Receptors	-0.394	0.010
FAM3B	Cytokines	GREM1	Cytokines	-0.572	0.007
FAM3B	Cytokines	TLR3	Antimicrobials	0.642	0.005
FCER1G	NaturalKiller_Cell_Cytotoxicity	THBS1	Antigen_Processing_and_Presentation	-0.709	0.003
GAL	Cytokines	PLA2G2A	Antimicrobials	0.432	0.006
INHBA	Cytokines	NRG4	Cytokines	-0.449	0.006
MMP12	Antimicrobials	OLR1	Antimicrobials	-0.440	0.005
MMP9	Antimicrobials	TNFRSF12A	Cytokine Receptors	-0.280	0.057
OASL	Antimicrobials	PGC	Antimicrobials	-0.541	0.006
REG1A	Cytokines	SPP1	Cytokines	-0.510	0.003

Then, based on the prognostic model, the risk score of each GC patients was calculated, and all patients were stratified into high- and low- risk groups according to the median of the risk score. As a result, the patients in the high- risk group have an extremely poorer OS compared with the low-risk group ($P=0.0001$) (Fig. 2A), and the specific risk score and survival status of each patient were shown in Fig. 2C-2D. The corresponding time-dependent area under the curves (AUCs) of ROC of 1-, 2-, and 3-years for OS were 0.781, 0.767, and 0.776, respectively, which means that the IRGPs signature can serve as a useful prognostic predictor for GC patients (Fig. 2B).

Validation of the expression of IRGs in Oncomine

In this study, 14 IRGPs were incorporated into the final OS signature, including 24 IRGs. The expressions of these IRGs in GC and normal gastric tissues were downloaded from the Oncomine database. As the result, there were 21 IRGs showed significantly different expression levels between GC and normal tissues (except for FAM3B, TNFRSF12A, and CHGA), which indicated the important role of these IRGs in the development of GC and guaranteed the accuracy of results (Supplementary Fig. 4).

External validation of the IRGPs signature

To study whether the IRGPs signature has similar prognostic value in different data sets, we applied the risk score at three different cohorts (GSE13861, GSE26901, and GSE66229) as external validation sets. The patients in each validation set were divided into high- and low-risk groups by the optimal cut off identified in the training set, and the survival time was compared. Similar to the results of the training set, patients in the high-risk group have a shorter OS than those in the low-risk group ($P=0.05$) (Fig. 3). More interestingly, we also find that patients in the high-risk group have worse relapse-free survival (RFS) than low-risk patients.

Potential mechanisms of IRGPs in GC

It was obvious that IRGPs was closely associated with the prognosis of GC patients, to further explore the potential mechanisms, we analyzed the expression of immune checkpoints and the distribution of immune cells in the two risk groups. As shown in Fig. 4B, the expression of five immune checkpoints were significantly different between the two groups. Among them, the expression of CD276 (B7-H3) and VTCN1 in the high-risk group was significantly higher than that in the low-risk group, while the expression of PD-1, PD-L1, and CTLA-4 was reversed. This result indicated that the high expression of CD276 and VTCN1 may promote the expression of IRGs, leading to a poor prognosis, which is consistent with previous studies [29, 30]. At the same time, it was shown that the fraction of CD8+ T cells, plasma cells, T cells CD4 memory activated, and macrophages M1 were significantly higher in the high-risk group than low-risk group (Fig. 4A). Among the 14 IRGPs, the risk score of 1 in GAL|PLA2G2A, CHGA|INHBA, and FAM3B|TLR3 was associated with high risk in GC, which suggested their potentially harmful role in GC patients' prognosis. And the remaining were opposite (Fig. 4C). At the same time, the N2-3 stage and T3-4 stage were enriched in the high-risk group, but there were no differences in sex and age between these two groups.

To understand the detailed relationship of risk score with immune microenvironment and clinical features. We found that a high level of CHGA|INHBA, CXCL9|GREM1, CXCL14|CXCL6, and INHBA|NRG4 was associated with a high level of B cells memory and low level of T cells CD4 memory activated (Supplementary Fig. 5A-5D). Then, it was shown that the risk score was higher in T3-4 and N1-2, which suggested the negative influence of risk score in the progress of GC (Supplementary Fig. 5E-5F).

To further explore the interaction between the related IRGs, a Circos diagram was developed, and the location of each IRG is shown in Fig. 4D. Although there were complex and diverse interrelationships between these genes, no link was found in each IRGP. Hence, it was more accurate to analyze the role of IRGPs in prognosis.

Construction of an IRGPs-clinical nomogram

Several clinical variables were found to be correlated with GC patients' prognosis like tumor site, tumor size, and TNM stage [31, 32]. Therefore, to predict the prognosis of GC patients more accurately, we used clinical variables and the risk score of IRGPs signature to establish a nomogram in the training set. The results indicated that age, T stage, N stage, and risk score were independently associated with OS (Fig. 5A). Then, a prognostic nomogram was developed based on these independent factors (Fig. 5B). The C-index for the OS nomogram was 0.719 (95% CI: 0.686 to 0.752), and the DCA and calibration curves of this nomogram for 1-, 2-, and 3-years were also established (Fig. 5C-F), which showed high credibility.

Discussion

GC is a highly malignant tumor, and its aggressiveness and high recurrence rate make the prognosis of GC patients still a challenge. Sensitive and reliable prognostic biomarkers are needed to identify those patients who have a poor prognosis and can benefit from the treatment. Although many gene-based prognostic signatures have been found to predict the prognosis of patients [33-35], their infeasibility and difficulty in measurement have led to lower clinical applicability. In the present study, a prognostic signature based on 14 IRGPs was developed and successfully validated in three independent datasets. Further research suggested that the differences between immune cells and immune checkpoints in different risk groups so that we supposed them to have predictive effects and to be used as new therapeutic targets. Finally, an IRGPs-clinical nomogram was established and showed an accurate predictive ability of the OS for GC patients.

Last year, Jiang et al [36] comprehensively studied the relationship between the prognosis of GC and IRGs and their corresponding immunological characteristics. Considering the technical deviations caused by different sequencing platforms, the previous prognostic model requires the standardization of expression profiles, which makes data analysis more complex. However, the prognostic signature in our study was based on gene pairs, not just on genes. This is a new

method of data analysis, which does not need to consider the technical deviation of different platforms [37], nor does it require scaling and normalization, but by pairwise comparison of gene expression values. This method was revealed to be reliable in colon cancer [38]. In the current study, IRGPs not only were used as a whole to predict the prognosis of GC patients but can simplify the detection procedure to some extent. For example, both CXCL9 and GREM1 are upregulated in GC. The increased level of CXCL9 is correlated with advanced disease stage and the recurrence of GC patients [39, 40], and GREM1 expression has been experimentally confirmed to be significantly associated with tumor growth and lymphatic metastasis of GC, leading poor survival [41]. These two genes form an IRGP in our study, and when the expression level of the former is lower than that of the latter, a high-risk score can be obtained resulting in a poor prognosis. The result only needs a simple test to compare the expression level, no specific expression data, and its feasibility is better than the traditional detection of IRGs.

IRGs were proved to have a positive correlation with some immune cells, which can affect the prognosis of patients [42, 43]. Thus, we studied the difference of immune cells distribution in high- and low-risk groups of the IRGPs signature. From the results, the infiltration level of plasma cells, T cells CD8, T cells CD memory activated, and macrophages M1 was significantly higher in the low-risk group, indicating that the immune response in tumor patients plays a protective role. CD4 + T cells can recognize tumor antigens and activate CD8 + T cells, thereby producing cytotoxic effects on tumor cells [44]. In previous studies, it was also found that the activation of T cells is positively correlated with the prognosis of GC patients [45, 46]. Plasma cells are a class of cells that can produce antibodies, and their number is significantly less in gastric cancer tissues compared to non-cancerous gastric mucosa, which suggested their inhibition role in tumor development [47]. Also consistent with our results, high infiltration of M1 macrophages in the tumor is associated with an increase in the patient's overall survival rate, while the effect of M2 macrophages is reversed [48, 49].

In our research, we also explored the immune checkpoints' role in risk groups. It was obvious that the expression of PD-1, PD-L1, and CTLA-4 was significantly higher in the low-risk group, which means that their high expression may improve patient prognosis. Immune checkpoints are the main targets of immunotherapy, which not only affect the tumor immune cell infiltration but also are related to the prognosis of patients. Previous studies reported that high levels of PD-L1 and CTLA-4 prolonged survival time of GC patients, which was associated with the increased infiltrating density of immune cells [50, 51]. However, good results for patients with increased expression of these immunosuppressive molecules are counterintuitive and contrary to the results reported by many malignancies [52, 53]. Therefore, we hypothesized that the upregulation of these immunomodulatory molecules may reflect an effect of inducing immune activation of immune cells. When the immune system is activated, it will still attack the tumor and show a survival advantage and non-activated immunity.

The nomogram is a practical and intuitive evaluation tool, which has been widely used in previous studies [54-56]. Clinical factors we included have been used as risk factors in other studies [57-59], indicating that the prognostic value of these characteristics has been widely recognized. After univariate and multivariate Cox regression analysis, we found that the risk score of IRGPs was also an independent risk factor for the prognosis of GC. Therefore, incorporating it into the establishment of the nomogram can more accurately predict the prognosis of patients, and DCA and calibration curves showed the effectiveness of this nomogram. Although there have been previous nomograms combining clinical variables and genomes like mRNAs and specific genes to predict the prognosis of GC [60, 61], this is the first study to combine IRGPs signature to predict the prognosis of GC patients, which provides new ideas for the nomogram study.

There are still several limitations to our study. On the one hand, although we applied different external data sets for verification, it was still a retrospective study that lacks long follow-up and corresponding clinical trials. On the other hand, guidelines of ASCO recommended evaluating all clinical-pathological risk factors as much as possible in the prognostic model to improve the risk prediction of patients [62]. Unfortunately, due to the limitations of public databases, only a fraction of clinical variables were available, and other variables that may affect the prognosis of patients were not

included, such as M stage and treatment status. Therefore, it is necessary to obtain more factors to construct a more accurate nomogram.

Conclusion

In summary, we comprehensively analyzed the prognostic role of the IRGPs signature, which could provide an effective method to assess the risk of GC patients. Besides, to understand the potential mechanisms and find the related targets of immunotherapy, the value of immune cells and immune checkpoints in different risk groups based on the IRGPs signature was explored. Finally, combined with the independent clinical factors, a nomogram was developed to accurately predict the prognosis, which will help in more effective treatment and management.

Abbreviations

IRGPs: immune-related gene pairs; GC: gastric cancer; DEIRGs: differently expressed immune-related genes; OS: overall survival; IRGs: immune-related genes; GEO: Gene Expression Omnibus; DEGs: differentially expressed genes; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; ROC: receiver operating characteristic; K-M: Kaplan-Meier; DCs: dendritic cells; C-index: concordance index; DCA: decision curve analyses; AUCs: area under the curves; RFS: relapse-free survival;

Declarations

Ethics approval and consent to participate

The research didn't involve animal experiments and human specimens, no ethics related issues.

Consent for publication

Not applicable.

Availability of data and materials

The data of this study are from GEO and ImmPort database.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

C L, B C, and CL Z conceived of and designed the study. C L, ZH H and C H performed literature search. ZH H and C H analyzed the data. B C ZH H and LQ J generated the figures and table. C L wrote the manuscript and CL Z critically reviewed the manuscript. CL Z and C H supervised the research. All authors have read and approved the manuscript.

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Figures

Figure 1

Establishment of the prognostic model for OS based on IRGPs. (A) Kaplan-Meier of the patients in the OS-prognostic signature; (B) time-dependent ROC curve analysis of the OS-prognostic signature; (C) risk score distribution and survival status scatter plots of GC patients.

Figure 2

Kaplan-Meier curves of OS and RFS among independent validation cohorts. (A, D) GSE13861; (B, E) GSE26901; (C, F) GSE66229.

Figure 3

The distribution of IRGPs that significantly associated with immune cells and immune checkpoints. (A) the differences among immune cells in high- and low-groups; (B) the differences among immune checkpoints in high- and low-groups; (C) the differences among IRGPs in high- and low-groups; (D) the annotated Circos diagram of IRGs. The outer circle indicates the position of IRGs in the chromosome. The inner circle represents the potential interaction between IRGs.

Figure 4

Development of an IRGPs-clinical nomogram. (A) univariate and multivariate Cox regression analysis of clinical factors. (B) a nomogram for the prediction of the OS of GC patients at 1-, 2-, and 3-year; (C) calibration curves of the nomogram; (D-F) DCA of the nomogram for 1-, 2-, and 3-years in the GC cohort.

Figure 5

Subgroup analysis of the nomogram in GC patients. (A) Age; (B) N stage; (C) Sex; (D) T stage.

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