

Prognostic Impact of Autophagy and Immunity Related Biomarker for Uveal Melanoma

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Research

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Abstract

Background: Autophagy and immunity related genes serve crucial roles in carcinogenesis, but little is known about the prognostic impact for uveal melanoma (UM).

Methods: Autophagy related and immunity related genes (AIRGs) expression data of 80 UM patients were obtained from the cancer genome atlas project (TCGA) database. Next, univariate cox regression analysis and the least absolute shrinkage and selection operator (LASSO) algorithms were applied to build a robust AIRGs signature in TCGA and validated in another two independent datasets. Besides, UM patients classified into two subgroups based on the risk model. Differences of clinical outcome, tumor microenvironment and the likelihood of chemotherapeutic response were further explored.

Results: In total, a 4-AIRGs signature was constructed and validated in various datasets, which can robustly predict patients' metastasis-free survival (MFS) and overall survival (OS) and is an independent prognostic factor in UM. The UM patients can be classified into high and low risk subgroups by applied risk score system. The high risk group have poor clinical outcomes, higher CD8+ T cell and macrophage immune-infiltrating and more sensitive to chemotherapies. In addition, Gene Set Enrichment Analysis (GSEA) analysis revealed that hallmark epithelial-mesenchymal transition and KRAS pathways are commonly enriched in high-risk expression phenotype.

Conclusion: Thus, our findings provide a new clinical strategy for the accurate diagnosis and identify a novel prognostic autophagy and immunity associated biomarker for the treatment of uveal melanoma.

Background

Uveal melanoma (UM), the most common primary intraocular malignancy which accounts for 70% of all ocular cancers. It became a growing global public health concern with about 50% of patients die of metastatic disease[1–3]. Despite exists certain advances in treatment of UM, the prognosis for patients is still poor[4, 5]. Thus, it's urgently required to explore the novel prognostic biomarkers of UM to unveil the underlying potential molecular mechanism as well as therapeutic targets.

Autophagy is an important process to mediate intracellular degradation which regulates cellular and biological homeostasis. Deregulation of autophagy has been proven in various pathological and disease processes, including differentiation, development and tumorigenesis[6]. As for malignant tumors, the function of autophagy in tumorigenesis like a double-edged sword, which may vary at different stages[7]. Recently, many researches revealed that autophagy can reduce cell damage and maintain chromosomal stability by eliminating damaged protein and organelles in early stages of cancer development[8]. In contrast, at the late stage of cancer, autophagy seems to promote tumor cells proliferation and escape from immune surveillance[9]. Moreover, autophagy is intimately associated with immune response, inflammation and therapeutic resistance[10, 11]. For instance, recent researches have showed that therapies target on autophagy will enhance the immune responses, anti-tumor effects and overcome drug resistances[12, 13]. Thus, it's undoubted that autophagy and immunity play crucial roles in a wide range of physiological conditions and diseases, especially for cancers. In addition, growing researches have demonstrated that autophagy and immunity related genes (AIRGs) biomarkers are associated with the prognosis of various types of cancer, including breast cancer, glioma, pancreatic ductal adenocarcinoma and hepatocellular carcinoma[14–16]. In spite of the significant role of AIRGs in various cancers have been proven, the prognostic value for uveal melanoma is poorly understood.

Luckily, largescale transcriptome datasets in public repositories, such as the cancer genome atlas (TCGA) and Gene Expression Omnibus (GEO), which afforded valuable resources to explore biomarkers and tumors in different organisms and biological conditions[17].

Therefore, in our research, AIRGs were firstly downloaded from The Human Autophagy Database and the ImmPort Database websites. Next, univariate cox analysis and LASSO model were performed to construct a robust AIRGs biomarker, which can distinguish different clinical outcomes, immune microenvironment and chemotherapy response of UM. The present study indicated that the expression of AIRGs takes pivotal roles in the prognosis of UM and could be considered as a prognostic marker for UM therapy.

Results

Survival associated AIRGs

Totally, 222 autophagy related genes and 1793 immunity related genes were selected for univariate cox analysis in TCGA-UVM dataset. According to the selection standard, 44 autophagy related genes were significantly associated with survival of UM patients, which contained 27 risk genes and 17 protect genes (Figure 2A). In addition, 365 immunity related genes are survival associated, which consist of 301 risk genes and 64 protect genes (Figure 2B). Apart from the overlapped genes, a total of 409 AIRGs was identified for subsequent analysis.

Identification and validation of prognostic AIRGs biomarker

Firstly, 1,000 iterations of LASSO modeling were performed to evaluate the qualified variables from the 409 prognostic AIRGs, and then these qualified AIRGs were used to construct prognostic biomarker. Totally, 12 qualified prognostic AIRGs were selected to subsequently build biomarker due to appear more than 990 times in LASSO modeling (Figure 2C). Moreover, based on the 5-year AUCs of different gene combinations, we found that a 4-AIRGs related biomarker (PRKCD, MPL, EREG and JAG2) was arrived the max value of 0.829 (Figure 2D,E). These four AIRGs were closely correlated with each other (Figure 2F). Finally, the 4-AIRGs biomarker was used to calculate risk score and the formula listed as follows: the risk score = . By applying this risk formula, a risk score for each sample will be generated and scaled to range from 0 to 1. Next, by applying the median cut-off value of the risk scores, UM patients were categorized into high-risk group (n = 40) and low-risk group (n = 40). Kaplan-Meier (KM) curves showed that patients in high-risk group have a shorter survival time than low-

risk with a log-rank test $p < 0.001$ (Figure 3A). The ROC curve was drawn and 3,5 years of AUCs were 0.916 and 0.829 respectively (Figure 3B). The distribution of risk score, OS, vital status, and expression of corresponding AIRGs in TCGA-UVM were shown in Figure 3C-E. In order to verify the robustness of the result, validated analyses were applied in another two independent datasets including GSE22138 and GSE84976. Patients in GSE22138 and GSE84976 datasets were accordingly classified into high-risk and low-risk groups. KM analyses manifested a similar result that patients in low risk group had significantly longer survival time than those in high-risk group (GSE22138 log-rank $p = 0.0019$ and GSE84976 log-rank $p < 0.001$ respectively) (Figure 4A and Figure 5A). The 3,5 years of AUCs in GSE22138 were 0.746, 0.713 (Figure 4B). Higher AUCs (3-year:0.836; 5-year:0.872) also observed in GSE84976 (Figure 5B). The distribution of risk score, MFS, vital status, and expression of 4 AIRGs in GSE22138 was shown in Figure 4C-E. Meanwhile, the same risk score heatmap of 4 AIRGs in GSE84976 was shown in Figure 5C-E.

Prognostic value of biomarker

To explore prognostic factors for OS or MFS in multiple datasets, the risk score of AIRGs biomarker and clinical variables were conducted by the univariate and multivariate cox regression analyses (Table 1). The results of univariate cox regression revealed that stage, age, metastasis, histological type and risk score were significantly associated with MFS or OS. According to the multivariate cox regression analyses, only the risk score was stably remained independent prediction for OS or MFS in TCGA-UVM dataset (HR = 34.951, 95% CI = 4.891–249.759, $P < 0.001$), GSE22138 dataset (HR = 45.623, 95%CI = 10.025–564.298, $P < 0.001$) and GSE84976 dataset (HR = 3.532, 95%CI = 1.783–9.276, $P = 0.042$). The 5 years AUCs of age, stage, metastasis, histological type and risk score in TCGA-UVM set were 0.741, 0.778, 0.658, 0.424 and 0.829 respectively (Figure 6A). In GSE22138, the 5 years AUCs of Chromosome 3 status and risk score were 0.762 and 0.713 (Figure 6B). In addition, the 5 years AUCs of Chromosome 3 status, metastasis and risk score in GSE84976 set were 0.912, 0.858, and 0.872 respectively (Figure 6C).

Associations between risk model with clinical characteristics and immune infiltration

The relationships between the risk score signature and various clinical characteristics were investigated in TCGA-UVM dataset. The box plot manifested that UM patients in stage IV, metastasis, epithelioid histological type and dead status have higher risk score levels than other subtypes (Figure 7A). Other clinical characteristics (age and gender) have no relationships with risk score. Thus, the risk model was intimately associated with tumor stage, metastasis, histological type and vital status. Through CIBERSORT analysis, the proportion of 22 different infiltrating immune cells was estimated in TCGA-UVM dataset. The stratifying analysis showed that the fractions of T cell CD8, T cells CD4, T cells gamma delta, B cells naïve, NK cells activated, Monocytes, Macrophages M1 and Neutrophils in high risk subgroup significantly different from those in low risk group (Figure 7B).

Gene set enrichment analysis (GSEA)

In order to explore the different hallmark pathways enriched in high and low risk group, GSEA analyses were performed. According to selected criterion, we confirmed that six positive correlated pathways were enriched in high risk group, which including epithelial mesenchymal transition, estrogen response late, myogenesis, estrogen response early, apical junction and KRAS signaling. moreover, six cancer hallmark pathways contained interferon gamma response, protein secretion, interferon alpha response, MTORC1 signaling, JAK STAT3 signaling and inflammatory response were actively associated with low risk group (Figure 7C).

High-risk subgroup more sensitive to chemotherapies

At present, chemotherapy is a common treatment for UM, therefore GDSC database was also applied to predict sensitivity to chemo drugs. Assessment by selecting criteria, 45 kinds of drugs were screened out in TCGA-UVM set. 34 and 14 kinds of drugs were sensitive to high risk group in GSE22138 and GSE84976 respectively (Table 2). The Venn plot of three datasets suggested that AMG.706 and JNK.Inhibitor.VIII were the common drugs (Figure 8A). We could observe a significant difference in the estimated IC50 between high and low risk group for these chemo drugs where high-risk group could be more sensitive to chemotherapies (Figure 8B-D).

Discussion

Like many kinds of tumor, the prognosis of uveal melanoma is largely depend on early diagnosis and treatment[3]. The growing clinical application of new therapeutic targets and the present studies are mainly focused on detection of novel prognostic biomarkers which are often used to assess disease risk and early diagnosis[19, 20]. Clinical application of prognostic biomarkers can early guide high-risk of patients undergone individual treatment and management, even prevent life-threatening metastases[21–24]. Hence, as far as we known, our study is the first time to investigate the prognostic role of AIRGs biomarker in UM. We also constructed a robust 4-AIRGs biomarker in UM prognosis and validated in multiple independent datasets. Our prognostic biomarker can subsequently classify patients into subgroups with different survival events. Compared with traditional clinical characteristics, our AIRGs biomarker achieved higher accuracy than most of clinical indicators (eg. stage, age, metastasis and histology type). Notably, the results of multivariate cox regression manifested that the risk score of 4-AIRGs biomarker can afford a robustly accurate prediction of OS or MFS in UM and could be considered as an independent prognostic model.

In this research, we developed a prognostic biomarker with four selected AIRGs (PRKCD, MPL, EREG and JAG2), which classified the OS or MFS of UM into high and low risk groups. Kaplan-Meier curves revealed that patients in high risk group have a poor survival. Among the four AIRGs, almost all of genes have been identified to be prognostic biomarker in other malignancies. For instance, PRKCD (Protein kinase C delta) is a member of protein kinase C (PKC) family which is a critical regulator of the chemosensitivity in cancers such as non-small cell lung cancer, ovarian cancer and prostate cancer[25–27]. Additionally, PRKCD has been reported to serve as a novel prognostic biomarker for ovarian cancer patients response to overall disease-specific survival[28]. MPL (thrombopoietin receptor) is a member of homodimeric class I receptor family, which mainly exerted its influence on regulation of megakaryopoiesis,

formation of immune synapses and enhancing anti-tumor function[29, 30]. Christopher et al recently reported that C-MPL can work as a novel immunotherapeutic target to promote anti-tumor activity of T cells by mediation of cytokine pathways[31]. Therefore, MPL will be regarded as a potential therapeutic target for UM patients in future. As for EREG (Epiregulin), which belongs to the epidermal growth factor family and benefits to wound healing, tissue repair, inflammation in normal physiology. While dysfunction of Epiregulin will increase the progression of different malignancies. Previous studies revealed that the overexpressed EREG closely correlates with many cancers, including prostate cancer, colon cancer, breast cancer and bladder cancer[32–35]. What's more, Asnaghi et al reported that JAG2 plays an important role in promoting tumor cells growth and metastasis in uveal melanoma, which could serve as a new therapeutic target to further investigation[36].

To better understand the underlying molecular functions, GSEA analyses were conducted to find the potential 4-AIRGs related pathways in cancer hallmarks database. The results showed that hallmark epithelial-mesenchymal transition (EMT) pathway positively enriched in high risk expression subgroup. It's generally recognized that EMT pathway is a crucial factor controlling the clinical outcome of patients with various tumors. Asnaghi et al proven that EMT related factors will promote invasive properties of uveal melanoma cell and finally lead to poor prognosis[37]. Interestingly, our result consistent with this conclusion indicated that high risk subgroups positive enriched in epithelial–mesenchymal transition with a poor survival in UM. Additionally, we also identified that KRAS signaling was also positively associated high risk group. Kirsten rat sarcoma viral oncogene homolog (KRAS) regards as the most lethal cancer-related proteins which takes a crucial role in human aggressive cancers. Although the current study about relationship between KRAS and UM is few, KRAS has been recognized an induction factor of lung tumorigenesis for more than 20 years[38]. By CIBERSORT assessment, we also observed that high risk group have a higher infiltration of CD8 + T cells and M1 macrophage. Notably, recent studies have demonstrated that the increased level of CD8 + T cells is a predictive factor for poor prognosis in UM and the accumulation of macrophages are poorly associated with the prognosis of melanoma patients[39–41]. Therefore, it is logical to speculate that various malignant hallmarks and tumor infiltrating immune cells lead to the poor outcome of high risk patients.

Furthermore, in order to explore the different chemotherapeutic response between high and low risk subgroups, GDSC drug database was performed and the results showed that high risk subgroup is more sensitive to chemotherapies. Moreover, the three datasets showed that AMG.706 and JNK.Inhibitor.VIII were the commonly enriched drugs. The estimated IC50 of AMG.706 and JNK.Inhibitor.VIII with high risk group is significantly lower than the low risk groups. AMG.706 (Motesanib) is a multikinase inhibitor which significantly inhibits VEGF-induced vascular permeability, angiogenesis and induces regression in tumor xenografts[42]. As for JNK.Inhibitor.VIII, that is a JNK inhibitor which can inhibit the cell apoptosis and block the phosphorylation of c-Jun and JNKAR1 which promote tumorigenesis and metastasis of cancers[43]. Hence, it's reasonable to believe that AMG.706 and JNK.Inhibitor.VIII could be considered as potential chemo drugs for further clinical treatment.

Conclusion

Sum up, our research constructed a robust 4-AIRGs prognostic biomarker in UM, which could be regard as an independent prognostic model in clinical application. The patients in the high risk score group could benefit more chemotherapy. Furthermore, basic and clinical studies are also needed to validate the accuracy for predicting prognosis and managing patients.

Materials And Methods

Gene expression profile and clinical information

Gene expression profiles of 80 UM and clinical information were obtained from TCGA-UVM database for training set. Another two datasets GSE84976 and GSE22138 consisting of 91 UM samples from Gene Expression Omnibus (GEO) were used as outside validation sets. The complete analysis workflow in this study is illustrated in Figure 1.

Autophagy & Immunity Related Genes (AIRGs)

The AIRGs were obtained from the Human Autophagy Database (<http://www.autophagy.lu/>) which contains 222 genes directly or indirectly associated with the autophagy process according to previous studies[18]. Moreover, the latest version of immunity related genes was acquired from the ImmPort Database (<https://immport.niaid.nih.gov>).

Identification of prognostic AIRGs model

The prognostic values of AIRGs were assessed by using overall survival (OS) time of patients in TCGA-UVM. Univariate cox-regression analysis was used to select AIRGs that were significantly correlated with uveal melanoma's OS (p values <0.05 & $HR \leq 1.10$). Next, we used LASSO algorithm to develop risk model via the prognostic AIRGs. Based on LASSO approach, the qualified prognostic AIRGs were selected out to construct the risk system and generate risk score for each patient of UM according to the corresponding coefficients. Afterwards, the patients were accordingly classified into high- and low-risk group by median cutoff. Kaplan-Meier survival curves (Log-rank) were applied to estimate the prognosis of high- and low-risk group. Receiver operating characteristic (ROC) analysis and area under the curve (AUC) were calculated to assess the sensitivity and specificity of risk model.

Relationships between risk score with clinical characteristics and immune microenvironment

To assess the relationship between risk score and clinical variables, the risk score distribution for subgroup of age, sex, stage, histological type, chromosome 3 status, metastasis and vital status was performed. Moreover, univariate and multivariate cox regression analysis for OS or metastasis-free survival (MFS) were carried out to evaluate the prognostic value of risk score and clinical parameters. The hazard ratios (HR) and 95% confidence intervals (95% CI) of the prognostic factors were calculated. Besides, to analyze the association between risk score and immune cell infiltration, "CIBERSORT" algorithm was performed to quantify the proportion of 22 different infiltrating immune cells in tumor immune microenvironment.

Gene set enrichment analysis

To illustrate the potential mechanism between the low- and high-risk groups, we performed Gene Set Enrichment Analysis (GSEA) via “clusterProfiler” R package. Firstly, the differentially expressed genes between low- and high-risk groups were ranked by the value of log₂ fold change. Then the cancer hallmark database (h.all.v7.0.symbols) was applied in GSEA to explore the signaling pathways correlated with different subgroups of UM. The FDR q-value < 0.05 was used to screen significant pathways in each phenotype.

Chemotherapeutic response prediction

To explore the likelihood of chemotherapeutic drugs, the Genomics of Drug Sensitivity in Cancer (GDSC) database (<https://www.cancerrxgene.org>) was used to predict the chemotherapeutic response for each sample and the significant chemo drugs were selected (P<0.05). This analysis process was conducted by R package “pRRophetic”.

Statistical analysis

Every statistical analysis was executed with R software(v.3.5.2) and corresponding statistical packages.

Abbreviations

TCGA—The Cancer Genome Atlas database

GEO — Gene Expression Omnibus

UM— Uveal melanoma

AIRGs—Autophagy related and immune related genes

GSEA — Gene set enrichment analysis

GDSC— The Genomics of Drug Sensitivity in Cancer

DEGs—differently expressed genes

OS —overall survival

AUC—The area under the curve

ROC— Receiver operating characteristic curves

Declarations

Ethics approval and consent to participate

not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and analysed during the current study available from the corresponding author on reasonable request

Competing interests

All authors declare that they have no competing interests.

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Author contributions

HBD was responsible for writing-original draft preparation; YH was responsible for writing-review and editing; WL were responsible for data curation; QW was responsible for project administration and funding acquisition. All the authors commented and approved the text.

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Tables

Table 1: Univariate and multivariate Cox regression analyses of clinicopathologic characteristics associated with overall survival in TCGA-UVM, GSE84976 and GSE22138 datasets. * P < 0.05, ** P < 0.01.

TCGA (n=80)	Univariate analysis				Multivariate analysis			
	unicox_p	HR	lower .95	upper .95	mutlicox_p	HR	lower .95	upper .95
marker								
Age	0.020*	1.058	1.009	1.109	0.056	1.055	0.999	1.115
Gender	0.354	1.747	0.537	5.686				
Stage	0.004**	8.557	1.998	36.653	0.906	0.902	0.163	4.990
M	0.024*	7.359	1.302	41.579	0.019*	16.395	1.599	168.124
N	0.086	0.364	0.115	1.155	0.001**	0.024	0.003	0.198
T	0.239	1.831	0.668	5.013				
Histological_type	0.017*	0.123	0.022	0.688	0.194	0.224	0.023	2.145
Recurrence	0.257	4.018	0.363	44.528				
Riskscore	0.000**	57.610	11.103	298.911	0.000**	34.951	4.891	249.759
GSE84976 (n=28)								
Age	0.142	1.030	0.990	1.071				
Chromosome 3 status	0.001**	9.444	2.636	33.832	0.821	1.253	0.179	8.783
Metastasis	0.000**	28.513	5.938	136.924	0.008**	20.930	2.249	194.820
riskscore	0.000**	99.53	9.230	1073.373	0.000**	45.623	10.025	564.298
GSE22138(n=63)								
Age	0.113	1.021	0.995	1.049				
Gender	0.316	0.702	0.352	1.400				
Chromosome 3 status	0.001**	5.990	2.061	17.406	0.159	3.803	0.591	24.474
Histological_type	0.065	2.123	0.954	4.724				
Riskscore	0.003**	5.340	1.313	10.532	0.042*	3.532	1.783	9.276

Table 2: The prediction of chemotherapeutic response for each datasets. The significant chemo drugs and P-values were listed.

TCGA		GSE22138				GSE84976			
Drug	p-value	Drug	p-value	Drug	p-value	Drug	p-value	Drug	p-value
A.443654	0.016267	Lenalidomide	5.19E-06	A.770041	0.000199	KU.55933	0.017693	AMG.706	0.04
A.770041	0.026679	Metformin	0.001474	AMG.706	0.041196	Lapatinib	0.005939	BI.D1870	0.04
ABT.888	2.19E-10	Methotrexate	1.50E-05	AP.24534	0.00145	Methotrexate	0.002012	Bleomycin	0.02
AICAR	0.001208	Mitomycin.C	0.014373	ATRA	0.004447	MK.2206	0.000409	Doxorubicin	0.01
AMG.706	0.001474	MK.2206	0.002871	AZD.0530	8.63E-06	Nilotinib	0.006702	Epothilone.B	0.00
AZ628	0.048548	MS.275	0.03403	AZD6482	0.015927	NVP.TAE684	0.000158	Etoposide	0.03
AZD.2281	2.08E-05	Nilotinib	0.000205	BMS.509744	0.002308	Pazopanib	0.046142	Gemcitabine	0.02
AZD6482	6.99E-07	Nutlin.3a	0.027288	BMS.536924	0.000805	PD.173074	0.003018	GSK.650394	0.00
BI.2536	0.007074	OSI.906	0.007271	CGP.60474	0.039783	PF.02341066	0.001835	JNK.Inhibitor.VIII	0.02
BMS.509744	1.92E-08	PD.173074	1.58E-06	Dasatinib	0.00504	PLX4720	0.039783	PF.562271	0.01
BMS.536924	1.50E-05	PF.02341066	0.011144	Elesclomol	0.031087	SB.216763	0.004263	Thapsigargin	0.04
BMS.708163	0.003243	PF.4708671	1.29E-07	Erlotinib	0.007851	Temsirolimus	0.000158	Vorinostat	0.00
BMS.754807	0.003881	PLX4720	0.000697	GDC0941	0.000409	WH.4.023	0.003295	X681640	0.00
CGP.60474	0.042948	RO.3306	4.45E-05	Gefitinib	0.004086	WO2009093972	0.032081	ZM.447439	0.01
Cyclopamine	0.019269	SB.216763	6.02E-09	GNF.2	0.036318	WZ.1.84	0.001751		
EHT.1864	0.006156	SL.0101.1	0.001574	GW.441756	0.018318	Z.LLNle.CHO	0.036318		
GDC.0449	0.000122	Sorafenib	0.014736	Imatinib	0.006702				
Gefitinib	0.003657	S.Trityl.L.cysteine	0.002871	JNK.Inhibitor.VIII	0.00048				
GW.441756	5.32E-11	Temsirolimus	5.25E-10						
GW843682X	0.005499	VX.680	4.65E-05						
JNK.Inhibitor.VIII	0.001056	VX.702	0.007074						
KU.55933	0.000132	X17.AAG	0.000697						
Lapatinib	0.000122								

Figures

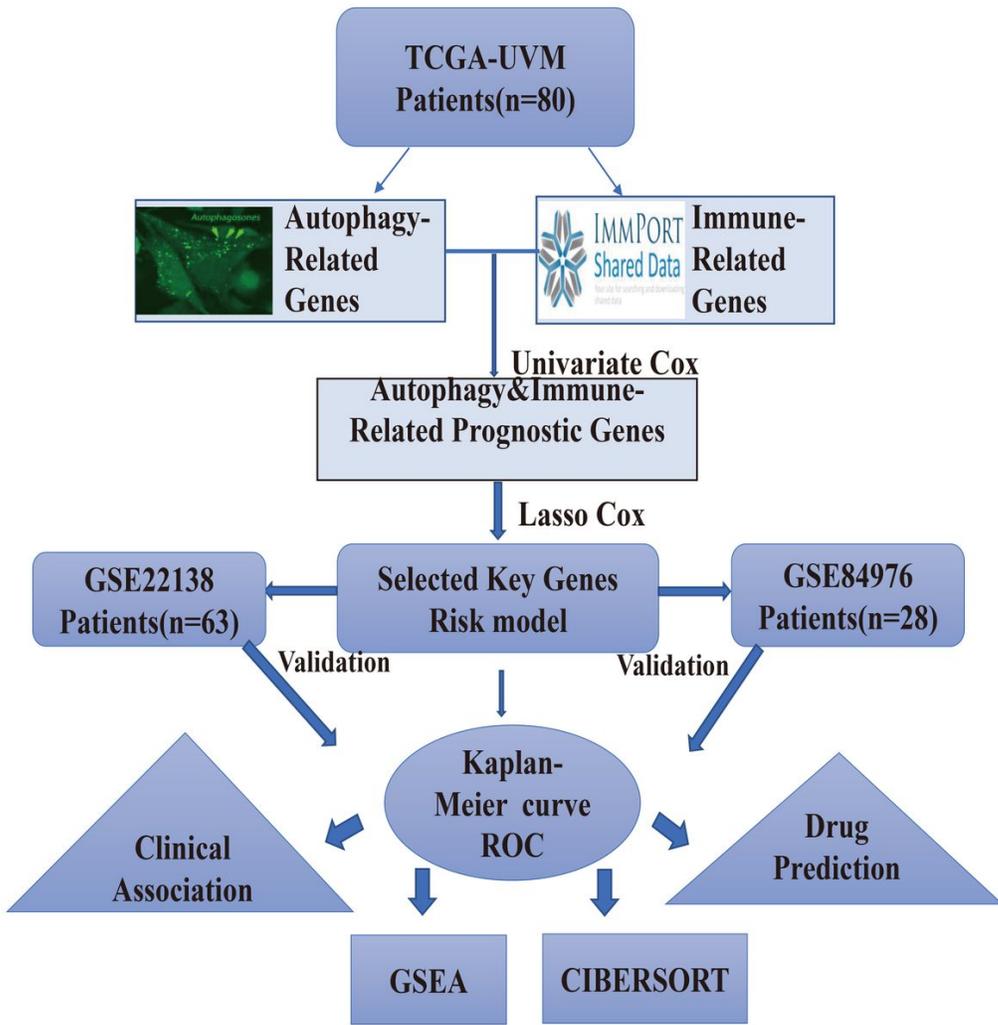


Figure 1

The complete workflow of the analysis in this study.

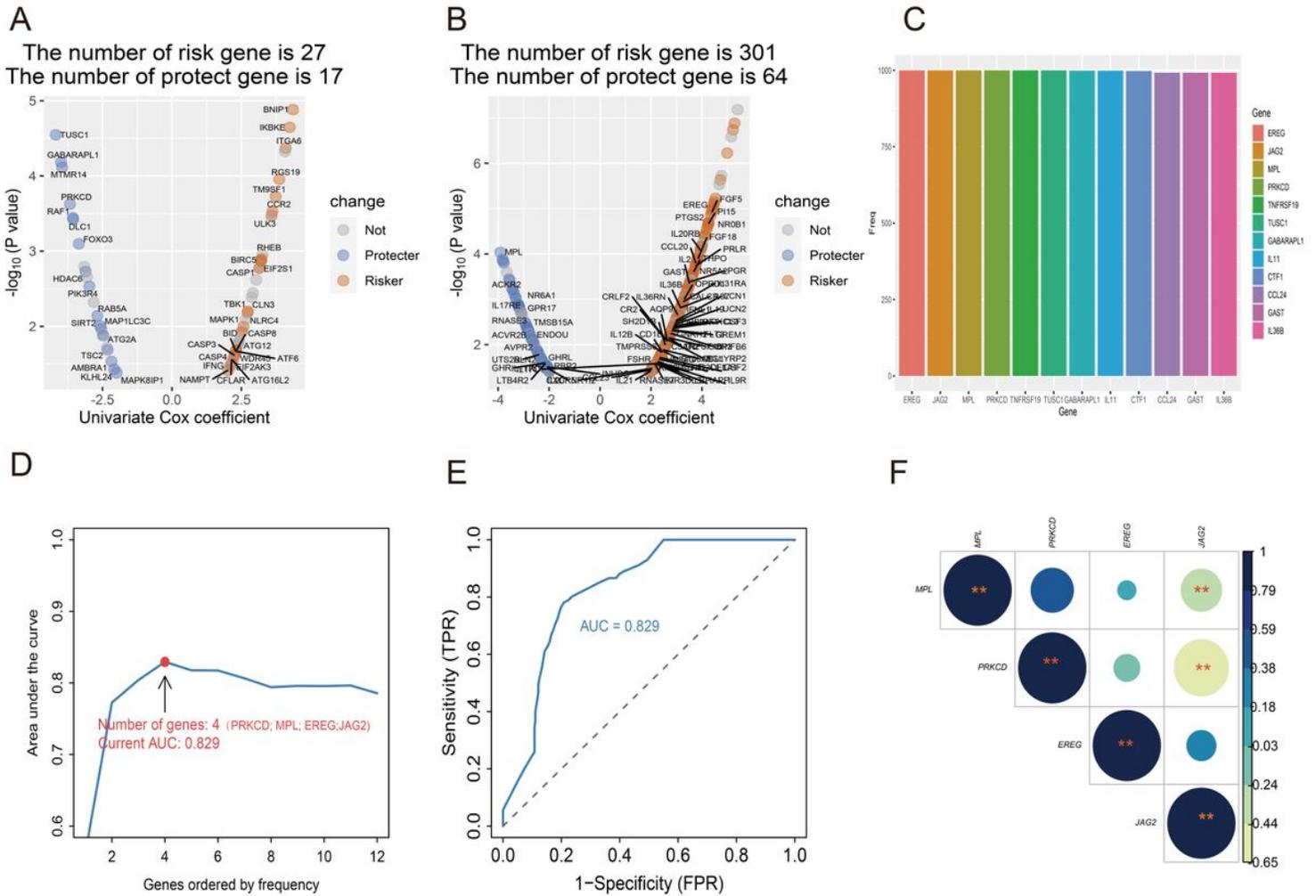


Figure 2
Identification of autophagy and immune related genes (AIRGs) biomarker for survival prediction. A: Volcano plot displayed the univariate Cox regression analysis of autophagy related genes. B: Volcano plot displayed the univariate Cox regression analysis of immune related genes. C: From 1000 iterations of lasso modeling, 12 AIRGs were reported as optimal for survival prediction more than 990 times; D: The AUC curves of AIRGs models, then the number of genes is four, the value of AUC is the highest (0.829). E: The 5 years AUC curve of 4-AIRGs biomarker. F: Correlation analysis of 4-AIRGs including PRKCD, MPL, EREG and JAG2. * $p < 0.05$; ** $p < 0.01$.

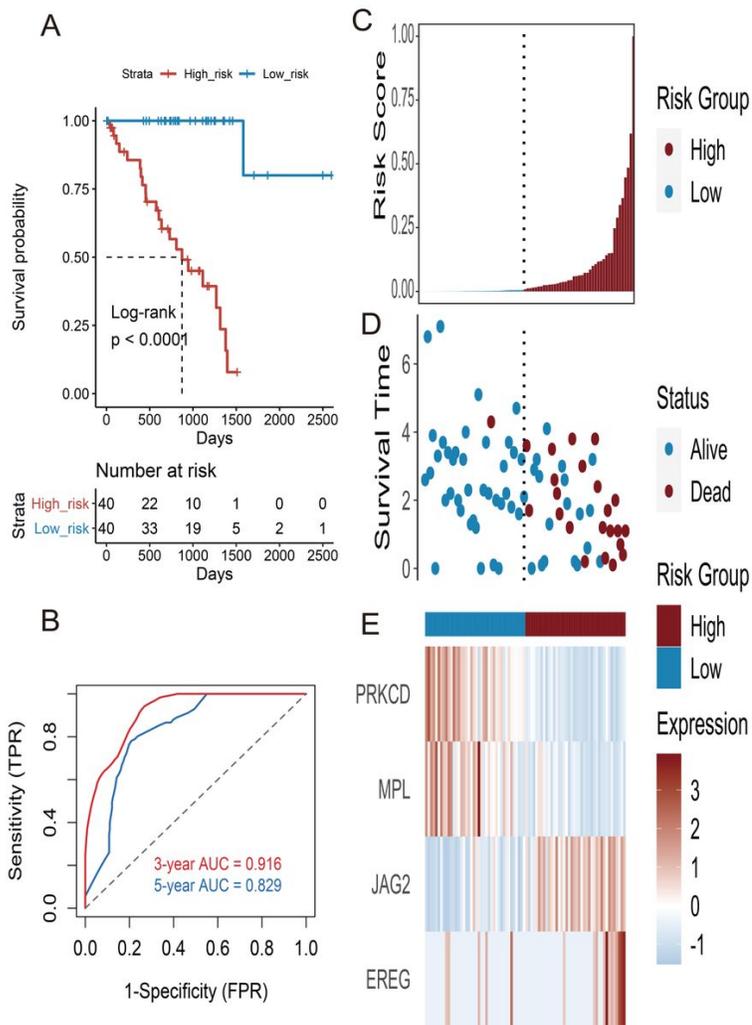


Figure 3

Construction of the 4-AIRGs biomarker in the TCGA-UVM set for predicting UM patients' overall survival (OS). A: The TCGA-UVM set was subjected to Kaplan-Meier analysis to compare OS between patients in the high-risk group and those in the low-risk group; B: The receiver operating characteristic (ROC) curves of 4-AIRGs biomarker in 3, 5 years; C-E: The distribution of risk score (C), OS and life status (D) and the prognostic 4- AIRGs expression patterns for the 80 patients in the TCGA-UVM set (E). The risk scores are arranged in ascending order from left to right.

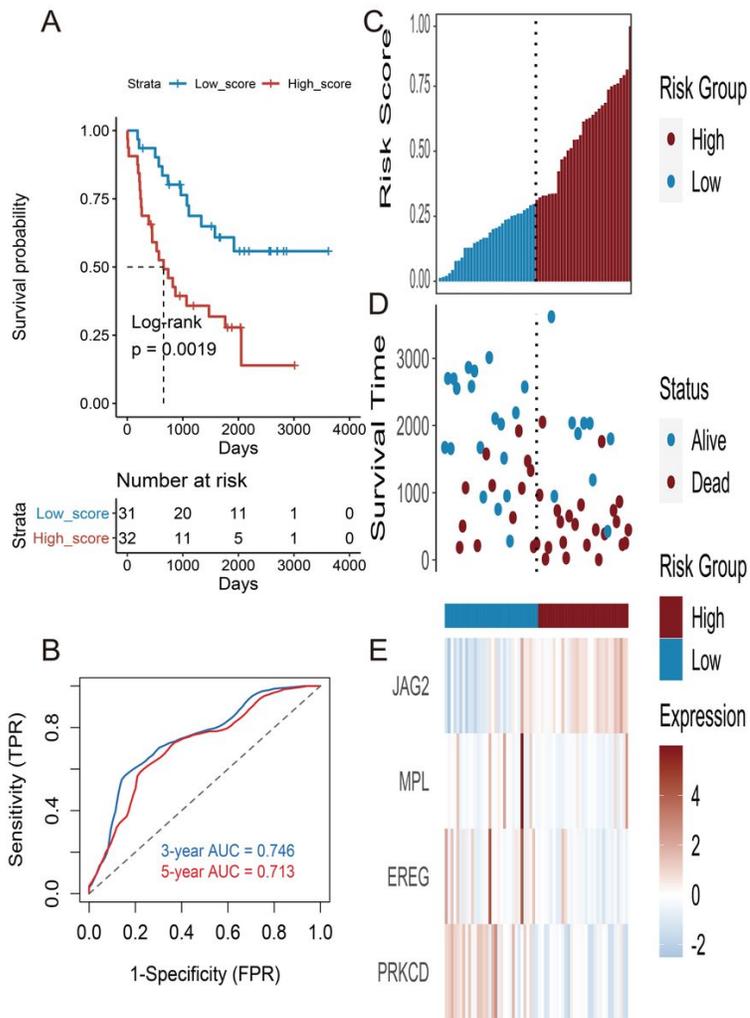


Figure 4

Validation of the prognostic value of the 4-AIRGs biomarker in the GSE22138 sets. A: The GSE22138 set was subjected to Kaplan–Meier analysis to compare metastasis-free survival (MFS) between patients in the high-risk group and those in the low-risk group; B: The receiver operating characteristic (ROC) curves of 4-AIRGs biomarker in 3,5 years; C-E: The distribution of risk score (C), MFS and life status (D) and the prognostic 4- AIRGs expression patterns for the 63 patients in the GSE22138 set (E). The risk scores are arranged in ascending order from left to right.

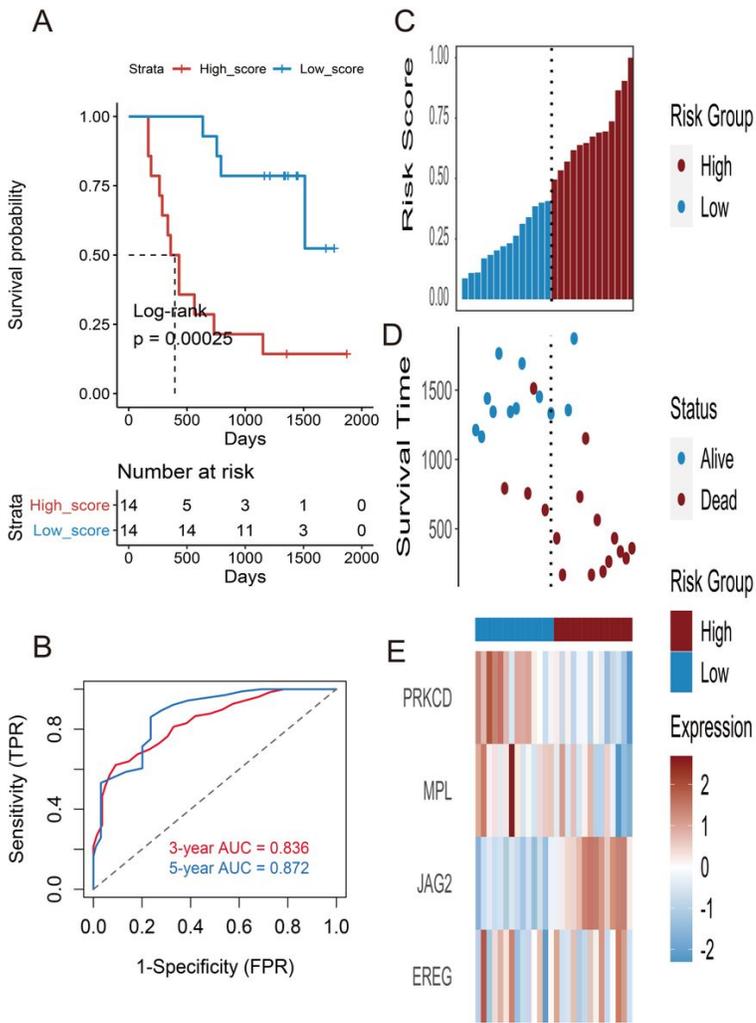


Figure 5

Validation of the prognostic value of the 4-AIRGs biomarker in the GSE84976 sets. A: The GSE84976 set was subjected to Kaplan–Meier analysis to compare overall survival (OS) between patients in the high-risk group and those in the low-risk group; B: The receiver operating characteristic (ROC) curves of 4-AIRGs biomarker in 3,5 years; C-E: The distribution of risk score (C), OS and life status (D) and the prognostic 4- AIRGs expression patterns for the 28 patients in the GSE84976 set (E). The risk scores are arranged in ascending order from left to right.

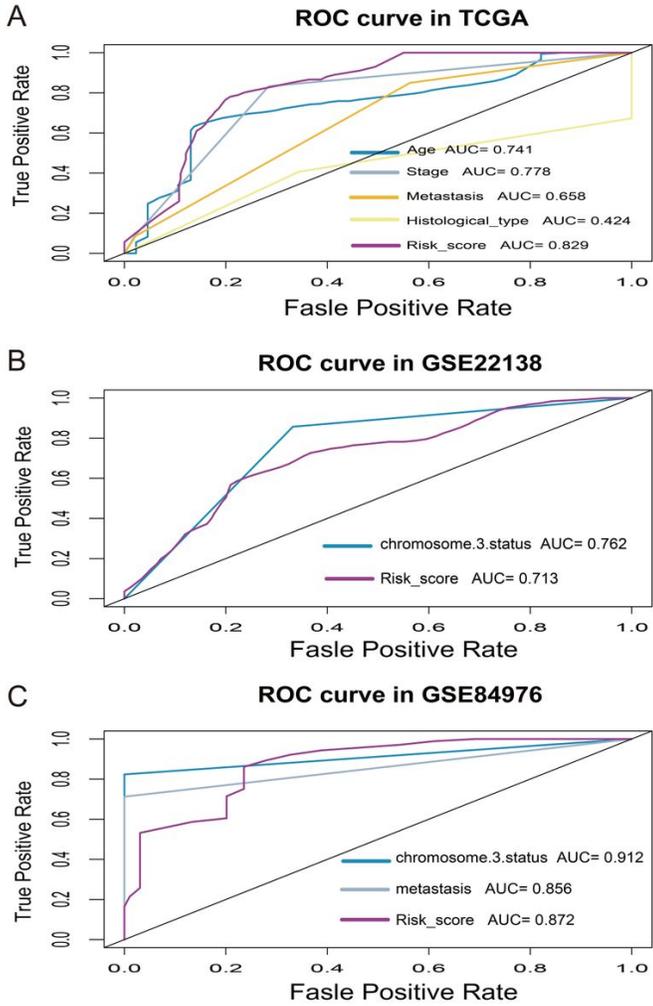


Figure 6

The 5 years area under the curve (AUC) of clinical characters associated with survival; A: The AUCs of age, stage, metastasis, histological type and risk score in TCGA-VUM set; B: The AUCs of chromosome 3 status and risk score in GSE22138 set. C: The AUCs of metastasis, chromosome 3 status and risk score in GSE84976 set.

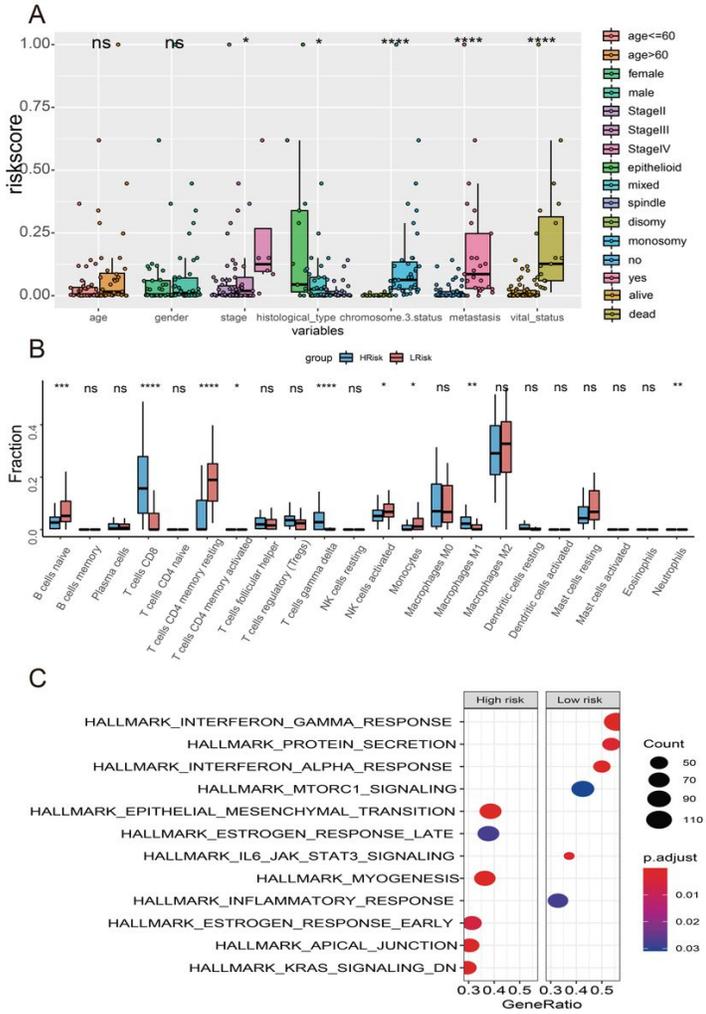


Figure 7

Relationships between risk score with clinical characteristics, immune infiltrating and Gene set enrichment analysis (GSEA). A: The risk score distribution of clinical characteristics including age, gender, stage, histological type, metastasis and vital status. * $p < 0.05$; **** $p < 0.0001$. B: The subgroup analysis of 22 immune infiltrating cells between high and low risk groups. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. C: Gene set enrichment analysis (GSEA) of high vs. low risk scores groups.

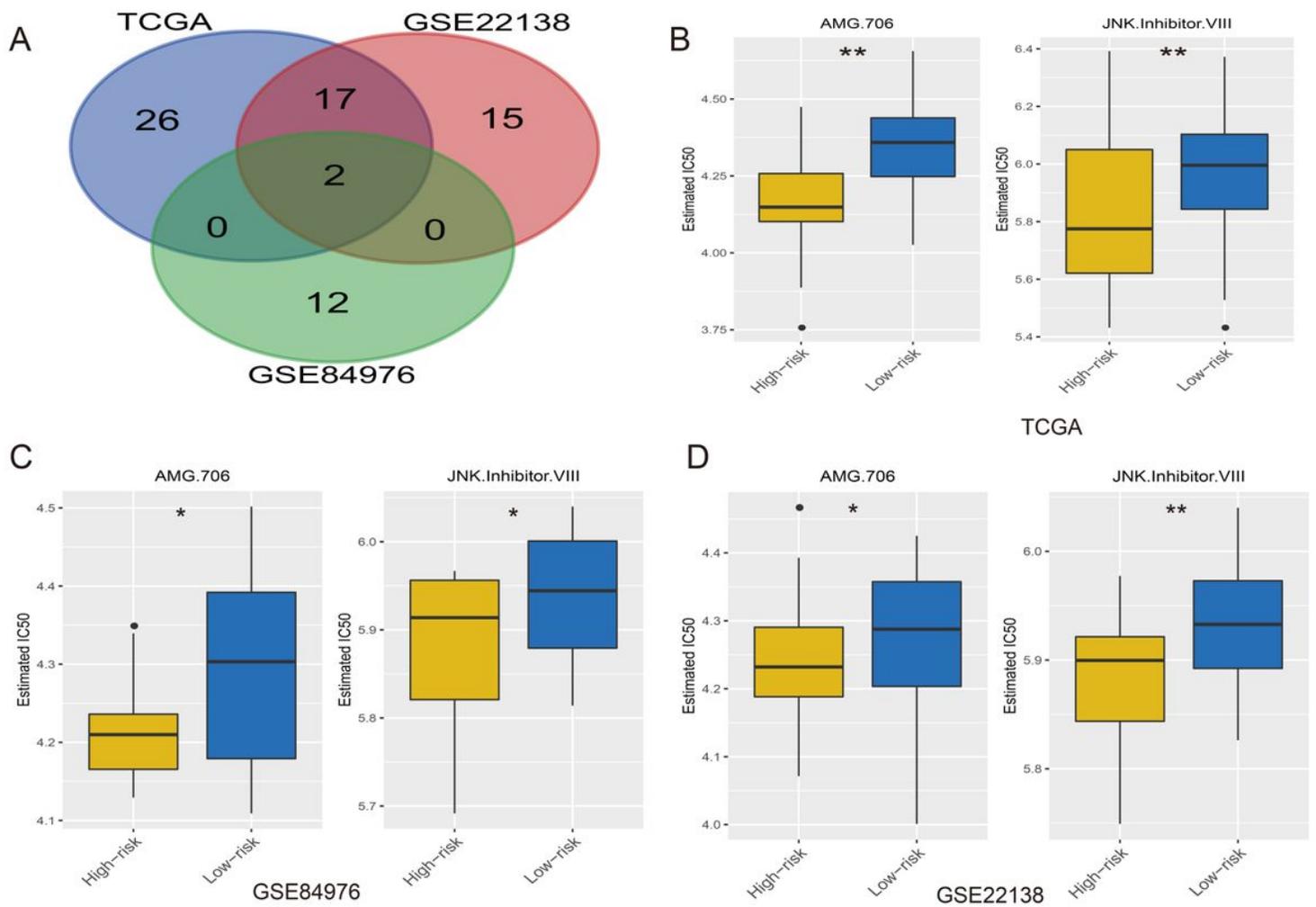


Figure 8

Differential putative chemotherapeutic response. A: The Venn plot of three datasets including TCGA-UVM, GSE84976 and GSE22138; B: The box plots of the estimated IC50 for AMG.706 and JNK.Inhibitor.VIII in TCGA-UVM; C: The box plots of the estimated IC50 for AMG.706 and JNK.Inhibitor.VIII in GSE84976. D: The box plots of the estimated IC50 for AMG.706 and JNK.Inhibitor.VIII in GSE22138. * $P < 0.05$, ** $P < 0.01$.