Comprehensive Analysis of Inflammation Response Related Genes and Immune Infiltrates in Osteosarcoma

Weilong Xu
Guangzhou University of Chinese Medicine
Wei Niu (niuwei@139.com)
Guangdong Hospital of Traditional Chinese Medicine

Research article

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Abstract

Background:

Osteosarcoma is one of the most common bone malignant tumors in children and young adults. Inflammatory response in the microenvironment which acts as active cross-talk signals between host and tumor may play a vital role in osteosarcoma. In present study, bioinformatics algorithms were applied to establish inflammatory response-related genes (IRG) signature to improve prognosis prediction in osteosarcoma.

Methods:

Clinical and mRNA expression profiles data of osteosarcoma patients were collected via Gene Expression Omnibus (GEO) and Therapeutically Applicable Research to Generate Effective Treatments (TARGET) databases. Prognostic values of IRG were evaluated via univariate and LASSO Cox regression analysis and combined to construct risk siganture. Relationship between immune cell infiltration and signature was investigated in present study. Single-sample gene set enrichment analysis was implemented to calculate immune related pathway activity and immune cell infiltration score.

Results:

We found 17 IRG were correlated with overall survival (OS). From LASSO Cox regression analyses, 11 IRG were identified as candidate genes to combine into risk score formulas. Patients were divided into high and low risk subgroups. patients in low-risk subgroup had a significantly better OS than patients in high-risk according to Kaplan-Meier curve result. In addition, gene set variation analysis of risk stratification may explain the different survival. Immune infiltration result demonstrated that high risk subgroups had lower levels of key antitumor infiltrating immune cells and antitumor immunity.

Conclusion:

The present study established IRG signature to act as a robust predictor of prognosis and a novel therapeutic target for treatment in osteosarcoma.

Introduction

Osteosarcoma is a frequent bone malignant tumor in adolescents. Despite the fact that bone malignant tumors are so prevalent, treatments are still far from satisfactory. High recurrence and metastasis rates are characteristics and leading mortality causes of osteosarcoma patients [1]. During the last four decades, empirical treatment intensification fails to improve osteosarcoma patients' survival rates substantially [2]. Effects of treatment options are limited mainly because of the paucity of effective therapeutics [3]. Immune inflammatory response system and its microenvironment are supposed to be taken into consideration when discussing osteosarcoma therapy [4]. Constant development of robust prognostic biomarkers and risk stratification approach are of high clinical importance.

Inflammation is ancient process which could mediate immunity and promote homeostasis. Inflammatory cells and cytokines are tended to contribute to tumor immunosuppression, progression and growth rather than their effective host antitumor response [5]. Cancer-related inflammation is associated with malignant conversion, tumorigenesis and antitumor immunity of solid cancers [6]. Inflammatory pathways may indicate a pivotal target to improve the efficacy of oncotherapy [7]. And these related studies include specific inhibition investigation of cytokines (IL-1β with canakinumab) to minimize risk [8] and inflammation inhibition study of non-steroidal anti-inflammatory drugs to reduce mortality in many tumors [9]. Inflammatory stress in tumor is reported to result in epigenetic alterations to promote neoplastic transformation [10]. Pro-tumorigenic inflammation may direct tumor-promoting signals and block anti-tumor immunity to promote cancer progress towards more tumor-permissive state [11]. In consideration of relationship between cancer development and inflammation, the prognostic values of inflammatory biomarkers are widely recommended in prognosis evaluation of several tumors [12]. Inflammatory biomarkers also showed significant prognostic ability in osteosarcoma. For example, systemic inflammatory biomarkers low PNI and high SII are proved to be independent prognostic biomarkers for overall survival in osteosarcoma [13]. However, inflammatory biomarker investigation of osteosarcoma is rarely reported and required for further investigantion.

In present study, bioinformatics algorithms were used to establish inflammatory response signature. Extential cohort was applied to validate the stability and reliability of the signature. Gene set variation analysis was carried out to investigate potential mechanism. Relationship between immune infiltrate and function types and signature was analyzed. The present study provided further insight into the therapeutic and prognostic role of inflammatory response.

Method

Data Acquisition, Processing, and Screening

Clinical and gene expression information of osteosarcoma patients were obtained from Therapeutically Applicable Research to Generate Effective Treatments (TARGET) database. Sample information lacking survival data was excluded. And 84 osteosarcoma samples were remained for further analysis. We downloaded GSE21257 dataset from Gene Expression Omnibus (GEO) database which contained 53 osteosarcoma samples. The platform of GSE21257 was GPL10295 platform (Illumina human-6 v2.0 expression beadchip). The expression and clinical data from TARGET and GEO database were both public and complied with data access policy and publication guidelines of database. Inflammatory response-related genes (IRG) list was collected from Molecular Signatures database [14]. IRG expression of TARGET was extracted as the modeling subgroup. And IRG expression of GSE21257 was extracted as the validation subgroup.

Establishment and Validation of Prognostic signature

IRG with prognostic value were identified via univariate Cox analysis. Then prognostic IRG signature was constructed from Least absolute shrinkage and selection operator (LASSO)-penalized Cox regression

analysis with “glmnet” R package. LASSO-penalized Cox regression analysis was variables selection algorithm which could minimize the risk of overfitting [15]. Prognostic scores of each osteosarcoma patient were calculated as previously described [14]. And these osteosarcoma patients were stratified into high and low risk subgroups according to median prognostic score value. Principal components analysis (PCA) and t-Stochastic Neighbor Embedding (t-SNE) analysis were utilized to investigate distribution of high and low risk subgroups via “Rtsne” and “ggplot2” R packages. The “survminer” R package was used to perform survival analysis between different risk subgroups. time-dependent receiver operating characteristic curve (ROC) curve analysis was conducted to assess predictive value of IRG signature via “timeROC” and “survival” R packages.

Evaluation of IRG signature

Univariate and multivariate Cox regression analysis of different clinicopathological characteristics were performed in osteosarcoma patients to further evaluate whether the potential application of IRG signature as is independent prognostic biomarker. Heatmap of clinical characteristics and risk signature was displayed. And risk scores comparison in different clinical subgroups were performed.

Functional Enrichment Analysis and Tumor Microenvironment investigation

Gene Set Variation Analyses (GSVA) analysis was conducted to compare signaling pathway variation score between different risk subgroups of osteosarcoma patients via GSVA packages. Significance threshold of analysis was adjusted P-value < 0.05. Single-sample gene set enrichment analysis (ssGSEA) was utilized to calculate infiltration scores of immune cells and activities of immune-related function between different risk subgroups with the “GSVA” R package. Stromal score and immune score were analyzed via estimate formula [16] to explored infiltration levels of stromal cells and immune cells in osteosarcoma. The relationship between these scores and risk score was tested via spearman correlation.

Results

Figure 1 displayed the flow chart of present study. The included information in the present study consisted of 84 osteosarcoma patients’ information in TARGET database and 53 osteosarcoma patients’ information in GSE21257. Detailed Clinical characteristics of osteosarcoma patients were summarized in Table 1.

Table 1
clinical charactics of TARGET and GEO cohorts.

	TARGET cohort	GEO cohort
Total number	84	53
Age		
Age < = 18	71	40
Age > 18	13	13
Gender		
Male	47	34
Female	37	19
Metastasis		
Yes	21	34
No	63	19
Status		
Alive	57	30
Dead	27	23

Identification of Prognostic IRG in TARGET database

17 IRG were correlated with OS according to univariate Cox analysis result (Fig. 2A). Figure 2B displayed the correlation between these IRG (Fig. 2B).

Establishment of prognosis signature in TARGET database

LASSO-Cox regression analysis was utilized to calculate the expression profiles of prognostic IRG (Fig. 2C, 2D). According to LASSO-Cox regression analysis result, we constructed the prognostic IRG signature. The risk score formula of each patient was listed as follows: risk score = 3.259*expression level of IL12B − 0.252*expression level of CCL2−0.001*expression level of CD82−0.292*expression level of CMKLR1−0.971*expression level of FPR1−0.618*expression level of MMP14 + 2.084*expression level of MYC + 0.045*expression level of PDPN + 0.380*expression level of RGS16−0.114*expression level of SELL + 1.089*expression level of SLC7A1.

Median risk score value was used to divide all the osteosarcoma patients into high and low risk subgroups. Patients in low-risk subgroup had a significantly better OS than patients in high-risk according to Kaplan-Meier curve result (Fig. 3A). Figure 3B and 3C displayed IRG heatmap and distribution in the

signature. The area under the curve (AUC) of prognosis signature were 0.870 at 1 year, 0.818 at 2 years, and 0.816 at 3 years (Fig. 3D). PCA (Fig. 3E) and t-SNE (Fig. 3F) analysis results demonstrated that patients in high and low risk groups were distributed in different directions.

Validation of the prognosis Signature in the GEO Cohort

To assess the stability of prognosis signature established from TARGET database, osteosarcoma patients in GSE21257 were also categorized into high and low subgroups. Patients in the low risk subgroup had a longer survival time compared with the high risk subgroup (Fig. 3G). Figure 3H and 3I displayed IRG heatmap and distribution in the signature in GEO cohort. And AUC of the prognosis signature in GSE21257 were 0.684 at 1 year, 0.708 at 2 years, and 0.763 at 3 years (Fig. 3J). PCA (Fig. 3K) and t-SNE (Fig. 3L) analysis results indicated a discrete distribution of osteosarcoma in different risk groups which was similar to the results in TARGET database.

Independent prognostic value of prognosis signature

To investigate whether risk score in IRG signature was an independent prognostic factor for osteosarcoma, univariate and multivariate Cox analysis of clinical characteristics in the TARGET cohort were performed. Risk score of IRG signature (HR = 6.067, 95% CI = 3.135–11.740, P < 0.001) and metastasis status (HR = 4.764, 95% CI = 2.220–10.220, P < 0.001) were significantly correlated with OS of osteosarcoma according to univariate Cox analysis result (Fig. 4A). And age (HR = 0.713, 95% CI = 0.335–1.520, P = 0.382) and gender (HR = 0.999, 95% CI = 0.919–1.086, P = 0.984) were no correlated with OS of osteosarcoma. In multivariate Cox analysis, risk score of IRG signature (HR = 6.154, 95% CI = 3.013–12.567, P < 0.001) and metastasis status (HR = 4.045, 95% CI = 1.856–8.813, P < 0.001) were still independent prognostic factors for OS after correction of other confounding factors (Fig. 4B). Heatmap of clinical characteristics and IRG signature was display in Fig. 4C. Next, GSVA was performed to elucidate the potential biological mechanisms between different risk subgroups. GSVA heatmaps (Fig. 4D) showed that cytokine receptor interaction, pathways in cancer, toll like receptor signaling pathway was dynamically correlated to risk stratification. These signaling pathways might be participate in the different risk subgroups. Percent weigh of different clinical characteristics in different risk score were display in Fig. 5. Risk score in metastasis status (P < 0.01) was significantly higher than that in non-metastasis status. Risk score in dead status (P < 0.01) was significantly higher than that in alive status. And no apparent significances of risk score in different age subgroups and gender subgroups were found. In the stratification survival analysis, no apparent significances of different risk score in age > 18 subgroup. And other clinical subgroups, patients in the low risk subgroup had a longer survival time compared with the high risk subgroup (P < 0.05).

Immune Status and Tumor Microenvironment Analysis

ssGSEA was utilized to quantify the enrichment scores of immune cell subpopulations, related immune functions. In TARGET cohort, the fractions of B cells, CD8 + T cells, DCs, Macrophages, Neutrophils, NK cells, pDCs, T helper cells, Tfh, Th2 cells, TIL and Treg in high risk subgroup were lower than that in low

risk subgroup (Fig. 6A). While in GEO cohort, the fractions of aDCs, B cells, CD8 + T cells, DCs, Macrophages, Neutrophils, pDCs, Tfh, Th1 cells, Th2 cells, TIL, Treg in high risk subgroup were lower than that in low risk subgroup (Fig. 6B). The results of fractions of B cells, CD8 + T cells, DCs, Macrophages, Neutrophils, pDCs, Tfh, Th2 cells, TIL, Treg were similar in both TARGET and GEO cohorts between different risk subgroups (adjusted $P < 0.05$, Figs. 6B, D). In both TARGET and GEO cohorts, function score of T cell co-inhibition, T cell co-stimulation, Type I IFN response, APC co-inhibition, APC co-stimulation, CCR, check point, cytolytic activity, HLA, inflammation promoting, parainflammation and MHC class I in high risk subgroup were significantly lower than that in low risk subgroup (Fig. 6D, 6E). To investigate the association between immune score, estimate score and risk score, the estimate formula was utilized to analyze immune and estimate score of osteosarcoma patients in TARGET cohort. And our results indicated negative correlations of risk scores with immunescore ($R=-0.68$, $P < 0.001$, Fig. 6C) and estimate score ($R=-0.54$, $P < 0.001$, Fig. 6F).

Discussion

The organ response to tumor has many parallels with wound healing and inflammation response. Inflammation contains recruitment, activation and action of cell of adaptive and innate immunity cell [11]. Around 15–20% of all tumor cases are originate from tissue infection, inflammation or autoimmunity [17]. Induced inflammation exists long to promote cancer formation in these tumor cases. However, the specific role of inflammation response in osteosarcoma is unclear. Genomic approaches have provided convenience to inflammation related analysis and to new classifications selection of cancers. There are no studies using IRG signature to predict the prognosis of osteosarcoma patients. In present study, we investigated IRG expression level in ostersarcoma and constructed a prognosis signature for survival prediction via univariate Cox analysis and LASSO Cox regression analysis. And this signature was validated in external GEO information. To further evaluate the potential value of this IRG signature, we compared the immune cell infiltration and activated pathways in different risk subgroups. And immune related analysis results indicated that low risk subgroup had increased expression level of inflitrating immune cells and immune pathways activity compared with high risk subgroup. Above-mentioned fingdings provide a timely and necessary study of possibility of IRG signature as a prognosis predictor. With further in-depth studies, IRG signature might be applied as additional supplement to osteosarcoma immunotherapy to achieve personalized, targeted therapy.

The signature established in present study consisted of 11 IRG. And The prognosis of osteosarcoma patients in the high and low risk subgroups show significant difference. The survival in the high risk subgroup was significantly worse than that in low risk subgroup. And ROC curve, PCA, t-SNE results and validation results in GEO cohort displayed reliability of signature. However, mechanism about these IRG affection on osteosarcoma remains to be explored. Based on the GSVA analysis, we found that cytokine receptor interaction, pathways in cancer, toll like receptor signaling pathway was dynamically correlated to risk stratification. And these pathways might be inner mechanism between high and low risk subgroups.

Some of these IRG expression and related influencing mechanisms have been reported in osteosarcoma. IL-12 which act as proinflammatory cytokine can target immune cells to mediate both innate and adaptive immunity [18]. IL-12 is an upstream immunomodulator that mediates anti-angiogenic and immunostimulatory activity. With regard to osteosarcoma, the vital role of IL-12 and its receptor has been reported in many studies. IL-12 is reported to trigger a potent antitumor immune response in primary and metastatic pediatric osteosarcoma [19]. Zhao et al. report that tumor cell-surface vimentin-targeted IL-12 can prolong overall survival and eliminate relapse and metastasis status through conversion of immune profile [20]. Encoded by the human PDPN gene, podoplanin is transmembrane lymphoid receptor glycoprotein which act as platelet aggregation regulator and lymphatic endothelial marker [21, 22]. Synthetic compounds, antibodies and biologics which could target PDPN are reported to inhibit septic inflammation and tumor progression in preclinical models [23, 24]. The expression of podoplanin is increased in various human cancer tissues and cell lines [24, 25]. High PDPN expression has been observed in human osteosarcoma tissues [26] and cell lines, including HOS, MG63 and U2OS [22]. And Previous research has indicated that expression of PDPN in these osteosarcoma cell lines could induce platelet aggregation [22]. Further survival analysis demonstrate that high expression of PDPN is significantly correlated with pulmonary metastasis and Enneking stage in patients with osteosarcoma [27]. Activating protein-1 could regulate podoplanin to promote osteosarcoma cell migration and platelet aggregation [22]. Recent studies related to PDPN have shown that blockade of interactions between PDPN and platelets C-type lectin-like receptor – 2 may prevent the lung metastasis in osteosarcoma [28]. As for CCL2, myeloid and astrocytoma cells can secrete CCL2 to recruits myeloid-derived suppressor cells and regulatory T cells. Increased expression of CCL2 is reported to promote the proliferation and invasion of osteosarcoma cells and tissue [29, 30]. MYC is also known as c-Myc. This proto-oncogene encodes nuclear phosphoprotein which is involved in cell apoptosis and cellular transformation. S1P/S1PR3 axis inhibites YAP phosphorylation and facilitate YAP nuclear translocation and YAP-c-MYC complex formation to promote aerobic glycolysis in osteosarcoma [31]. SLIT2/ROBO1 axis is also reported to activate SRC/ERK/c-MYC/PFKFB2 pathway to exert cancer-promoting effects in osteosarcoma [32]. In clinical samples, the evaluation of C-MYC and dihydrofolate reductase at diagnosis is proved to be an early identification of risk subgroups in osteosarcoma [33]. Although our results show these IRG are associated with tumor prognosis. However, there are few reports about undermentioned IRG in osteosarcoma. CD82 is a metastasis suppressor gene which encodes transmembrane 4 superfamily membrane glycoprotein. CMKLR1 has been showed to promote the progression of inflammatory diseases [34]. however, uncharacterized interaction between CMKLR1 and its protein–ligand chemerin limited its drug exploitation and further clinical trial investigation 33705662. FPR1 is a known G-protein-coupled receptor that involved in activation of immune inflammation [35]. Encoded by the human MMP14 gene, matrix metalloproteinase 14 is a transmembrane protein which is also the first member of the matrix metalloproteinase family [36]. In sarcoma, MMP14 is related to ECM degradation and metastasis formation [36]. RGS16 is member of RGS gene family which play vital role in many aspects of tumorigenesis [37]. SELL is also called CD62L. CD62L localizes to the T cell surface and exert promotive effects of T cell homing. Downregulated CD62L is reported in chronic lymphocytic leukemia and such downregulated expression is correlated with cancer progression [38]. Additionally, CD62L is supplement

therapy which can enhance the efficacy of tumor immunotherapy for cancer treatment [39]. The combination of CD62L targeted drugs and immunotherapy is viable in enhancing the antitumor efficacy [39]. SLC7A1 is a transporter that responsible for the uptake of cationic amino acids uptake function, and such function is essential for cellular growth [40]. At present, studies on these IRG genes in osteosarcoma is less reported. A further exploration between these IRG and tumor biomolecules is needed

Inflammation is a known hallmark of cancer. Inflammatory cells and their released factors are identified as the main mediators of tumor progression, metastasis and resistance to therapy [41]. And chronic inflammation is related to poor therapy outcomes to immunotherapy [42]. Inflammation treatment is conducive to extend the benefit of tumor immunotherapy. In TARGET cohort, high risk subgroups had lower levels of key antitumor infiltrating immune cells. We found the similar result in GEO cohort. Our study also displayed a significant negative correlation of risk scores with immune score and estimate score. Patients with higher risk score had a lower immune score and estimate score. These results indicated an overall impairment of immune function in osteosarcoma of high risk subgroup. According to the above analysis, the better OS result of osteosarcoma in low risk subgroup may result from the increased levels of antitumor immunity. Due to remarkable diversity and specificity of immune system, more investigations of immune resistance mechanism are warranted. inflammation has emerged as a vital determinant to shape immune-resistance. Further investigation to explore the immune regulatory network between these IRG and immune is needed.

There are some limitations in the present study. Firstly, patient's clinical information downloaded from the TARGET database is incomplete, especially radiotherapy and chemotherapy information. Detailed therapy information may be helpful to understand the relationship between IRG and treatment. Secondly, we cannot confirm the mechanism between inflammation response and precise process in osteosarcoma and this question deserves further in-depth studies. Lastly, we performed a systematically analysis in osteosarcoma based on the national database (TARGET and GEO), more work is warranted to verify this prognostic signature in large-scale and multicenter cohort. More in vitro and in vivo experiments are required to elucidate biological function of these IRG in osteosarcoma. In despite of these limitations, the present study does investigate comprehensive analysis of IRG profiles in osteosarcoma, and in-depth studies in the future will further explore the prognostic value of these IRG.

Conclusion

The present study proposed IRG signature to serve as risk factor for prognosis prediction in osteosarcoma. Current evidence suggests that inflammation response signature may serve as an available predictor role for the prognosis and promote development and progression of diagnosis and treatment in osteosarcoma. These results provide a novel IRG signature and lay the foundation for further investigation of relationship between immunity and inflammation response in osteosarcoma.

Abbreviations

CI: confidence interval

GEO: Gene Expression Omnibus

GSVA: Gene Set Variation Analyses

HR: hazard ratio

IRG: Inflammatory response-related genes

LASSO: Least absolute shrinkage and selection operator

OS: overall survival

PCA: principal components analysis

ROC: receiver operating characteristic curve

SNE: Stochastic Neighbor Embedding

ssGSEA: Single-sample gene set enrichment analysis

TARGET: Therapeutically Applicable Research to Generate Effective Treatments

Declarations

Availability of data and materials

All the data of the manuscript are presented in the paper or additional supporting files. GEO dataset (GSE21257) were obtained from the Gene Expression Omnibus database. The RNA-Seq and corresponding clinical data were downloaded from Therapeutically Applicable Research to Generate Effective Treatments repository.

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Authors' contributions

Conception and design: Weilong Xu, Wei Niu. Data collection, analysis and interpretation: Weilong Xu, Wei Niu. Original draft: Weilong Xu. Article reviewing, editing, and diction analysis: Wei Niu. The authors read

and approved the final manuscript.

Ethics declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figures

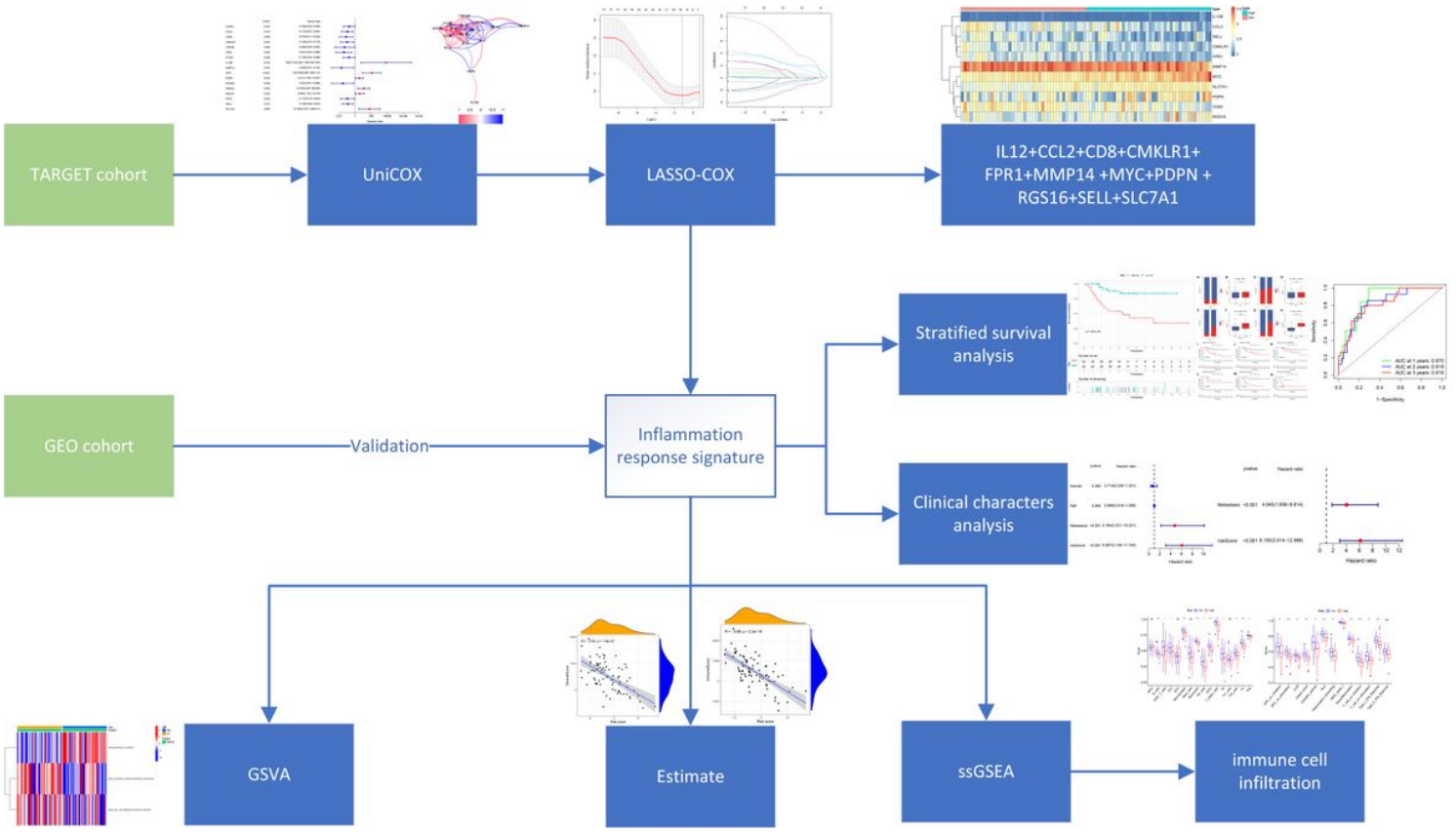

Figure 1

The flow chart of present study

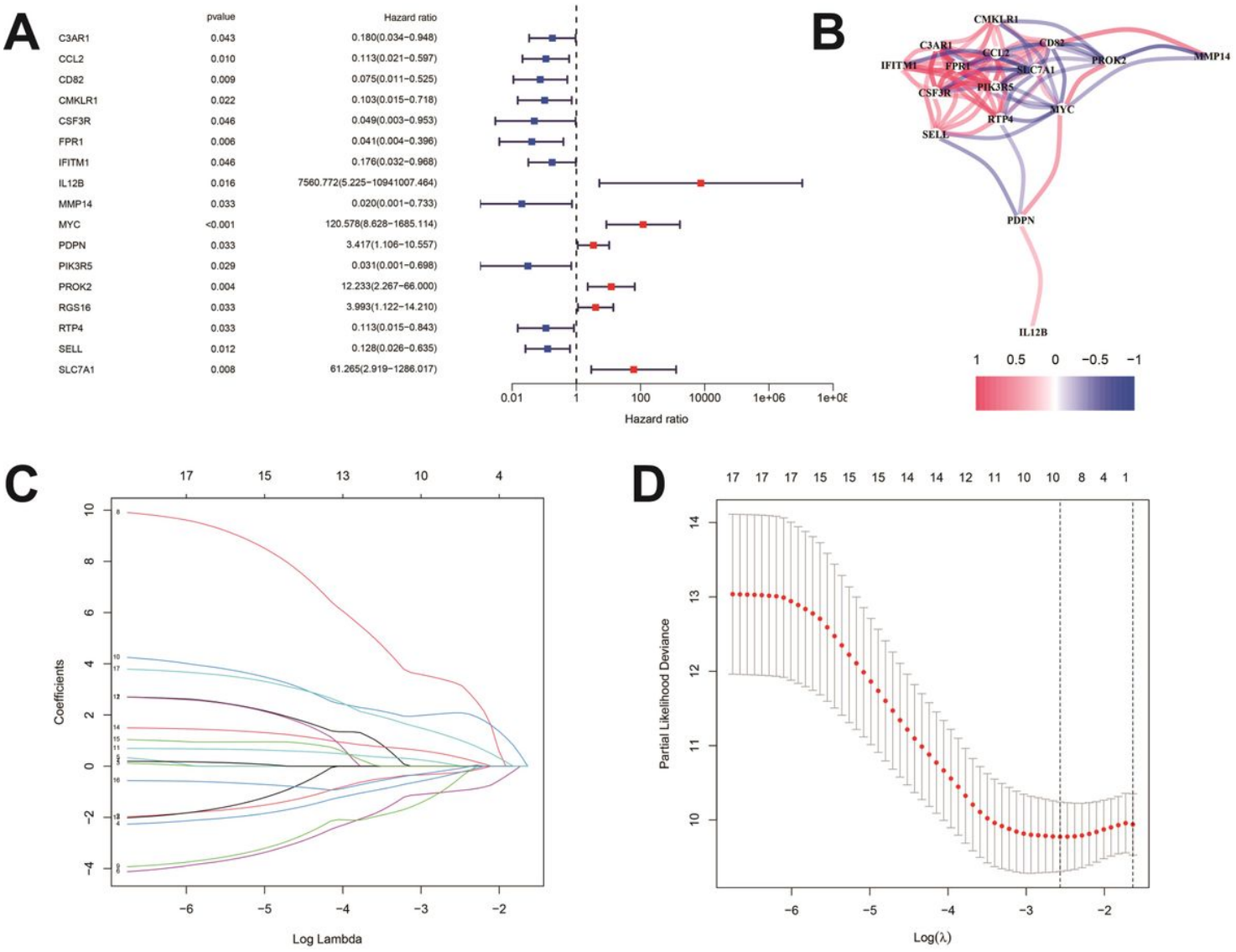

Figure 2

A Forest plots results of the relashionship between 17 IRG expression and OS. B The correlation network of prognosis IRG. C LASSO coefficient profiles of prognostic IRG. D cross-validation for tuning parameter selection in the LASSO model.

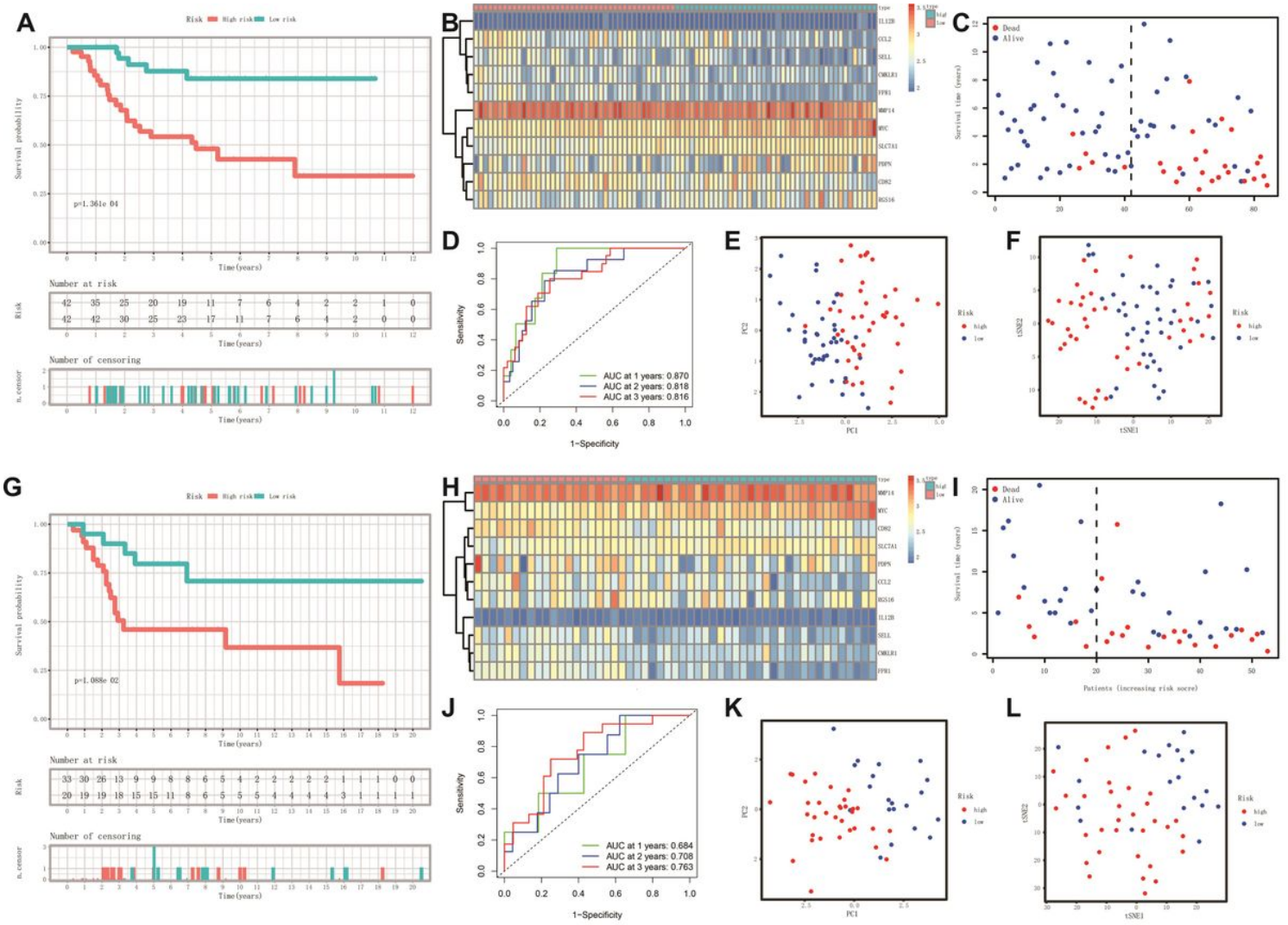

Figure 3

Prognostic analysis of the IRG signature model in the TARGET cohort (A-F) and GEO cohort (G-L). A Kaplan-Meier curves for OS of osteosarcoma patients in different risk subgroups in the TARGET cohort B heatmap of IRG in the TARGET cohort C The distribution of OS status in the TARGET cohort D ROC curves for OS in the TARGET cohort E PCA plot in the TARGET cohort F t-SNE analysis in the TARGET cohort G Kaplan-Meier curves for OS of osteosarcoma patients in different risk subgroups in the GEO cohort H Heatmap of IRG in the GEO cohort I The distribution of OS status in the GEO cohort J ROC curves for OS in the GEO cohort K PCA plot in the GEO cohort F t-SNE analysis in the GEO cohort

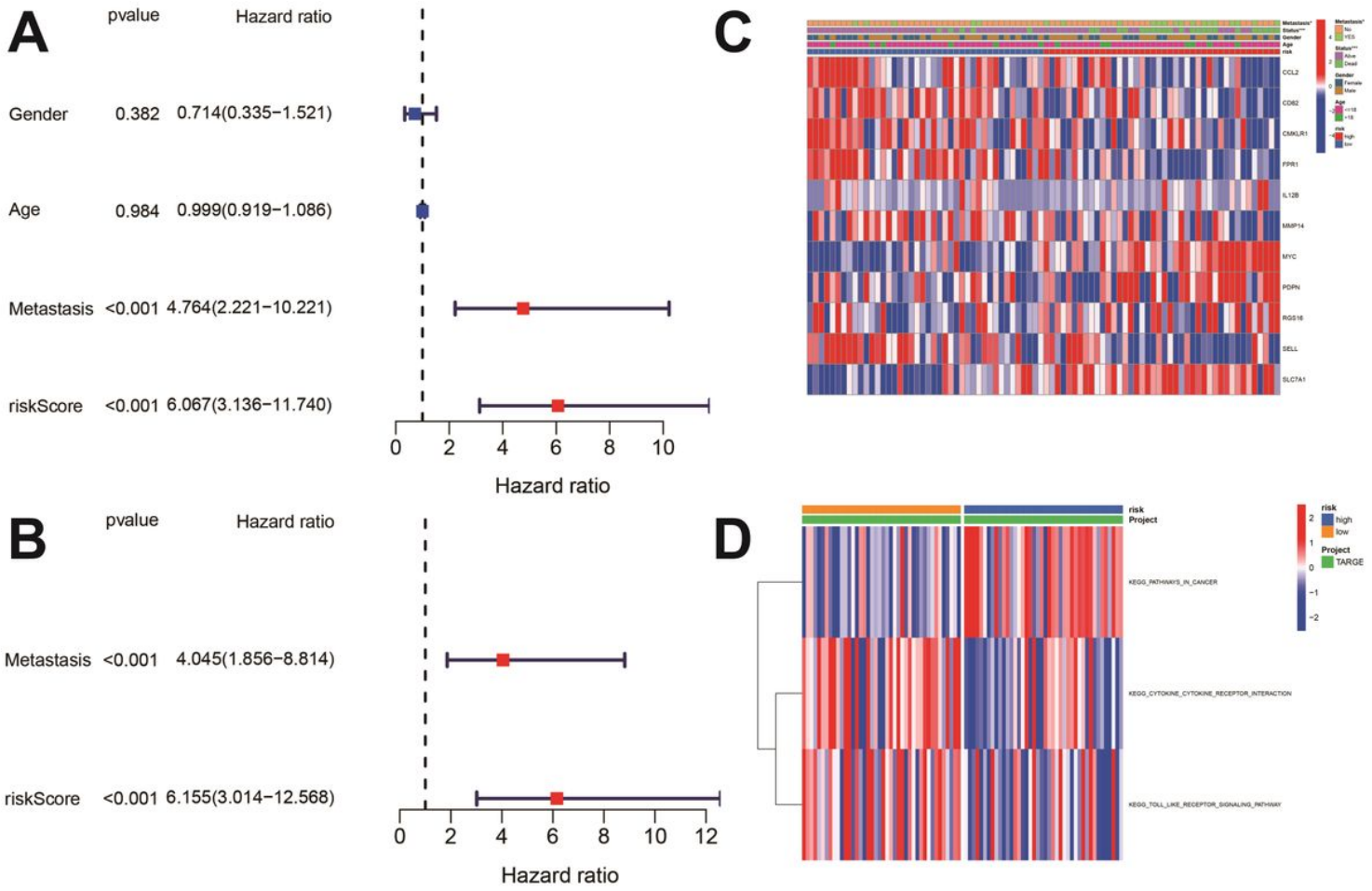

Figure 4

A OS-related factors were screened by Univariate Cox regression analysis in the TARGET cohort B OS-related factors were screened by Multivariate Cox regression analysis in the TARGET cohort C heatmap of clinical factor and expression of IRG in the TARGET cohort D GSVA result in the TARGET cohort

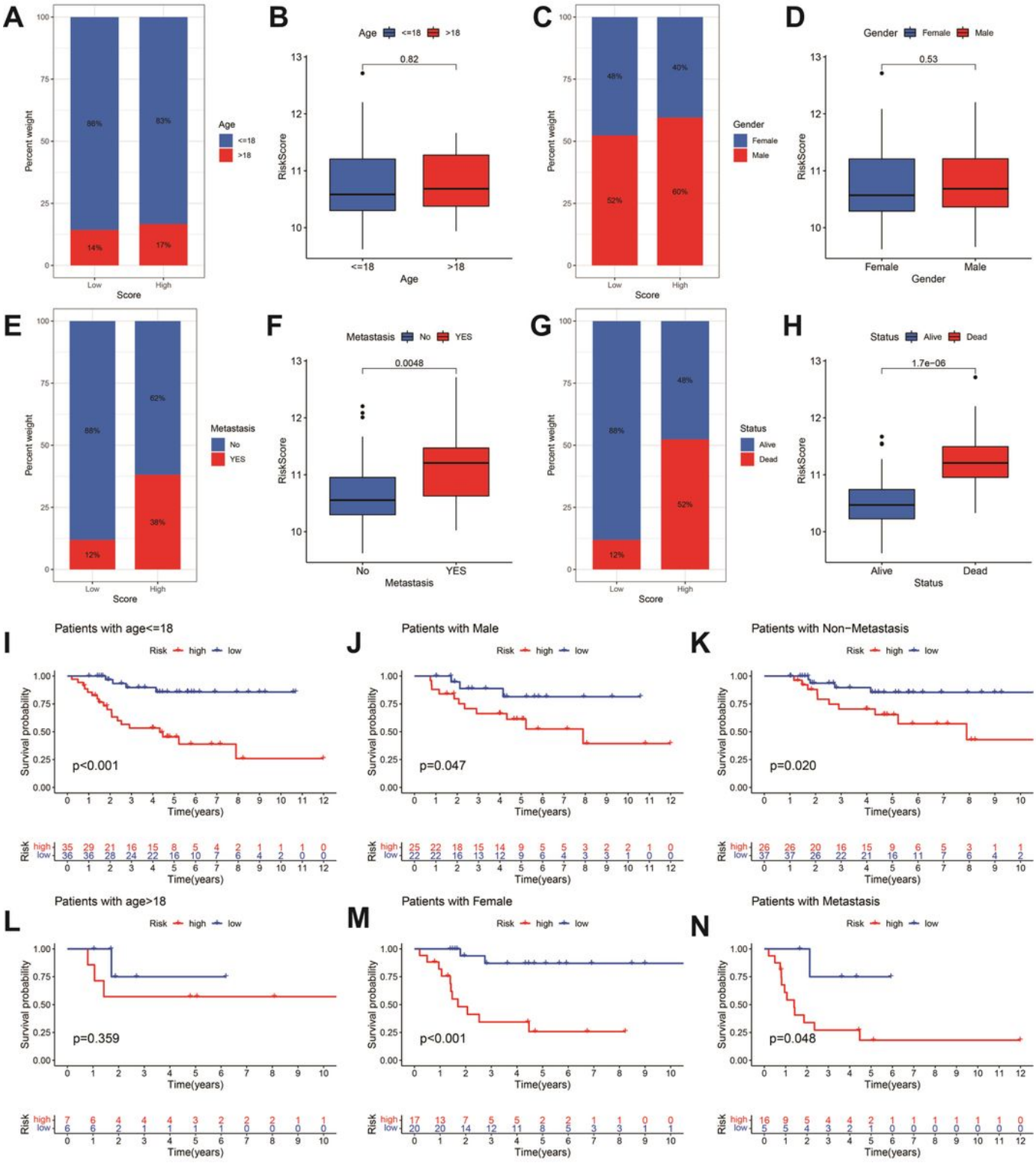

Figure 5

A percent weight of different age subgroups B comparison of riskscore in different age subgroups C percent weight of different age subgroups D comparison of riskscore in different age subgroups E percent weight of different age subgroups F comparison of riskscore in different age subgroups G percent weight of different age subgroups H comparison of riskscore in different age subgroups I Kaplan-Meier curves for OS of osteosarcoma patients in age<=18 subgroups J Kaplan-Meier curves for OS of

osteosarcoma patients in male subgroups K Kaplan-Meier curves for OS of osteosarcoma patients in non-metastasis subgroups L Kaplan-Meier curves for OS of osteosarcoma patients in age>18 subgroups M Kaplan-Meier curves for OS of osteosarcoma patients in female subgroups N Kaplan-Meier curves for OS of osteosarcoma patients in metastasis subgroups

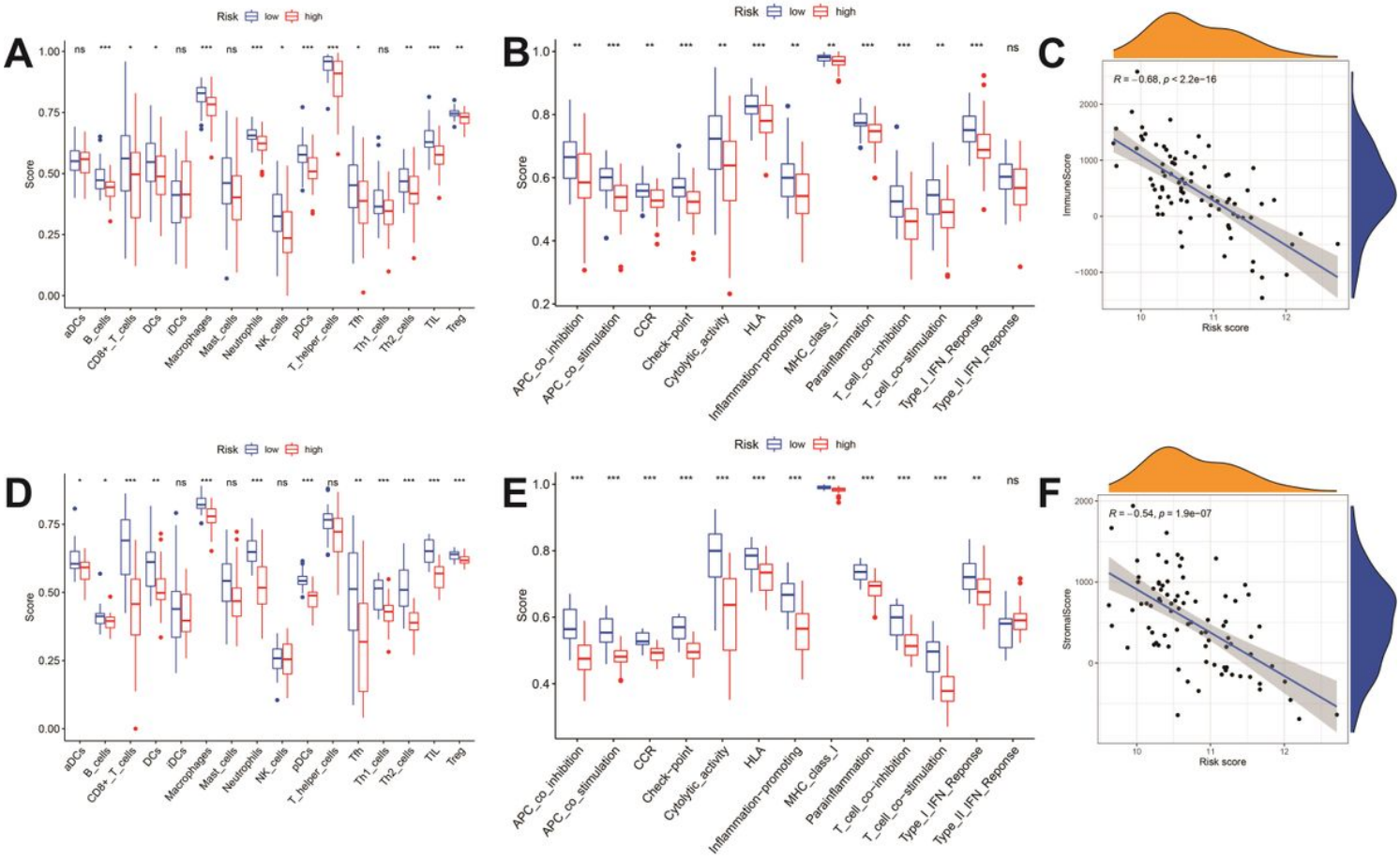

Figure 6

A The scores of immune cells distribution in the TARGET cohort B the immune-related functions distribution in the TARGET cohort C The relationship between risk score and immunescore in the TARGET cohort D The scores of immune cells distribution in the GEO cohort E the immune-related functions distribution in the GEO cohort F The relationship between risk score and stromalscore in the TARGET cohort P values were showed as: ns, not significant; *P < 0.05; **P < 0.01; ***P < 0.001.