Alternative Polyadenylation Regulatory Factors Signature for Survival Prediction in Kidney Renal Cell Carcinoma

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ABSTRACT

BACKGROUND: Alternative polyadenylation (APA) plays a vital regulatory role in various diseases. It is widely accepted that APA is regulated by APA regulatory factors.

OBJECTIVE: Whether APA regulatory factors affect the prognosis of renal cell carcinoma remains unclear, and this is the main topic of this study.

METHODS: We downloaded the transcriptome and clinical data from The Cancer Genome Atlas (TCGA) database. We used the Lasso regression system to construct an APA model for analyzing the relationship between common APA regulatory factors and renal cell carcinoma. We also validated our APA model using independent GEO datasets (GSE29609, GSE76207).

RESULTS: It was found that the expression levels of 5 APA regulatory factors (CPSF1, CPSF2, CSTF2, PABPC1, and PABPC4) were significantly associated with tumor gene mutation burden (TMB) score in renal clear cell carcinoma, and the risk score constructed using the expression level of 5 key APA regulatory factors could be used to predict the outcome of renal clear cell carcinoma. The TMB score is associated with the remodeling of the immune microenvironment.

CONCLUSIONS: By identifying key APA regulatory factors in renal cell carcinoma and constructing risk scores for key APA regulatory factors, we showed that key APA regulators affect prognosis of renal clear cell carcinoma patients. In addition, the risk score level is associated with TMB, indicating that APA may affect the efficacy of immunotherapy through immune microenvironment-related genes. This helps us better understand the mRNA processing mechanism of renal clear cell carcinoma.

KEYWORDS: Renal clear cell carcinoma, alternative polyadenylation, survival, Lasso regression, immunotherapy

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Introduction

Kidney tumors are one of the top 10 most common malignant tumors, accounting for 3.7% of newly diagnosed tumors. Renal cell carcinoma (RCC) is the most common type, accounting for 85% of cases.[1] The median age at diagnosis is 64 years, and it is more commonly seen in men than in women. The 5-year survival rate of RCC has been constantly improving, but the overall prognosis remains poor, especially in patients with a later-stage disease.[2] In the past 12 years, RCC has been targeted by cytokines, targeted therapy and immunotherapy, however, therapeutic benefits are limit. The most common pathological type of RCC is renal clear cell carcinoma (KIRC), which has poor prognosis and a high degree of malignancy.[3]

mRNA-processing events which include alternative splicing, m6A methylation and alternative polyadenylation (APA), are crucial in the regulation of most human genes in various diseases, such as brain cancer, lung cancer, liver cancer and COVID-19.[4-9] However, there is limited research on the relationship between alternative polyadenylation (APA) and renal clear cell carcinoma. APA is regulated by core polyadenylation trans-factors, including cleavage and polyadenylation specificity factor (CPSF), cleavage stimulatory factor or cleavage stimulation factor (CSTF), cleavage factor (CFim and CFIIm), poly(A) binding protein nuclear 1 (PABPNl), cytoplasmic poly(A) binding proteins (PABPC1 and PABPC4), Factor Interacting With PAPOLA And CPSF1(FIP1L1), Symplekin(SYMPK), and Cleavage and polyadenylation factor subunit(PCF11).[10] Presently, numerous relevant studies have found that APA causes abnormal changes in a variety of tumors.[6,11-15]

Cancer development is highly associated with the physiological state of the tumor microenvironment.[16] Tumor gene mutation burden (TMB) pertains to the number of mutations

in the cancer cell genome and TMB score was associated with multiple immune components and signatures in tumor microenvironment.[17] APA have been reported to be closely associated with the tumor microenvironment in breast cancer.[18] However, few studies have focused on the relationship between APA and tumor microenvironment in kidney cancer.

In this study, we constructed a (least absolute shrinkage and selection operator) lasso regression model using transcriptomic and clinical data of APA regulatory factors in The Cancer Genome Atlas (TCGA) database and found that 5 APA regulatory factors (CPSF1, CPSF2, CSTF2, PABPC1, and PABPC4) play more important roles in renal cell carcinoma. Lasso is a regression analysis method that combines feature selection and regularization to enhance the predictive accuracy and interpretability of statistical models. We found above 5 regulatory factors mainly regulate the mRNA expression of immune-related genes. To further analyze the relationship between APA regulatory factors and clinical features in KIRC and confirm the specific pathway regulating APA regulatory factors, we analyzed the dynamic changes between the expression levels of these APA regulatory factors and clinical features by applying multi-omics data from TCGA. At last, we validated our result in independent datasets of GEO.

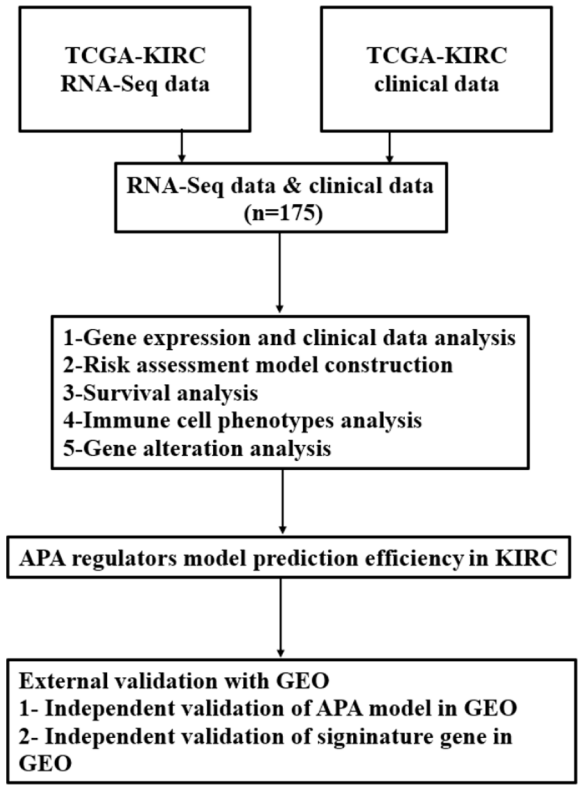

Figure 1. The pipeline of our method.

Methods

Patient dataset collection

Gene expression data of Kidney Renal Clear Cell Carcinoma were downloaded from the TCGA database (https://cancergenome.nih.gov/).[19] RNA-Seq and clinical data were obtained for 538 samples. The downloaded clinical data included information on age, pathological stage, sex, chemotherapy status, follow-up date, and survival status. Those with missing survival data, missing follow-up date, and survival less than 1 month duration were excluded, and the remaining data were further matched with gene expression data. Finally, 175 patients with both gene expression and clinical data were obtained. To validate our results analyzed from TCGA, independent RNA-Seq data for Renal Cell Carcinoma from the cancer and normal tissue groups were downloaded from the GEO database GSE76207 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE76207)[20] and GSE29609 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE29609).[21]

Immune cell phenotypes analysis

All analyses involved in this study were performed using the R software (version 3.5.1). The method flow of the KIRC classification model and the prognosis model construction is shown in Figure 1. First, the immune cell infiltration levels of KIRC tumor tissue and normal tissue were evaluated using TIMER (https://cistrome.shinyapps.io/timer/).[22] Then, the immune cell score composed of 8 immune cells was constructed using lasso regression combined with clinical characteristics to construct the KIRC model by Cox regression. TIMER can recognize 22 immune cell phenotypes, including B cells, T-cells, natural killer cells, macrophages, dendritic cells, and bone marrow subpopulations, with high sensitivity and specificity.

Gene expression and clinical data analysis

Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/), developed by Peking University, is an interactive web server that integrates and helps analyze cancer expression profile data, including RNA sequencing expression data of tumor samples and normal samples from 33 kinds of tumors in the TCGA and GTEx.[23,24] In this study, the GEPIAs database was used to plot survival curves. Cbioportal (https://www.cbioportal.org/study/clinicalData?id=kirc_tcga) is a TCGA online data analysis platform that includes data on gene mutation, transcriptome, and proteomics. Oncoprints were performed using Cbioportal software. Differential gene expression analyses were performed by DESeq2 according to the read counts, read counts of each gene determined by HTSeq. Genes with $P \leqslant .05$ and mean CPM (Couts per Million) > 100 were determined to be significantly differentially expressed genes, as we descripted previously.[25]

Risk assessment model construction

Lasso regression helps obtain a more concise model by constructing a penalty function to compress some regression

coefficients and set some regression coefficients to zero.[26] In this study, the R-package "glmnet" was used to select the penalty parameter with the minimum error through 10-fold-change cross-validation λ, to select the most effective prognostic marker and its corresponding regression coefficient in the construction of the risk score. According to the risk model, samples in TCGA were assigned a score and then divided into high-risk and low-risk groups with the median risk score as the threshold. The survival curves of the patients in the high- and low-risk groups were drawn with the R-package "Survival," and the survival times of the 2 groups were compared using log-rank test. We developed a computational framework through integrating gene expressions and clinical data in TCGA and GEO (Figure 1).

Results

Construction of APA regulator signature-based risk assessment model in KIRC

Analysis of the gene expression of APA regulatory factors in TCGA renal cell carcinoma samples revealed that CPSF1, CPSF2, CPSF3, CPSF4, CPSF6, CPSF7, FIP1L1, CSTF1, CSTF2, CSTF2T, PCF11, SYMPK, PAPOLG, PABPC1, PABPC4, and PABPN1 were significantly different between tumor tissues and normal tissues (Figure 2A). CPSF4L, CSTF3, PAPOG were not significantly different between tumor tissues and normal tissues. Lasso Cox regression analysis was performed on the above significantly differently expressed APA regulators. The penalty parameter lambda was selected by the cross-validation method to obtain relatively independent feature genes for subsequent model analysis (Figure 2B and C). The risk score for each patient was calculated using the following formula: Risk score = 0.01927*EXP(CPSF1) + (−0.00165)*EXP(CSTF2) + (−0.00927)*EXP(CPSF4) + (−0.01672)*EXP (PABPC1) + (−0.00255)*EXP (PABPC4) (Figure 2B and C). All kidney cancer patients from TCGA were classified as high or low risk according to the optimum cut-off risk score in the KIRC cohort. Interestingly, GO enrichment analysis of differently expressed genes between high-risk group and low-risk group indicated that APA regulators were associated with immune microenvironment-related genes in KIRC (Figure 2D). We speculated that APA regulatory factors might regulate immune microenvironment-related APA to affect the immunotherapy of KIRC.

Hallmarks and survival analysis of APA regulators

To further investigate the relationship of immune microenvironment and APA regulators, all patients classified as high or low risk were used to performed TMB score. TMB score partly explains the clinical response to immunotherapy, and we found that the high-risk group had low TMB (Figure 3A), indicating lower levels of neoantigens that can be recognized by the immune system. OS was also compared between the 2 groups using Kaplan-Meier analysis. The results (Figure 3B) showed a different survival curve based on the variables selected by the Cox model in the KIRC, suggesting the significance of the APA regulators in the separation of the 2 risk groups. Furthermore, we downloaded the most frequently mutated genes (VHL, PBRM1, SETD2, BAP1, MTOR, PTEN, KDM5C, ARID1A, TP53, and SPEN) in KIRC. The patients were ranked by the risk score (formula given earlier). The mutation status of these genes is shown in Figure 2C. Interestingly, the mutation rates of VHL, PBRM1, MTOR, and SETD2 were low in the high-risk score group. However, BAP1 mutations were mutually exclusive with PBRM1, MTOR, and SETD2. In addition, we did not find an anomalous trend in the mutation status of PTEN, KDM5C, ARID1A, TP53, and SPEN. This could be due to the low mutation rates of these genes.

APA regulators risk assessment model for predicting the immune status of KIRC

Just as we expected, higher expression levels of CPSF1, CPSF2, CSTF2, PABPC1, and PABPC4 were significantly associated with increased tumor infiltration of CD4+ T-cells and neutrophils (Figure 4). CPSF1 was associated with tumor infiltration of CD4+ T-cells and neutrophils in the KIRC. CPSF2, PABPC1, and PABPC4 were associated with purity, B cells, CD8+ T-cells, CD4+ T-cells, macrophages, neutrophils, and dendritic cells. However, we found that CSTF2 had no relationship with the purity of KIRC.

Survival analysis of five genes in KIRC

Furthermore, 3 (CPSF1, CPSF2, and CSTF2) of the 5 genes were closely correlated with the overall survival (OS) in KIRC. The high expression levels of CSTF2 and CPSF2 were associated with superior OS in KIRC, indicating that CSTF2 and CPSF2 are risk factors for KIRC. The low expression level of CPSF1 is associated with poor survival time in KIRC, indicating that CPSF1 is not a risk factor for KIRC. Thus, most key APA regulatory factors were significantly correlated with prognosis (Figure 5).

Dynamic APA to APA regulatory factor network and risk model for predicting survival in KIRC

In fact, Hu et al have constructed a multi-omic data based 7-gene model in KIRC previously. To compare with this model, we plotted the receiver operating characteristic (ROC) curve of the true-positive rate (sensitivity) as a function of the false-positive rate for 7-gene model and our APA model. We found the AUC of their 7-gene model was 0.64 in our data. Our APA model shows better performance comparing with previously published 7-gene model (Figure 6A). Therefore, the results show that our risk model has a very

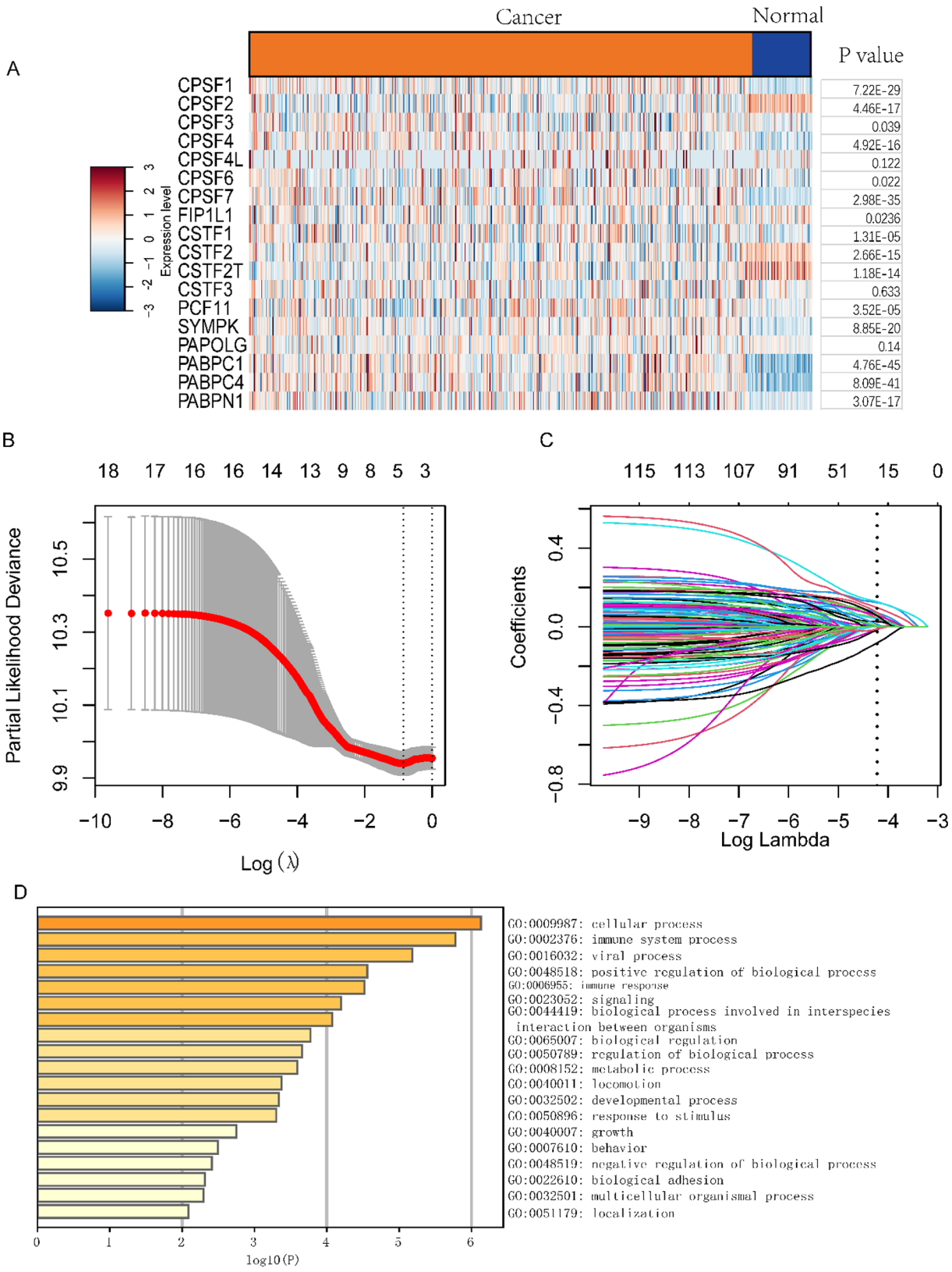

Figure 2. Feature selection using the LASSO Cox regression model. (A) Heatmap showing the expression level of APA regulator in cancer and normal tissue. (B) The partial likelihood deviance was plotted versus log (lambda). The y-axis indicates the partial likelihood deviance, while the lower x-axis indicates the log (lambda) and the upper x-axis represents the average number of predictors. (C) LASSO coefficient profiles. The coefficients (y-axis) were plotted against log (lambda) and 5 features with nonzero coefficients were selected to build the radiomics signature. (D) GO-Term analysis of differently expressed genes between high-risk group and low-risk group.

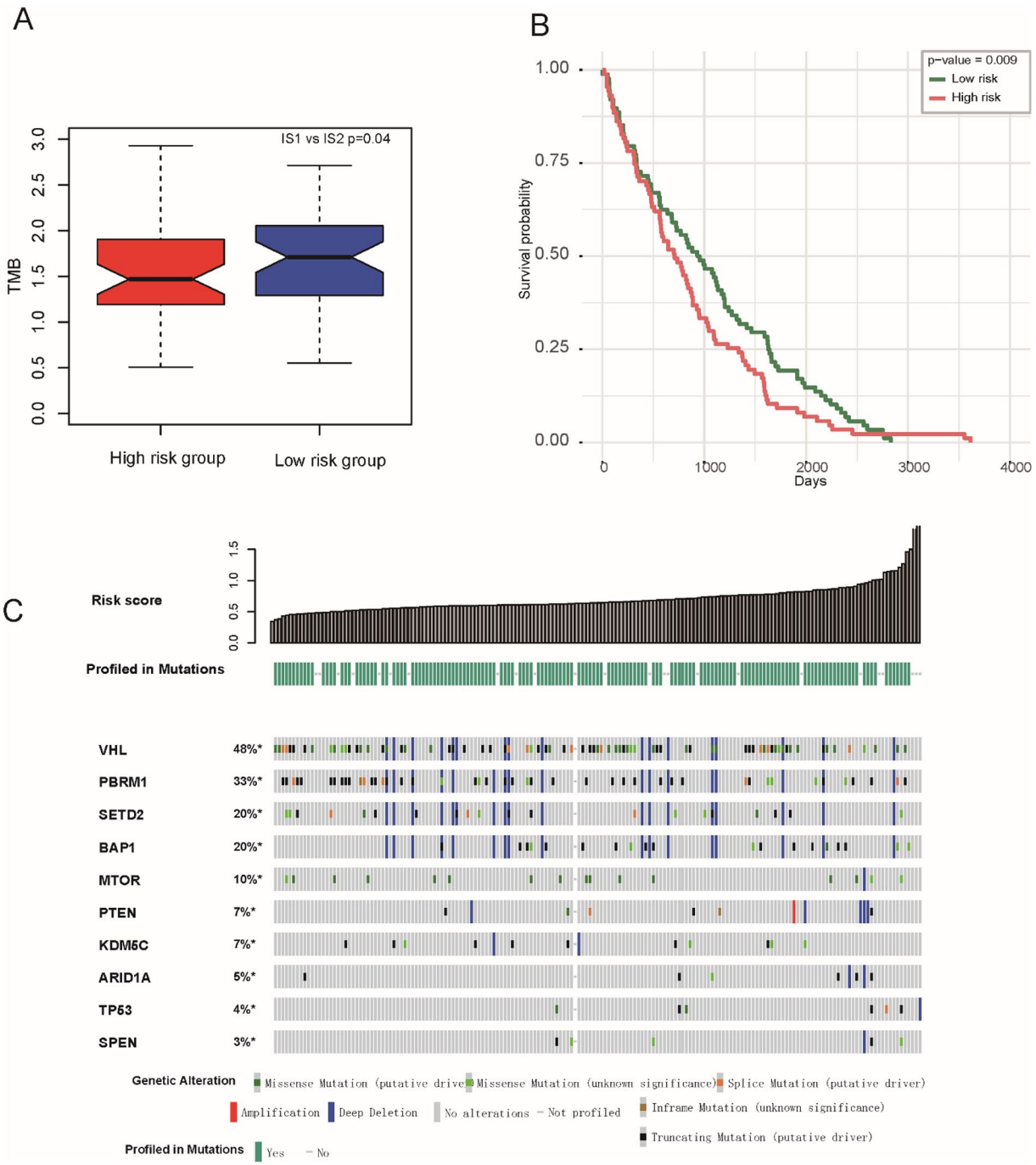

Figure 3. LASSO Cox regression model predicted TMB and gene alternative status of KIRC. (A) Mutation burden in high-risk score group versus low-risk score groups. (B) Survival curves obtained for the genes exclusively selected by the COX method, when analyzed individually. (C) Oncoprint plot showing key genes mutated in KIRC. Each column denotes an individual tumor and each row represents a gene. Colors indicate type of gene alternative as indicated in the legend below the oncoprint.

good predictive efficiency in KIRC. To further validate our results concluded in TCGA datasets, we analyzed RNA-Seq data of other independent RNA-Seq data for Renal Cell Carcinoma from the cancer and normal tissue groups and observed the same results (Figure 6B),[20] suggesting 5 APA regulatory factors (CPSF1, CPSF2, CSTF2, PABPC1, and PABPC4) were significantly associated with development of KIRC.

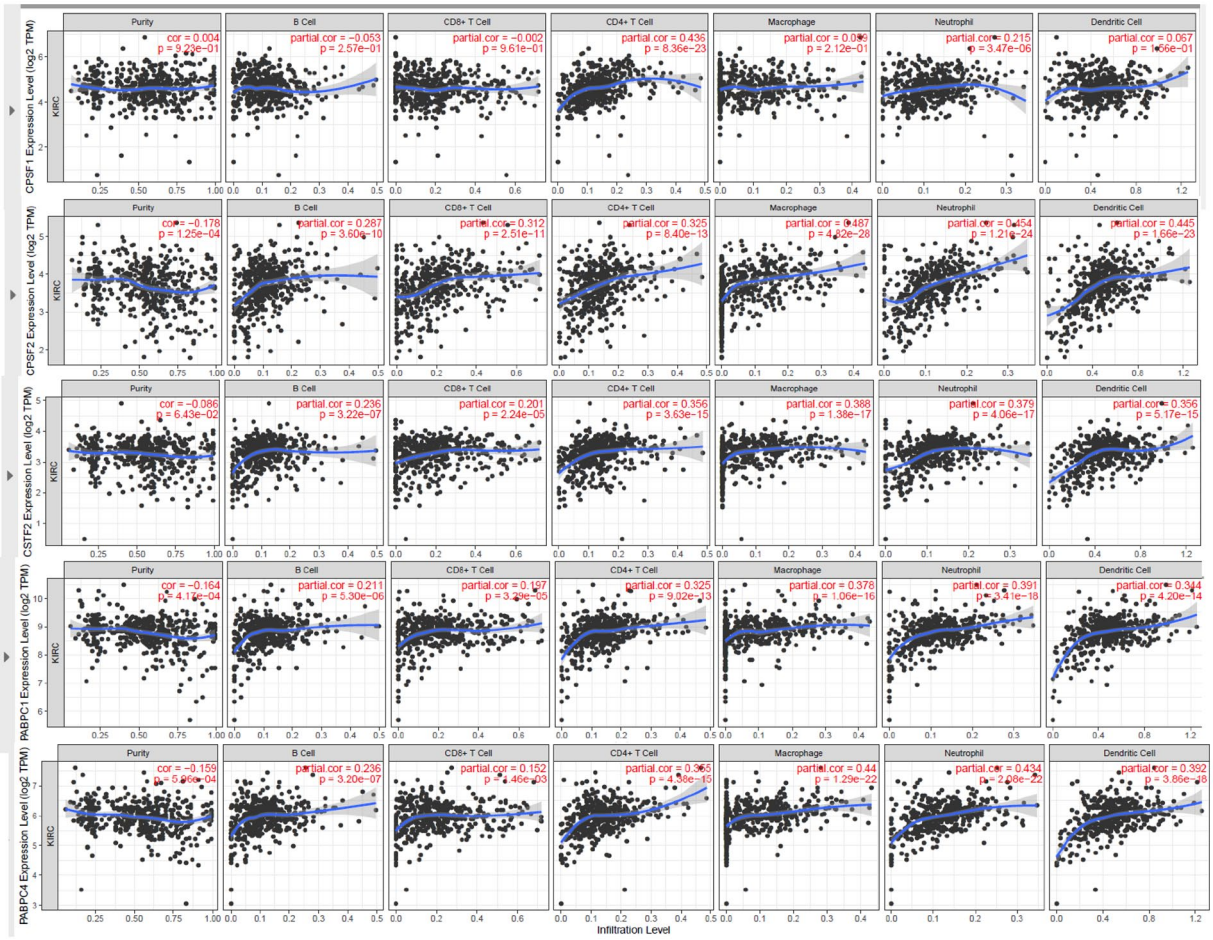

Figure 4. Expression level of 5 APA regulators CPSF1, CPSF2, CSTF2, PABPC1, and PABPC4 is correlated with the level of immune infiltration in KIRC. *P* value and correlation coefficient are indicated.

Discussion

APA has been reported to drive oncogenic gene expression in many cancers, particularly kidney cancer.[27] Therefore, APA regulators which regulated APA may be an excellent predictor of survival in renal cell carcinoma. The research of APA regulators including CPSF, CFim and CFIIm, PABPC1 and PABPC4, CSTF, PABPNl, FIP1L1, PCF11, and SYMPK[28-32] is limit. To date, there has been no research focusing on the role of APA regulators in KIRC.

Previous research has found that APA plays an important role in renal cancer using bioinformatics analysis.[27] There are 2 potential mechanisms of APA regulation during tumorigenesis. APA is regulated in *cis* through genetic aberrations or in *trans* by regulatory proteins.[10,33-35] Our research highlights the role of regulatory proteins in APA. In this study, we constructed a Lasso regression model using transcriptomic data and clinical data of APA regulatory factors in the TCGA database and found that 5 APA regulatory factors (CPSF1, CPSF2, CSTF2, PABPC1, and PABPC4) play more important roles than other APA regulators in renal cell carcinoma. These 5 regulatory factors may mainly regulate the APA of immune-related genes. Previous research has reported that APA is associated with immune-related genes in breast cancer,[36,37] and this mechanism was confirmed by our research in KIRC. Furthermore, our results indicate that APA in renal cell carcinoma may be mainly regulated by these 5 regulatory factors rather than other APA factors and that immune cells of KIRC are associated with APA regulators. Even though, these results may not be further investigated by wet experiments, bioinformatics analysis in 2 independent databases make our results reliable.

The results of this study will enhance our understanding of the underlying roles of APA in KIRC. The conclusions might be meaningful to improve the understand of mechanism of KIRC and provide directions for future treatment trends.

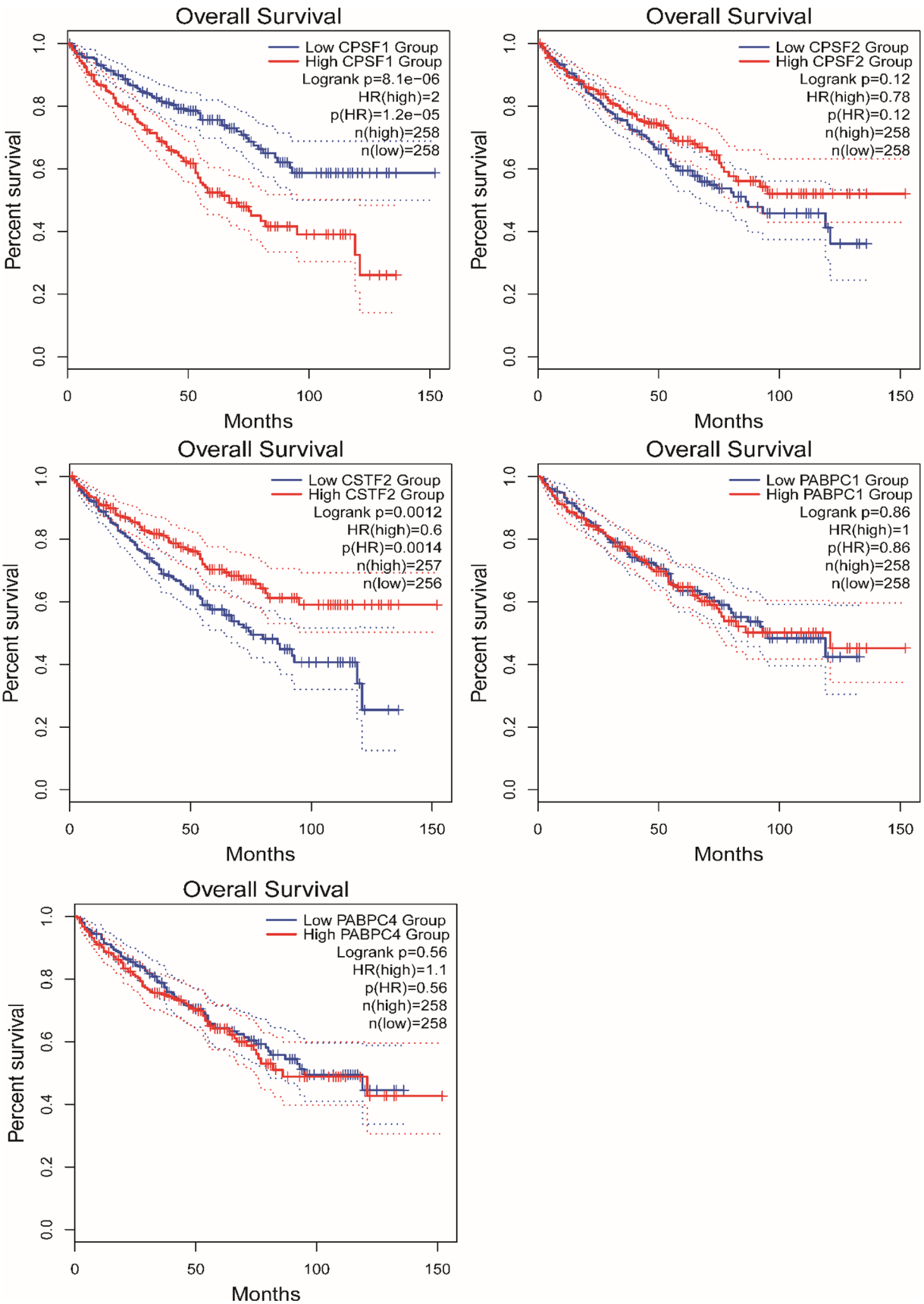

Figure 5. Kaplan-Meier survival plots representing the correlations between the expression level of 5 APA regulators expression levels in KIRC. P value is indicated.

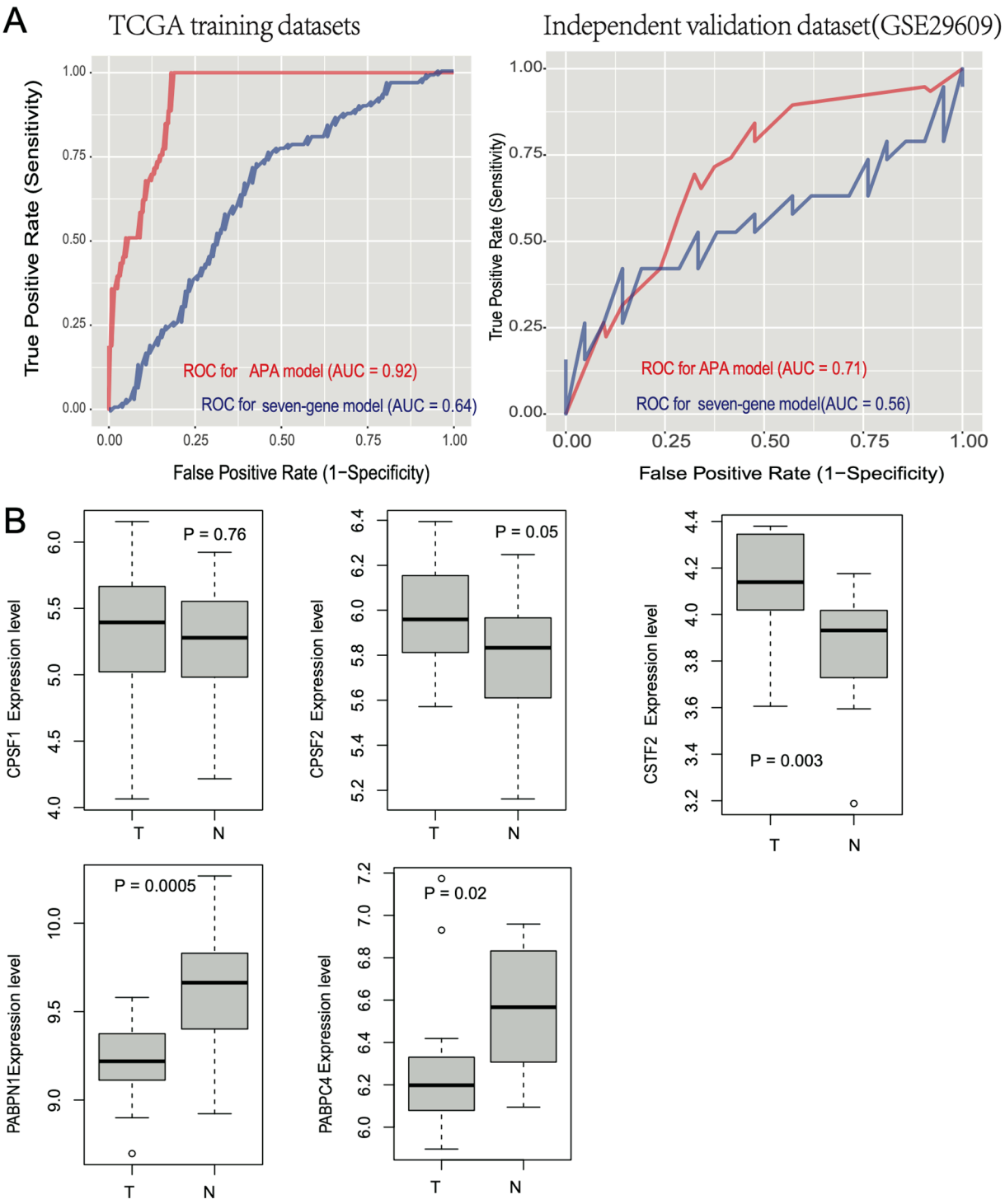

Figure 6. ROC curve and data validation. (A) TCGA and GSE29609 receiver operating characteristic (ROC) curve for APA risk model (red line) and 7-gene model (blue line) for the prognosis of KIRC. (B) Boxplot of gene expression level of 5 APA regulators CPSF1, CPSF2, CSTF2, PABPC1, and PABPC4 in validation datasets. *P* value of 2-tailed *t* test is indicated.
Abbreviations: N, normal; T, tumor.

Author Contributions

Xiaoyu Wang, Yueqi Li and Mingcong Chen designed the project; Yueqi Li and Yao Lin performed data collections and conducted the analysis; Xiaoyu Wang, Yueqi Li, Zheng Li and Yao Lin participated in writing the manuscript; Mingcong Chen revised the paper. All authors read and approved the final manuscript.

Data Availability Statement

The datasets analyzed during the current study are available in TCGA database (https://cancergenome.nih.gov/), TIMER datebase (https://cistrome.shinyapps.io/timer/), GEPIA database (http://gepia.cancer-pku.cn/), and Cbioportal datebase (https://www.cbioportal.org/study/clinicalData?id=kirc_tcga).

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