Research Square

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Development of a Novel Immune-Related Prognostic Signature for Prostate Cancer

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Abstract

Purpose: Prostate cancer (PCa) has a high incidence in older men. In the field of tumor immunology therapy, there are no strong characteristics to predict the survival of PCa in tumor immunology. Therefore, it is necessary to explore the characteristics of PCa in immunology.

Methods: In this study**,** RNA-seq and clinical data of 499 PCa tissue samples and 52 normal tissue samples were obtained from The Cancer Genome Atlas (TCGA). Finally, 193 differentially expressed immune-related genes (IRGs) between PCa and normal prostate tissues were identified based on TCGA. Functional enrichment analyses showed that immunology may act in a tumor-suppressive role in the initiation of Pca, and then we identified different expressed transcription factors (TFs) and construction of the correlation network between TFs and IRGs. Univariate Cox analysis and multivariate Cox regression analysis were performed to identify 5 key prognostic IRGs (S100A2, NOX1, IGHV7-81, AMH, AGTR1). Furthermore, the predictive nomogram was established and verified.

Results: As a result, we successfully constructed and validated an immune-related prediction model for PCa. In this model, 5-genes model showing more stable than other gene groups. Consistent with our expectations, the signature can independently predict the survival outcome of PCa patients. Patients with high-immune risk were found correlated with advanced stage. We also found that high S100A2 gene expression has a lower biochemical recurrence, high AMH gene expression has a higher gleason score, lymph node metastasis rate and tumor grade, a lower lymphnodes positive, high ATGR1 gene expression has a lower psa value.

Conclusion: our study showed that our 5-gene immune-related signature could treated as an independent prognostic indicator for PCa.

Introduction

According to the latest data, PCa is the most common non-skin cancer diagnosed among men and the second leading cause of male cancer deaths in the United States[1, 2]. Most PCa are that originate in the peripheral zone and develop polycentric of prostate[3]. Current studies have identified risk factors such as genetics, diet, hormones, etc[4]. Radical prostatectomy or radiation are the standard primary treatments for patients with localized PCa, while recurrent disease or advanced staged PCa, the main therapy is with or without therapy intensification[5-7]. Endocrine therapy for PCa includes antiandrogen therapy and castration therapy. In recent years, gene therapy for individuals has become more and more popular, and some results have been achieved in the application of clinical cases[8]. In addition, PCa patients are often excluded from molecular investigations and randomized clinical trials for PCa. Therefore, there is still lack of solid data for further investigation the molecular profiling of PCa, which may unearth novel targets for diagnosis, treatment, and prognosis of PCa[9-11].

Despite initial effective responses with androgen suppression therapy (AST), almost all patients ultimately progress to metastatic castration-resistant prostate cancer (mCRPC)[12, 13]. Docetaxel, abiraterone, cabazitaxel, and (Sip-T) are approved by the FDA for the treatment of mCRPC, however each of these regimens provides only limited 2-4 months median survival benefit[14-18]. The median overall survival (OS) for mCRPC patients ranges from 13-32 months with a 15 percent of 5-year survival rate. Therefore, efforts to explore new therapeutic modalities for mCRPC are urgently needed.

In the last decade, there have been significant milestones achieved for immunotherapies. In 2010, the FDA approved the first dendritic cell based vaccine, Sip-T for the treatment of non-symptomatic metastatic prostate cancer[19]. Following this approval, the immune checkpoint CTLA-4 inhibitor, ipilimumab, was approved for the treatment of metastatic melanoma in 2011[20, 21]. Shortly thereafter, immune checkpoint PD-1/PD-L1 inhibitors were approved starting in 2014 for a variety of cancers including lung cancer, kidney cancer, urothelial carcinoma, Hodgkin's disease, breast cancer, as well as for microsatellite high and mismatch repair deficient solid tumors[22-24]. Other than Sip-T, no other immunotherapeutic modality is approved for use in PCa. However, there are currently many ongoing immunotherapeutic clinical trials to assess their immune and clinical efficacy. In the present review, we will discuss advances made in preclinical trials and the tools used in this research and recent clinical trials.

In this study, we utilized the transcriptome data from TCGA to develop and validate an immune-related risk signature consisting of 5 IRGs for PCa. To evaluate the clinical value of the immune signature, we analyzed the correlation between the signature and clinical factors.

Materials And Methods

Data colletion and immune-related genes

Clinical and transcriptomic data of PCa samples were collected from UCSC-TCGA (https://tcga-data.nci.nih.gov/tcga/). The transcriptome profiling of RNA expression was obtained by RNA-seq. Log2-based transformation was used for the normalization of RNA expression profiles.We did an overlap by comparing the obtained RNA-seq data with the HADb database and then standardize the RNA-seq data using the R programming language.

Differentially expressed immune-related genes enrichment analysis

Limma package in R statistical software was applied to estimate differentially expressed IRGs between PCa and normal samples. Genes exhibiting at least 2-fold changes corresponding to an adjusted P-value less than 0.05 were selected as the significantly differentially expressed IRGs. First, heatmap and valcano figure were used for filtrating differentially expressed IRGs between PCa and normal prostate tissues. Then, we performed a series of gene functional enrichment analyses to find the major biological attributes of these genes, including gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). The Database for Annotation, Visualization, and Integrated Discovery (DAVID, https:// david.ncifcrf.gov/), a widely used functional annotation tool, was used to identify enriched GO and KEGG themes. To provide high-dimensional information, the GOplot package of R was performed to concentrate on the visualization of enrichment terms.

Construction and validation of a novel immune-related prognostic signature for prostate cancer

To analyze the IRGs ontology (GO) terms of the immune-related risk signature, gene set enrichment analysis (GSEA) was performed between high-risk and low-risk phenotypes (https://pypi.org/project/gseapy/). 22 Gene ontology gene sets were downloaded from Molecular Signatures Database (MSigDB) (http://software.broadinstitute.org/gsea/ downloads.jsp). We considered the enriched gene sets to be statistically significant in GSEA when the nominal P value was less than 0.05, and the false discovery rate (FDR) was less than 0.25.

In order to identify the key IRGs, univariate and multivariate COX regression analysis were performed by respectively to exclude some IRGs with little prognostic value. According to the weight of each gene in multivariate COX regression analysis, we obtained the correlation coefficient in the model formula for predicting the prognosis of patients. Combined with the expression of various prognosis-related genes, we established an independent prognostic model. The prognostic index was calculated using the following formula $\beta_1 \times gene_1$ expression + $\beta_2 \times gene_2$ expression + ····· + $\beta n \times gene_n$ expression, where β corresponded to the correlation coefficient.

Identification of differently expressed transcription factors (TFs) and construction of the correlation network between TFs and IRGs

We applied the Cistrome database (http://www.cistrome.org/) to predict TF targets and extract enhancer profiles in cancers. The prediction was from integrative analysis of TCGA expression profiles and public ChIP-seq profiles. Then we identified differently expressed TFs by use of “Lima”package in R statistical software between PCa and normal prostate tissues. Correlation test between differently expressed TFs and IRGs was conducted by R programming language. Moreover, we set correlation coefficient at least 0.4 with a P-value less than 0.01 as the remarkably correlated.

Evaluation of the immune-related prognostic signature for prostate cancer

According to our prognostic model, each patient will get a riskscore and we set the median riskscore as the cutoff value for dividing PCa patients into a high-risk group and a low-risk group, respectively. Kaplan-Meier (K-M) method was utilized to plot the survival curves, and the log-rank test was performed to assess differences in the survival rates between high-risk group and low-risk group. The receiver operating characteristic curves (ROC) were created by the“survivalROC” package, and the area under the curve (AUC) values was calculated to evaluate the specificity and sensitivity of the model. The riskscore distribution of patients in different risk groups, survivalstate program showed that patients with high riskscore have high dead rates. The number of censored patients, and the heatmap of prognosis-related immune-related genes were also displayed. A prognostic nomogram was also performed to visualize the relationship between individual predictors and survival rates in patients with immune-related genes based on the Cox proportional hazard regression model by means of “rms” package of R software.

Correlation of the immune-related risk signature with clinicopathologic features

To further improve accuracy of prognostic signature in predicting OS of PCa patients, we integrated age, race, T, N, lymphnodes_positive, gleason_score, psa_value and biochemical_recurrence to further evaluation.

Statistical analysis

The heatmaps were generated by applying R package “ComplexHeatmap” R package. The boxplots were conducted using the R package called “ggplot2”. We calculated c-index with R package “survcomp”.The Student’s t test was used for statistical comparison of paired data. The ANOVA test was conducted for comparison of more than two scores. Pearson’s chi-square tests were performed for comparison of categorical variables. Exact test was performed using R package “stats” version 3.5.1. The statistical analysis of this research was conducted by R language (https://www.r-project.org/). A P value <0.05 was thought to be statistically significant.

Results

Differentially expressed immune-related genes

The research process was showed in Figure 1. Altogether RNA-seq and clinical data of 499 PCa tissue samples and 52 non-tumor samples were downloaded from TCGA. Among these patients, a total of 499 primary PCa patients with gene expression data and clinical follow-up information was involved in the current study. According to the result, we initially screened 193 differentially expressed genes (Figure 2A-2B).

Network analysis of TF-IRG interaction

To explore the interaction between transcription factors (TF) and IRGs, we extracted 22 differentially expressed TFs by analyzing the RNA-seq data described above. The heatmap of TFs with markedly different expression between the PCa tissues and normal prostate tissues were shown in Figure 3A, and we obtained 11 up-expressed and 11 down-expressed TFs (Figure 3B-C). The TF-IRGs interaction network was detailed in Figure 3D.

Functional annotation of the differentiallyexpressed immune-related genes

Functional enrichment analysis of the 193 differentially expressed IRGs offered that the biological understanding of these genes. The GO terms function and KEGG pathway enrichment of these genes were summarized in Table 1. According to the results of DAVID, we found that the top enriched GO terms for biological processes were: positive regulation of response to external stimulus, positive regulation of cell migration and leukocyte migration, and for cellular components were:immunoglobulin complex, circulating, external side of plasma membrane, and immunoglobulin complex. On the basis of molecular function, genes were mostly enriched in terms of receptor ligand activity, growth factor activity, and cytokine activity. The overview schematic of the analysis

results is displayed in Figure 4. Besides, in the KEGG pathway enrichment analysis for the differentially expressed immune-related genes, these genes were shown to be notably associated with Pathways in Cytokine-cytokine receptor interaction, Ras signaling pathway, Neuroactive ligand-receptor interaction, MAPK signaling pathway, EGFR tyrosine kinase inhibitor resistance, and so on. As shown in Figure 4A, the Z-score of enriched pathways less than zero indicated that most of the cancer pathways were more likely to be decreased (Figure 4B).

Construction and validation of the immune-related risk signature

These survival-related genes were subjected to a univariate Cox regression analysis and multivariate Cox regression analysis to remove the genes that might not be an independent indicator in prognosis monitoring. Finally, several prognostic IRGs were obtained. The relationships between the expression profiles of 193 differentially expressed IRGs were obtained from TCGA, resulting in six prognosis-related IRGs(Figure 5A). In order to improve the robustness, six prognosis-related immune-related (S100A2, NOX1, IGHV7-81, AMH and AGTR1, BIRC5) were selected for further multivariate Cox regression model by SPSS 24.0 (Figure 5B). However, the gene of AGTR1 showed no significant prognostic value with $P>0.05$. Finally, five genes including S100A2, NOX1, IGHV7-81, AGTR1 and AMH were identified to develop the model (Table 2).

Evaluation of the immune-related prognostic signature for prostate cancer

After constructing our prognostic model, we validated and evaluated this model. The results from K-M analysis indicated that the high-risk group had shorter survival time than the low-risk group, and *p* value of the log-rank test was 1.163e−03, and the heatmap of prognosis-related IRGs were also displayed(Figure 6A-6B). To evaluate how well the immune-related prognostic signature predicts the prognoses of PCa, the time-dependent ROC curve analysis was carried out. The receiver operating characteristic curves (ROC) were created by the“survivalROC” package, and the area under the curve (AUC) values was calculated to evaluate the specificity and sensitivity of the model. The AUC for the prognostic signature was 0.985, demonstrating the competitive performance of the prognostic signature for survival prediction in the TCGA dataset(Figure 6C). The riskscore distribution of patients in different risk groups showed that the high-risk group has high risk score, and survivalstate program showed that patients with high riskscore have high dead rates(Figure 6E-6F). A prognostic nomogram was also performed to visualize the relationship between nomogram and survival rates in patients with immune-related genes based on the Cox proportional hazard regression model by means of “rms” package of R software(Figure 6D).

Integrated prognostic signature by combining with clinical parameters

Results showed that high S100A2 gene expression has a lower biochemical recurrence, high AMH gene expression has a higher gleason score, lymph node metastasis rate and tumor grade, a lower lymphnodes positive, high ATGR1 gene expression has a lower psa value(Figure 7A-7F).

Disscussion

PCa is the most commonly diagnosed malignancy and ranked the second leading cause of cancer related death in US men. In the United States, the incidence of PCa has surpassed that of, and has become the most common cancer that affects men's health[25]. The incidence of PCa in Asia is much lower than in Europe and the United States, but it has been on the rise in recent years, and the increase is faster than in developed countries in Europe and the United States[26]. However, there are no strong characteristics to predict the survival of PCa in tumor immunology.

In this research, TCGA was used to obtained RNA-seq and clinical data of PCa tissue samples and normal tissue samples, which have provided effective measures for selecting gene signatures. In this research, we deeply mined the expression profiles of IRGs from TCGA and aimed to search prognostic signature for detecting the prognosis of PCa patients. We first screened 193 differentially expressed IRGs between PCa and non-tumor tissues by the the volcano map and the heat map. Based on the results, To explore the interaction between TFs and IRGs, we extracted 22 differentially expressed TFs by analyzing the RNA-seq data described above and The TF-IRGs interaction network was showed in our results. Considering these genes may be depth involved in the initiation of PCa, we performed GO and KEGG analysis of these genes. To identify the key IRGs prognostic signature, univariate and multivariate COX regression analysis were performed, which showed five key IRGs (S100A2, NOX1, IGHV7-81, AMH, AGTR1) could used be prognostic signature for PCa in the TCGA database. On this basis, we validated and evaluated this model and Linked the model to clinical factors.

At present, several studies have built prediction models using database. For example, Wang et al analyzed the RNA-Seq data of 411 BC patients and 19 non-tumor samples from TCGA, and managed to obtain a identification and validation of an individualized autophagy-clinical prognostic index, which exerted a prognosis predicting value[27]. An et al analyzed the RNA-Seq data of 117 serous ovarian cancer tissues and 52 normal ovarian tissues from GEO datasets[28]. Howerer, this is the first study to build a immune predictive model for Pca, and the first correlation of the immune-related risk signature with clinicopathologic features. Due to the lack of enough cases, we failed to validate the expression of five key prognostic IRGs (S100A2, NOX1, IGHV7-81, AMH, AGTR1) between PCA tissue and normal tissues.

In a word, we developed an IRGs expression model that could independently predict the overall survival of PCa patients. Furthermore, our results expressed that use of a targeted IRGs therapy might be a promising future strategy for treating PCa. Further investigations into the molecular mechanisms of IRGs will demonstrate how IRGs affects survival, and provide new suggestions for treating PCs. Thus, our five IRGs signature may predict the overall survival of PCa patients, and also guide the therapeutic approaches used for those patients.

Conclusion

In conclusion, based on the comprehensive analyses with IRGs expression profiles from TCGA, five prognostic IRGs (S100A2, NOX1, IGHV7-81, AMH, AGTR1) were identified could be treated as an independent prognostic indicator for PCa.. Integrated prognostic signature by combining with clinical parameters, we

found that high S100A2 gene expression has a lower biochemical recurrence, high AMH gene expression has a higher gleason score, lymph node metastasis rate and tumor grade, a lower lymphnodes positive, high ATGR1 gene expression has a lower psa value.

Abbreviations

PCa:Prostate cancer; IRG:immune-related gene; TFs:transcription factor; AST:androgen suppression therapy; mCRPC:metastatic castration-resistant prostate cancer; OS:overall survival; GO:gene ontology; ROC:operating characteristic curves; AUC:the area under the curve.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All the authors have consented for the publication.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interest.

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Author's Contribution

Chen Ming and Chen Shuqiu designed the study; Cheng Hong, Wang Yi and Wu Jianping conducted the study and maintained the data; Jing Jibo and Wu Tiange analyzed the data and made the figures; all authors drafted and revised the paper; all authors approved the final version of the manuscript.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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Tables

Table 1: GO terms function of IRGs for PCa patients

ONTOLOGY	ID	Description	pvalue	p.adjust	Count
BP	GO:0032103	positive regulation of response to external stimulus	3.47E-20	1.06E-16	29
BP	GO:0030335	positive regulation of cell migration	1.44E-18	2.19E-15	34
BP	GO:0050900	leukocyte migration	4.38E-18	4.46E-15	33
BP	GO:0042742	defense response to bacterium	6.88E-18	5.25E-15	27
BP	GO:0006959	humoral immune response	1.31E-16	8.01E-14	27
BP	GO:0050727	regulation of inflammatory response	4.10E-15	2.09E-12	28
BP	GO:0060326	cell chemotaxis	1.58E-14	6.87E-12	23
BP	GO:0002460	adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	3.09E-13	1.18E-10	23
BP	GO:0050920	regulation of chemotaxis	4.80E-13	1.63E-10	19
BP	GO:0030595	leukocyte chemotaxis	5.10E-12	1.56E-09	18
CC	GO:0042571	immunoglobulin complex, circulating	2.51E-08	2.38E-06	8
CC	GO:0009897	external side of plasma membrane	3.82E-08	2.38E-06	16
CC	GO:0019814	immunoglobulin complex	4.13E-08	2.38E-06	8
CC	GO:0072562	blood microparticle	4.56E-08	2.38E-06	13
CC	GO:0031012	extracellular matrix	0.000111229	0.004649379	15
CC	GO:0043025	neuronal cell body	0.000670151	0.023343588	13
CC	GO:0034774	secretory granule lumen	0.001628514	0.048622789	10
MF	GO:0048018	receptor ligand activity	1.86E-48	6.31E-46	60
MF	GO:0008083	growth factor activity	9.51E-32	1.62E-29	32
MF	GO:0005125	cytokine activity	2.06E-22	2.33E-20	28
MF	GO:0005126	cytokine receptor binding	1.01E-20	8.55E-19	29
MF	GO:0005179	hormone activity	9.94E-15	6.76E-13	17
MF	GO:0001664	G-protein coupled receptor binding	1.17E-13	6.65E-12	22
MF	GO:0003823	antigen binding	2.68E-11	1.30E-09	17
MF	GO:0042379	chemokine receptor binding	8.61E-11	3.66E-09	11
MF	GO:0070851	growth factor receptor binding	1.31E-10	4.95E-09	14
MF	GO:0008009	chemokine activity	1.89E-09	6.43E-08	9

BP: biological processes CC:cellular components MF:molecular function

Table 1: KEGG pathway enrichment of IRGs for PCa patients

ID	Description	pvalue	p.adjust	Count
hsa04060	Cytokine-cytokine receptor interaction	2.03E-18	3.86E-16	32
hsa04014	Ras signaling pathway	7.05E-10	6.70E-08	20
hsa04080	Neuroactive ligand-receptor interaction	8.91E-08	5.64E-06	21
hsa04010	MAPK signaling pathway	2.25E-07	1.07E-05	19
hsa01521	EGFR tyrosine kinase inhibitor resistance	4.84E-07	1.71E-05	10
hsa04061	Viral protein interaction with cytokine and cytokine receptor	5.40E-07	1.71E-05	11
hsa04015	Rap1 signaling pathway	1.30E-06	3.53E-05	15
hsa04024	cAMP signaling pathway	1.65E-06	3.92E-05	15
hsa04062	Chemokine signaling pathway	1.05E-05	0.000220858	13
hsa04151	PI3K-Akt signaling pathway	1.40E-05	0.000265748	18

Table 2: Univariate Cox regression analysis to filtrate key ARGs for BC patients

id	HR	HR.95L	HR.95H	pvalue
S100A2	1.056105264	1.008492562	1.105965846	0.020380834
NOX1	0.019298037	0.000428891	0.868320058	0.042086476
BIRC5	1.211058502	1.089847975	1.345749799	0.00037223
IGHV7-81	2.550963691	1.525108226	4.266855059	0.000359584
AMH	1.261444149	1.124176792	1.415472506	7.77E-05
AGTR1	1.033184764	1.001946872	1.065396567	0.037148163

Multivariate Cox regression analysis to filtrate key ARGs for BC patients

id	coef	HR	HR.95L	HR.95H	pvalue
S100A2	0.095097498	1.099766075	1.040001191	1.162965418	0.000850669
NOX1	-5.852129483	0.002873773	1.79E-05	0.460277302	0.023848854
IGHV7-81	1.029487511	2.799630687	1.262231918	6.209581514	0.011310899
AMH	0.220039707	1.24612621	1.085665335	1.430303134	0.001756294
AGTR1	0.039940648	1.040749001	0.994116914	1.089568509	0.087694566

Table 3: Clinical correlation analysis between these 5 prognostic IRGs, our established riskscore and clinical features;

id	age	race	T	N	lymphnodes_positive	gleason_score	psa_value	biochemical_rec
S100A2	-0.597(0.551)	0.399(0.819)	-0.868(0.386)	-0.864(0.391)	0.849(0.399)	-0.305(0.760)	-0.017(0.986)	1.993(0.049)
NOX1	-1.193(0.235)	0.263(0.877)	-1.379(0.169)	-0.154(0.878)	0.141(0.888)	-0.861(0.390)	0.093(0.927)	-0.086(0.931)
IGHV7-81	-1.914(0.058)	2.319(0.314)	0.021(0.983)	1.185(0.238)	-1.024(0.307)	-1.845(0.067)	-0.25(0.804)	-0.812(0.419)
AMH	-1.736(0.085)	0.486(0.784)	-3.391(7.813e-04)	-2.562(0.012)	2.903(0.005)	-4.56(9.136e-06)	0.063(0.950)	-1.019(0.311)
AGTR1	-1.954(0.053)	0.197(0.906)	-0.953(0.341)	1.603(0.111)	-1.694(0.092)	1.956(0.051)	2.573(0.013)	0.007(0.994)
riskScore	-0.492(0.623)	0.21(0.900)	-0.352(0.725)	-0.819(0.416)	0.816(0.418)	-1.536(0.127)	1.411(0.159)	1.528(0.128)

Figures

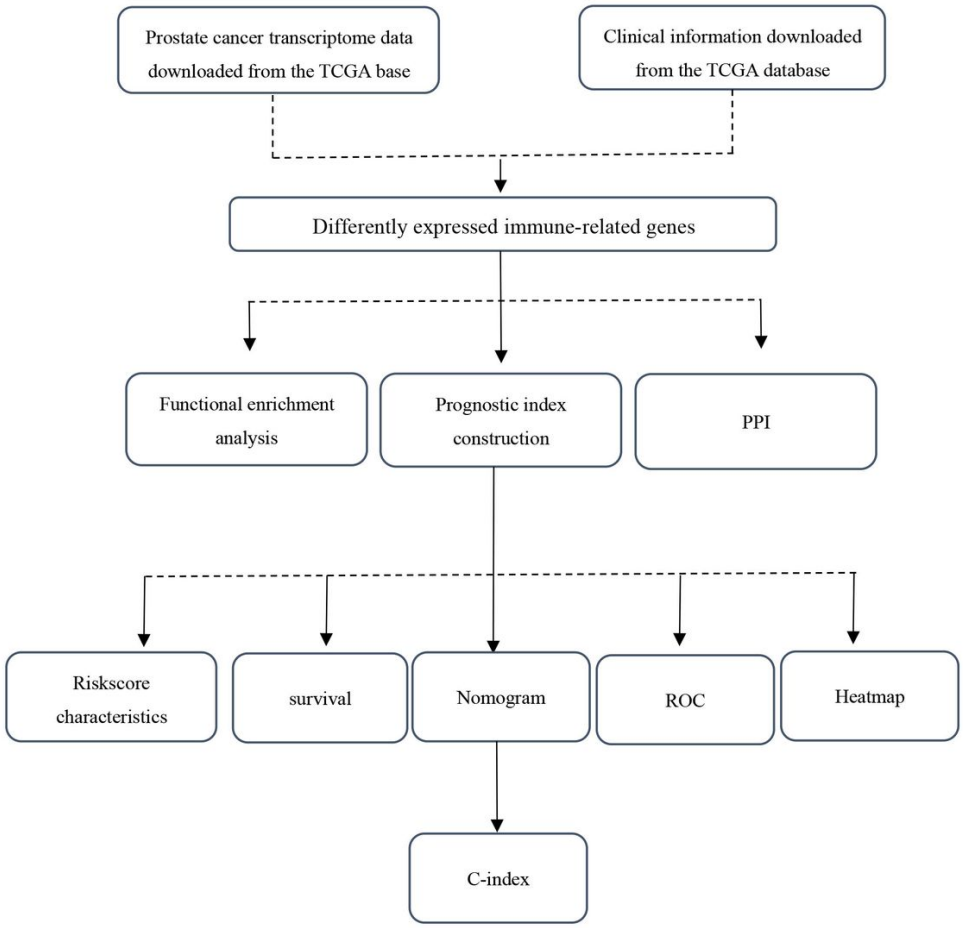


Figure 1

The flow chart of the whole research process

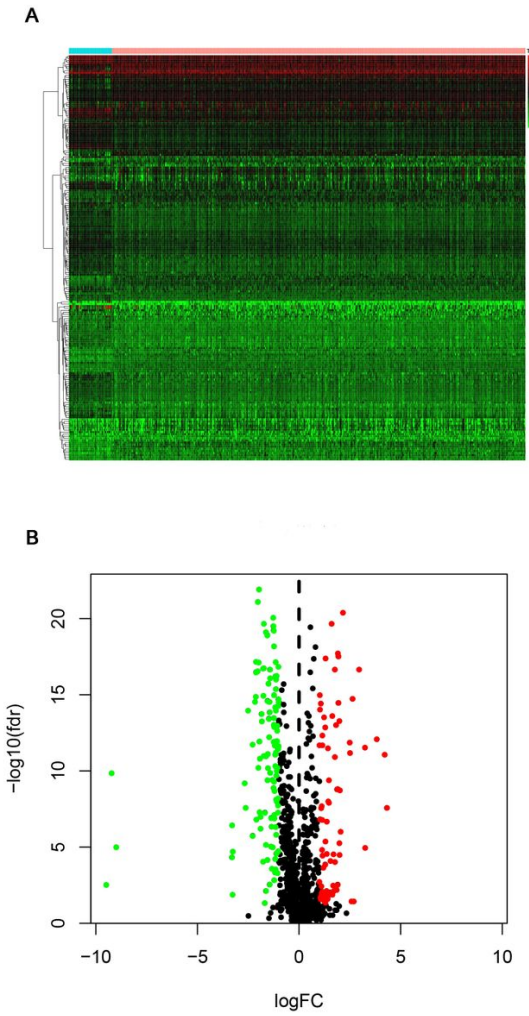


Figure 2

Differentially expressed immune-related genes (IRGs); (A) Heatmap of differentially expressed IRGs; (B) Volcano map of differentially expressed IRGs;

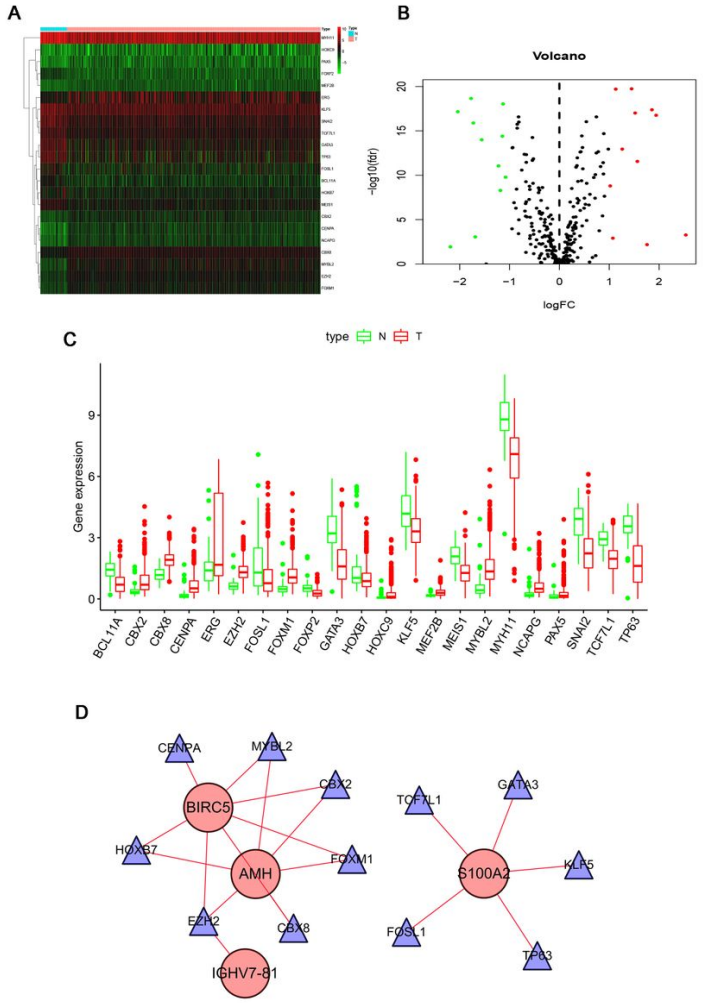
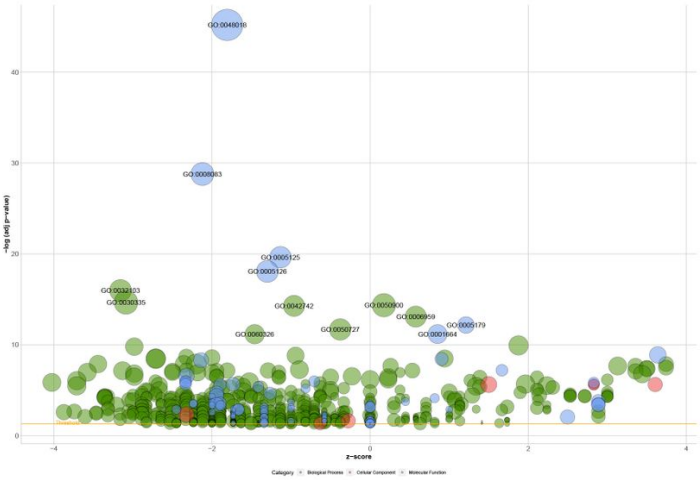


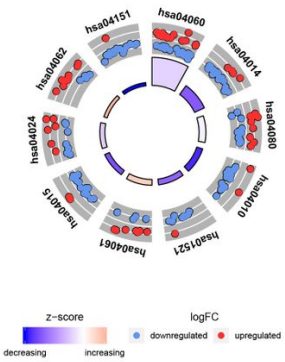
Figure 3

Differentially expressed transcription factors (TFs); (A) Heatmap of differentially expressed TFs; (B) Volcano map of differentially expressed TFs; (C) boxplot map of differentially expressed TFs; (D) A network shows the relationship between TFs and ARGs;

A



B



ID	Description
hsa04060	Cytokine−cytokine receptor interaction
hsa04014	Ras signaling pathway
hsa04080	Neuroactive ligand−receptor interaction
hsa04010	MAPK signaling pathway
hsa01521	EGFR tyrosine kinase inhibitor resistance
hsa04061	Viral protein interaction with cytokine and cytokine recept
hsa04015	Rap1 signaling pathway
hsa04024	cAMP signaling pathway
hsa04062	Chemokine signaling pathway
hsa04151	PI3K−Akt signaling pathway

Figure 4

Functional annotation of differentially expressed immune-related genes (IRGs); (A) The bubble plot of enriched GO terms. Greed circles correspond to the biological process, red indicates the cellular component, and blue shows the molecular function category. (B) Circle diagram of KEGG pathways. Red circles display up-regulation and blue ones down-regulation;

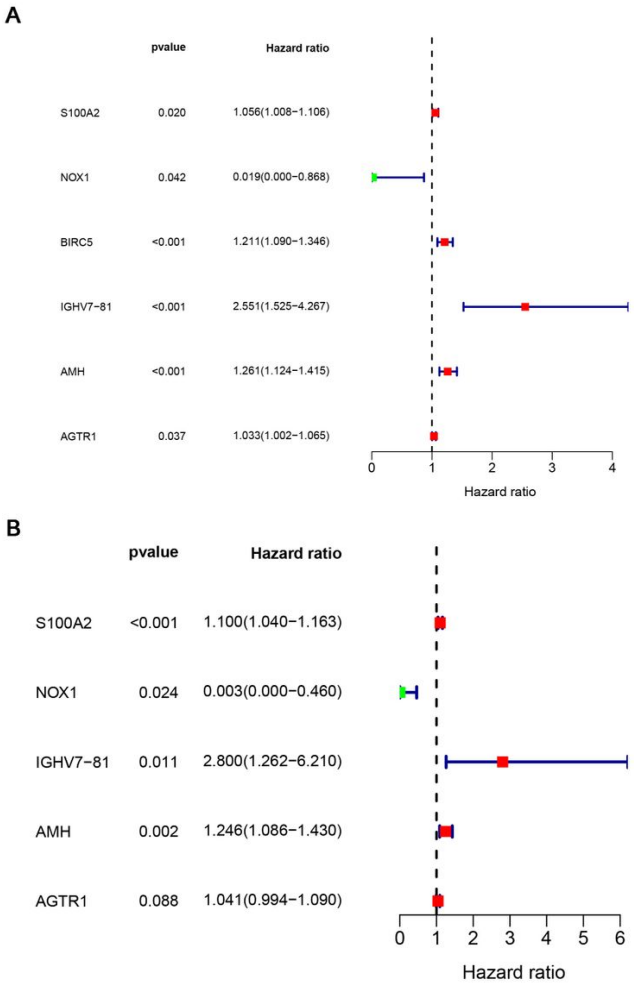


Figure 5

Identification of prognostic IRGs; (A) Univariate cox regression analysis; (B) Multivariate cox regression analysis;

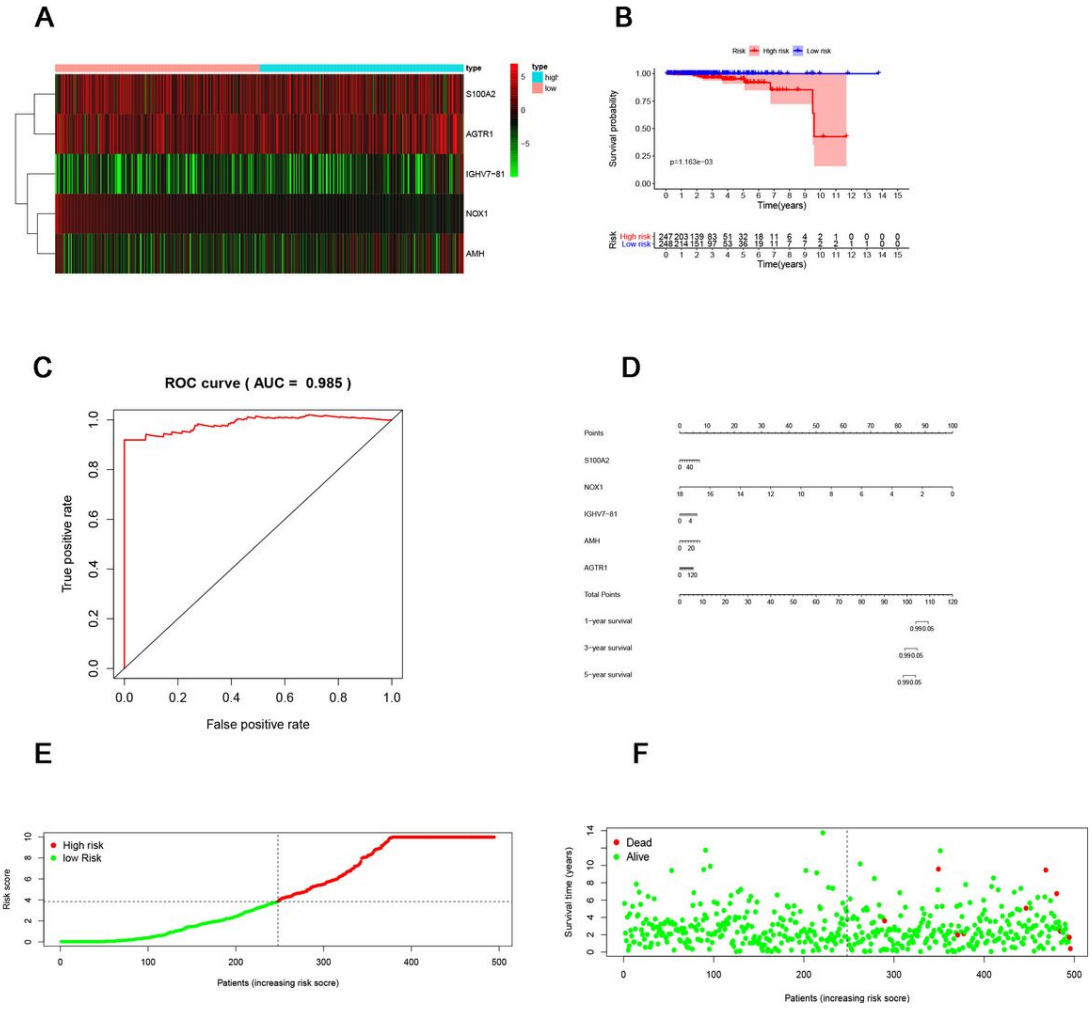


Figure 6

Evaluation of prognostic index based on IRGs for PCa patients; (A) Heatmap; (B) Kaplan–Meier plot; (C) ROC curve; (D) Prognostic nomogram; (E) Riskscore; (F) Survstat;

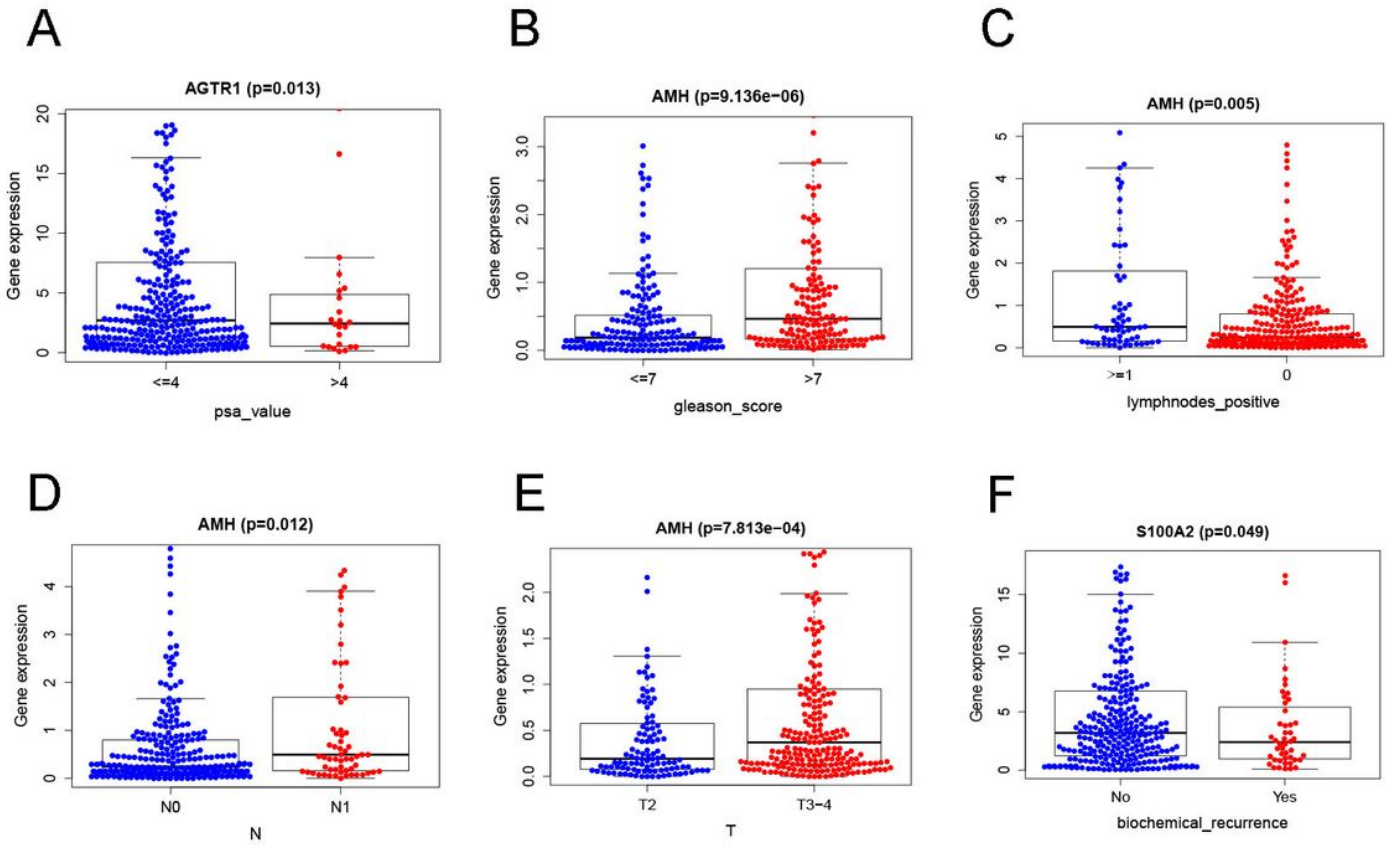


Figure 7

Correlation of the immune-related risk signature with clinicopathologic features (A) ATGR1 and psa_value (B) AMH and gleason_score; (C) AMH and lymphnodes_positive;(D) AMH and N; (E) AMH and T; (F) S100A2 and biochemical_recurrence.